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Special Lecture on Teaching Physiology

P.002

Evolutionary biology in the medical curriculum: Darwinian evolution and cardiovascular remodeling

Bernard Swynghedauw
U942-INSERM, Hôpital Lariboisière, Paris, France

Evolutionary medicine, is a tool which can be utilized to decipher certain aspects of cardiovascular, CV, diseases such as cardiac remodeling, CR, and the ensuing heart failure, HF, but also arterial remodeling and its consequences on arterial impedance and atherogenesis. Mechanics is crucial in cardiology and 'the cellular responses to physical forces', also called mechano-transduction, MT, is central to CV pathophysiology.

Mechano-transduction, MT, is an ancient evolutionary legacy existing in every living species which involves complex rearrangements of multiple proteins in response to a mechanical stress. MT includes three different interrelated steps: mechano-sensation, mechano-transmission and mechano-response. Each step is specifically adapted to a given tissue and stress. Both cardiac and arterial remodeling involves MT.

Physiological or pathological cardiac remodeling, CR, is firstly a beneficial mechano-response, MR, which allows the heart to recover a normal economy better adapted to the new working conditions. Nevertheless, exercise-induced cardiac remodeling is more a coming-back to normal conditions than a superimposed event. On a longer term, the MR creates fibrosis which accounts, in part, for the reduced cardiac output in CR.

In the hypertension-induced arterial remodeling, arterial MR allows the vessels to maintain a normal circumferential constraint before an augmented arterial pressure. In atherogenesis: (i) the presence of atheroma in several animal species and atherosclerosis in ancient civilizations suggests more basic predispositions. (ii) The atherosclerotic plaques preferably develop at predictable arterial sites of disturbed blood flow showing that MT is involved in the initial steps of atherogenesis.

FEPS Teaching Symposium: New Challenges in Teaching Physiology to Medical Students

SYM.001

Practical courses in the medical curriculum: pros and cons

Levente Kiss
Department of Physiology, Semmelweis University, Budapest, Hungary

Traditionally, practical courses were core parts of the curriculum just like lectures. Nowadays, when the relevancy of lectures is questioned the financial constraints and curriculum trends are questioning the importance of practical courses as well. The disproportionately increased number of students in medical schools made it more difficult to maintain practical courses while the trend of problem based learning raised the argument that these programs of pre-clinical departments are expensive and outdated components of the curriculum. This assertion is also financially tempting as it might be argued that the cost-benefit ratio of practical courses are not feasible because 21st century doctors need different skills than doing some experiment proving an information already found in textbooks. The costs necessary to maintain a proper practical course are high indeed. Apart from the direct costs of the practical course such as the salary of the assistance staff the indirect costs could also be quite high. Without proper staff numbers the wear and tear of the involved tutors can potentially lead to less scientific output which could damage the reputation of the university. Proponents of practical courses state that it is still important - perhaps more imperative than ever – as this is the key part of the curriculum which ingrains and reinforces the sound, evidence based thinking, exactly what is needed in properly trained doctors.

The lecture aims to give context on why to consider maintaining or reinstalling practical courses in the curriculum and delineates what is necessary to make them worthwhile.

SYM.002

Simulations as teaching tools for medical students

Stefan Titz
Institute of Physiology and Pathophysiology, University of Heidelberg, Heidelberg, Germany

Simulations can be versatile tools especially to teach procedural skills in medical education. Hence a variety of high and low fidelity simulation tools are commercially available. On the other hand, one of the most important tasks in teaching physiology, is to facilitate the comprehension of the mechanisms of body function. Therefore, and in light of the sometimes immense costs of simulating tools, the benefit of such tools for the students has to be carefully weighted. Simulations might allow self-directed learning and -assessment, replace the use of *in vitro* preparations, allow access to pathophysiological examples and provide an additional motivation to be engaged with a certain topic. At the same time technical features can distract students and teachers from the actual learning goals and/or resources are spent to a small part of the curriculum and lack therefore in others.

Over the last 10 years we used a variety of simulations in different curricular settings to teach physiology to preclinical medical students. Among these were self-constructed low fidelity tools, online simulations and more sophisticated tools like the cardiopulmonary patient simulator 'Harvey'. In our hands the success and acceptance of a simulation depends critically on several factors which are independent from the simulation tool itself. These include the way of integration in the curriculum, the student perception of an additional benefit and the support given by the teacher.

SYM.005

Advantages of iBooks learning in physiology education (from theory to practice)

Bruno Chenuel
Physiology Department – EA DevAH – Medical School and University Hospital of Nancy, Vandoeuvre-Les-Nancy, France

Textbook-based dDidactic lectures have been the gold standard way of imparting knowledge to students for centuries. But it was a passive way, requiring experiments to relate theory to practice. Nowadays, the use of new technologies allows mixing theory and practical training, enhancing student's interactivity and their active learning experience. Computers are now exceptionally powerful resources that can be used for the robust multimedia presentation of physiological concepts. However the methods by which professors elaborate and distribute course content is undergoing significant change through resources. Consequently, how to efficiently blend into one document, the advantages of paper content for a course, which enables students to personalize content through highlighting and note taking, and electronic content, which incorporates multimedia elements for students to gain a greater perspective, is becoming a pedagogical challenge.

Some new digital contents and online environments seem to be particularly well designed for teaching of physiology such as iBooks and the online learning platform « Lt » from AD instruments, for example. iBooks is a tool from Apple to create interactive, multi-touch digital books for iPad. Readers can flick through rich multimedia contents such as photo galleries, rotate 3D objects, or play video and audio. This iBooks format allows highlighting and note-taking procedures, appearing automatically on study cards. « Lt » is a specialized cloud-based platform, combining multimedia life science content and real time data recording and analysis together, and enhancing practical training in Physiology.

We report our most recent experience using both systems at the Medical School of Nancy.

FEPS Opening Ceremony

Plenary Lecture

P.003

Signaling properties of homeoprotein transcription factors in the nervous system

Alain Prochiantz
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Homeoproteins represent a family of a few hundred transcription factors first identified through the analysis of developmental mutants. However, they are also expressed in the adult where they probably serve several physiological functions in and outside the nervous system.

Most homeoproteins contain two conserved regions allowing intercellular transfer, a rather unexpected finding that led to the proposal that homeoproteins are not only cell autonomous transcription factors but also non-cell autonomous signaling entities.

During brain development, this novel signaling mechanism participates in the formation of brain compartments, cell migration and axon guidance. In the post-natal and adult mouse, homeoprotein signaling regulates important aspects of cerebral cortex plasticity, in particular the ability for external and internal stimuli to modify neuronal circuits morphology and physiology.

The fact that homeoproteins are internalized by live cells suggests that blocking or, alternatively, enhancing their capture through pharmacological or genetic manipulations could be of therapeutic interest. This will be illustrated with animal models of neurological and psychiatric diseases.

Finally, it will be shown that these novel signaling proteins, at least for the few that have been studied, display several non-cell autonomous modes of action that include the regulation of protein translation and gene transcription, plus the control of the chromatin epigenetic status.

Future of Neuromodulation in Neuropsychiatric Diseases

SYM.008

Brain-machine interfaces for Parkinson's disease and tremor

Peter Brown
Medical Research Council Brain Network Dynamics Unit at the University of Oxford, Oxford, UK

Think of your central heating being on at full blast in every room at home, all of the time, winter or summer. That is the state of current electrical brain stimulation therapy for Parkinson's disease and Tremor. Patients undergo fixed, continuous, regular high frequency stimulation of key brain targets through an implanted pacemaker irrespective of their clinical state at any given moment. Just as domestic central heating tracks temperature using a thermostat, we should be able to identify the core brain circuit changes underpinning symptoms and continually monitor these to optimally control stimulation. Understanding these circuit changes may also tell us with what pattern to best stimulate for any given symptom complex. We are just beginning to understand the aberrant circuit dynamics in Parkinson's disease and Tremor sufficiently well to pilot such closed loop and intelligently patterned stimulation regimes. Using pathological circuit dynamics to guide the delivery of therapeutic brain stimulation should heighten specificity and may improve efficacy, efficiency and reduce side-effects. I will demonstrate how recordings in patients have led to the development of temporally patterned forms of electrical stimulation for improved therapy in Parkinson's disease and Tremor, and discuss their implementation thus far. These advances offer a potentially highly selective form of electrical brain stimulation that can be extended to other disorders as underlying causal circuit mechanisms become clear.

Update on Renal Hemodynamics

SYM.011

Capillary filtration in the kidney glomerulus and why it matters

Marcus Moeller
Nephrology, University Hospital Aix-La-Chapelle (Aachen), Pauwelsstrasse, Germany

The glomerular filter produces about 180 l of protein-free primary urine, and never clogs. It is still not completely resolved how nature accomplishes this miracle. If we do not understand how the filter functions, we cannot explain why and how it fails in disease: The pathogenesis of proteinuria or why it predisposes to cardio-vascular mortality is still not known.

The generally accepted *pore-model* for glomerular filtration is probably incomplete because it cannot explain multiple unresolved characteristics. We have shown that an electrical field is generated by glomerular filtration (termed *streaming potential*). Extra-cellular streaming potentials are a novel biophysical phenomenon in capillary filtration. Their electrical field is proportional to filtration pressures (=glomerular filtration rate, GFR). It drives the plasma proteins back into the blood by electrophoresis. This novel *electrokinetic model* of glomerular filtration can provide mechanistic explanations for most of the remaining unresolved characteristics of the filter, including the pathogenesis of proteinuria. We propose that the *electrokinetic model* is also valid in filtering capillaries outside of the kidney glomerulus (explaining the need for a blood–brain-barrier).

In the second part, we propose that the electrical field also drives charged soluble growth factors across filtering capillaries. We show that VEGF-A backfiltration may be driven across the glomerular filter by electrophoresis. Since the electrical field is directly proportional to filtration pressures, we provide evidence that this novel mechanism may couple GFR to glomerular hypertrophy. Outside the kidney, the same mechanism is predicted to couple capillary filtration to neoangiogenesis.

SYM.012

Determinants of hypertension during chronic kidney disease: results from the nephrotest cohort study

Emmanuelle Vidal-Petiot[1], Marie Metzger[2], Jean-Jacques Boffa[3], Jean-Philippe Haymann[4], Eric Thervet[5], Pascal Houillier[6], Bénédicte Stengel[2], François Vrtovsnik[7], Martin Flamant[1]

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Hypertension is highly prevalent during chronic kidney disease (CKD) and in turn worsens CKD prognosis. We aimed to describe the determinants of blood pressure (BP) control and resistant hypertension during CKD.

We performed a cross-sectional analysis from the baseline visit of the tricentric nephrotest cohort study (n = 2084). Patients with CKD stage 1–5 underwent thorough renal exploration including measurement of GFR (clearance of ^{51}CrEDTA) and of extracellular water (ECW, volume of distribution of the tracer). Hypertension was defined as BP (average of three office measurements) above 140/90 mmHg or the use of antihypertensive drugs. Resistant hypertension was defined as uncontrolled BP (>140/90) despite three drugs including a diuretic or the use of four or more drugs, regardless of BP level.

In 1938 patients with complete dataset (mean age 59 years, 67% male, mean GFR 41 mL/min/1.73 m^2), prevalence of hypertension was 94%. In the 1821 hypertensive patients, prevalence of uncontrolled and resistant hypertension were 42.2 and 31.7%, respectively. On multivariate analysis, older age, higher albuminuria, increased ECW, hypokalemia, african origin, diabetic nephropathy and the absence of aldosterone blockers were independently associated with uncontrolled BP. Lower GFR, higher albuminuria, ECW, uricemia and BMI, African origin, diabetes, diabetic nephropathy, and previous cardiovascular disease were associated with resistant hypertension.

CKD is associated with hypertension severity but not with BP control. ECW is an independent determinant of both resistant and uncontrolled hypertension during CKD, which advocates for the large use of diuretics in this population.

SYM.013

Alterations of renal hemodynamics: the cornerstone of renal disease?

Christos Chatziantoniou

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Regulation of renal hemodynamics is a major physiological function of the organism controlling homeostasis, blood pressure and urine formation. The composition of the primitive urine is the consequence of the ultrafiltration of plasma depending on several interdependent mechanisms such as renal blood flow, hydrostatic pressure of glomerular capillary, and glomerular coefficient of ultrafiltration. Under physiological conditions, the renal blood flow (RBF) and the glomerular filtration rate (GFR) are stable parameters regulated by the intrinsic vascular and tubular autoregulation, the balance between paracrine and endocrine agents acting as vasoconstrictors and vasodilators, and the effects of renal sympathetic nerves.

The mechanisms controlling renal hemodynamics are highly complex. This is due to the variety of vasoactive agents and their targets, and multiple interactions between them. Nevertheless, the stability of RBF and GFR during important variations of systemic haemodynamics and volaemia is due to three major operating mechanisms: autoregulation of renal vascular tone, local synthesis and action of angiotensin II, and the sensitivity of renal resistance vessels to respond to NO release.

Alterations of renal hemodynamics are involved in early phases of renal injury and participate in the subsequent progression towards chronic disease in almost all models of renal injury. In addition, several novel for the kidney mediators have been identified that are activated during dysfunction of renal vessels to subsequently induce inflammation and fibrosis. Some of them have the potential to be pharmacologically targeted providing thus, additional prospects of therapy against chronic kidney diseases.

Vascular Functions of Connexins

SYM.014

Connexin-specific differences in gap junction channel gating and permeation

Janis M Burt, Tasha K Pontifex, Nicole L Jacobsen, Jose F Ek Vitorin

Department of Physiology, University of Arizona, Tucson, AZ, USA

Connexins comprise a family of proteins with 21 members. They facilitate coordinated function of

tissues by providing an intercellular path, gap junction channel, for the diffusive exchange of ions, messenger molecules such as IP_3 and cyclic nucleotides, and metabolites. Interaction of connexins with multiple intracellular proteins regulates channel functions as well as cell behavior in growth, injury and disease settings. Based on studies of the structure and function of gap junction channels, it is widely assumed that the mechanisms underlying their gating and permeation properties are similar and comparably regulated, irrespective of connexin composition and despite considerable, connexin-specific differences in channel properties. With the goal of defining their potential contribution to the development and response of arterial vessels to injury and disease, we have used electrophysiologic, imaging and molecular approaches to compare the gating and permeation functions of channels composed of either Cx37 or Cx43, connexins alternatively expressed in arterial endothelium. Cx37 forms large conductance, cation selective, transjunctional voltage-sensitive channels that only reside in the fully open conductive state when specific sites in its carboxyl terminus (CT) are phosphorylated by growth factor activated kinases. Cx37 is also able to regulate cell cycle arrest/progression and apoptototic death behavior of cells through phosphorylation events in its CT. These unique characteristics of Cx37 involve the entire protein; no region of the Cx37 sequence confers onto Cx43 similar channel or growth regulatory properties, which suggests that the three dimensional structure and integrated function of the channel are essential to these properties of Cx37.

SYM.016

Role of gap junctions in vasodilation

Kim A Dora
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Introduction: Gap junctions between cells within the wall of small arteries and arterioles facilitate the transfer of homocellular and heterocellular signals. Hyperpolarizing current readily transfers from endothelial to smooth muscle cells through myoendothelial gap junctions, and can also pass longitudinally between cells to underlie coordinated local and conducted dilation. The aim of this study was to establish the role of gap junctions in local and conducted dilation.
Materials and methods: Experiments were performed using freshly isolated arteries and arterioles, which were cannulated and pressurized. Ca^{2+} indicator dyes were selectively loaded into the endothelium and/or smooth muscle cells and imaged using confocal microscopy. Local and conducted vasodilation were stimulated by either bath or focal application of agonists. In addition, vasoconstrictor agonists were applied to the bath to demonstrate heterocellular signalling from smooth muscle to endothelial cells.
Results: Endothelium-dependent agonists were able to stimulate hyperpolarization of smooth muscle cells and dilation that was prevented in either the presence of non-selective gap junction uncouplers or inhibitory antibodies to connexins. Conducted dilation relies on the intercellular passage of current via the endothelium. Smooth muscle-selective agonists were able to stimulate hyperpolarization of endothelial cells secondary to the movement of a Ca^{2+} signal, most likely via myoendothelial gap junctions, forming a feedback pathway against vasoconstriction.
Discussion: The gap junctions within the walls of resistance arteries and arterioles are not only vital in the electrical control of arteriolar diameter, but also by intercellular Ca^{2+} signalling.

SYM.017

Channel independent functions of connexins

Kristin Pogoda
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Connexins are a family of 21 transmembrane proteins, best known as essential constituents of Gap Junctions. Connexins have a similar structural organization, consisting of a channel-building N-terminal part, highly conserved between members of the connexin family and a cytosolic carboxyl tail, which varies in length and composition. Post-translational modifications of the carboxyl tail and the ability to interact with other proteins determine the functional status and modify channel permeability. However, recent investigations using deletion mutants or connexins incompetent for proper channel forming clearly demonstrated that biological functions of connexins are not restricted to the ability to form gap junction channels or hemichannels. Growth inhibiting and tumor suppressive functions of connexins as well as stimulating effects on cell differentiation seem to be regulated channel independently. Effects on gene expression of pathways including cell growth and apoptosis have also been shown by over-expression or deletion studies. A channel independent role of cell migration has been reported for Cx43 *in vitro* and *in vivo* in different cell types. The principal mechanism which underlies this non-channel action is the capability of the C-Terminus to interact with proteins involved in the control of cell migration. For instance a Cx43/ZO-1 interaction seems to be necessary for a faster wound healing of endothelial cells. Our group could show that Cx43 enhances cell migration and migration related changes of actin dynamics via activation of PAK1/p38/Hsp27 signalling in a channel independent manner. Thus, via such protein interactions connexins can potentially act as part of multiple signal transduction pathways.

Workshop

WS.001

PHENOMIN, your French partner for understanding mammalian gene function in mouse disease models

Elodie Bedu[1], Philippe André[1], Abdelkader Ayadi[1], Marie-Christine Birling[1], Ghina Bou About[1], Marie-France Champy[1], Philippe Charles[1], Frederic Fiore[2], Jean-Pierre Gorvel[2], Dalila Hali-Hadji[1], Philippe Hoest[2], Hugues Jacobs[1], Sylvie Jacquot[1], Stéphanie Lerondel[3], Karelia Lipson[3], Patricia Lopes-Pereira[3], Marie Malissen[2], Hamid Meziane[1], Guillaume Pavlovic[1], Patrick Reilly[1], Gilles Warcollier[2], Marie Wattenhofer-Donze[1], Olivia Wendling[1], Cécile Fremond[3], Bernard Malissen[2], Tania Sorg[1], Ana Zarubica[2], Yann Hérault[1]

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PHENOMIN is a National Infrastructure to promote research in mouse functional genomics. PHENOMIN contributes to International Mouse Phenotyping Consortium to develop an encyclopedia of mammalian gene function.

Generating precise and target genome mutations in the mouse genome is one of the most common approach in functional genomics. It helps to better understand the gene function, basic biological mechanisms, physiopathology, to identify and validate new drug targets and to perform risk assessment in drug development.

PHENOMIN offers state-of-the-art specialized services and consultancy: Ready-to-use genetically engineered mouse models and comprehensive, standardized or advanced characterization of gene function by identification of anatomical, physiological, and behavioral phenotypes in mouse models.

PHENOMIN drives the expertise and sets resources for French researchers through its involvement in the European infrastructure for mouse disease models 'INFRAFRONTIER' and the International Mouse Phenotyping Consortium 'IMPC'. Both are composed of major mouse genetics research institutions, national funding organizations and corporations formed to systematically invalidate each of the 20 000 genes in the mouse in order to decipher the main functions of the genes coding for proteins. The 'loss of function' or 'knockout' mouse models generated are characterized according to a standardized phenotyping protocol.

PHENOMIN actively collaborates with national and international partners to promote access to open resources (data, mouse models, standard operating procedure, trainings...), provide genotype-phenotype annotation, to develop innovative tools (imaging, cancer and rare disease models, genetically engineered rat models...).

PHENOMIN opens access to its known-how through calls for proposal to nominate, generate and/or phenotype mouse models.

WS.002

Review of a pilot study, introducing Lab Tutor™ software to final year nursing students (n = 95) with the aim of improving their simulated learning experience and introduction of Lt™ into the new curriculum

Anthony Wales, Jack Simpson

University of the West of Scotland – Lecturer (Adult Nursing), Hamilton, UK

Online simulation provides an alternative approach to hands-on simulation and a number of software packages are becoming available to educators.

Lt™ is an online, immersive learning platform, which aims to bridge the gap between theory and practice, by using real patient case studies along with interactive exercises.

We introduced Lab Tutor™ software as an integral part of our year 3 clinical skills module, alongside the use of the SimMan 3G Advanced Human Patient Simulator (AHPS), with the aim of providing students with a more immersive simulation experience. Lt is now almost completely integrated into all pre-registration nursing courses within the University of the West of Scotland

We introduced LabTutor™ to final year nursing students at the beginning of an 11-week clinical skills module. On completion of the module we evaluated the use of the software and whether the students considered that using the interactive software improved their knowledge of disease processes and management of patients.

We evaluated results from this pilot study. Early feedback from students indicates that the introduction of LabTutor™ improved understanding of disease processes as well as their management. Following the pilot we contacted ADInstruments and worked closely with them to produce a more nurse related platform with fewer experiments and more nursing intervention

We are also in the process of developing a number of patient scenarios, using existing Lt™ patients, for use on the SimMan 3G AHPS.

Feeding Behavior: New Concepts and Regulations

SYM.022

Deconstruction of a neural circuit for hunger

Deniz Atasoy
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Synaptic connectivity and molecular composition provide a blueprint for information processing in neural circuits. Detailed structural analysis of neural circuits requires nanometer resolution, which can be obtained with serial-section electron microscopy. However, this technique remains challenging for reconstructing molecularly defined synapses. We used a genetically encoded synaptic marker for electron microscopy (GESEM) based on intra-vesicular generation of electron-dense labeling in axonal boutons. This approach allowed the identification of synapses from Cre recombinase-expressing or GAL4-expressing neurons in the mouse and fly with excellent preservation of ultrastructure. We applied this tool to visualize long-range connectivity of AGRP and POMC neurons in the mouse, two molecularly defined hypothalamic populations that are important for feeding behavior. Combining selective ultrastructural reconstruction of neuropil with functional and viral circuit mapping, we characterized some basic features of circuit organization for axon projections of these cell types. Our findings demonstrate that GESEM labeling enables long-range connectomics with molecularly defined cell types.

SYM.023

Food palatability and neuronal circuit

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Feeding behavior is exquisitely regulated by homeostatic and hedonic neural substrates that integrate energy demand as well as the reinforcing and rewarding aspects of food. Understanding the net contribution of homeostatic and reward-driven feeding has become critical due to the ubiquitous source of energy-dense foods and the consequent obesity epidemic. Hypothalamic, agouti-related protein-secreting neurons (AgRP neurons) represent primary orexigenic drives of homeostatic feeding. Using a models of neuronal inhibition or ablation we demonstrate that the feeding response to a fast, ghrelin or serotonin receptor agonist relies on AgRP neurons; however, when palatable food is provided, AgRP neurons are dispensable for an appropriate feeding response. In addition, AgRP-ablated mice present exacerbated stress-induced anorexia and palatable food intake – a hallmark of comfort feeding. These results demonstrate that hedonic circuitry can solely operate feeding and override the homeostatic circuitry especially in conditions where positive response to energy demands is chronically defective.

SYM.024

Taste for fat: the 6th taste modality

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There exists five basic taste modalities, for example, sweet, sour, bitter, salty and umami. Recent compelling evidence from rodent and human studies raise the possibility for an additional *sixth taste* modality devoted to the perception of lipids. A number of studies have recently suggested that lingual CD36 and GPR120 mainly expressed by circumvallate papillae of the tongue, might be implicated in the oro – sensory perception of dietary fat.

Our recent studies have not only supported the existence of the 6th taste modality, destined for the perception of fat, but also explored the intracellular signalling mechanisms, involved in this phenomenon. We have shown that lingual CD36, after activation by free fatty acids, induces increases in free intracellular calcium concentrations, ($[Ca^{2+}]^i$), phosphorylation of protein-tyrosine kinase (PTK) and release of the neurotransmitters like serotonin and nor-adrenaline into synaptic clefts. This signalling cascade is likely responsible for physiologic responses, induced by the detection of lipids in the oral cavity. The lipid-mediated regulation of feeding behaviour which is very critical in the development of several diseases like obesity and other metabolic disorders. Our studies show that fat taste signaling is altered in obese animals and there is a polymorphism of CD36 in obese subjects.

New Insights into Aldosterone and the Mineralocorticoid Receptor

SYM.027

Mineralocorticoid receptor (MR) in the adipose organ, pathophysiological and translational considerations

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Aldosterone is the primary ligand for the mineralocorticoid receptor (MR), and has been considered for years a 'renal' hormone, acting at this site as a key regulator of plasma volume, electrolyte homeostasis and blood pressure. In addition to its well-documented expression and activity in the kidney, in the last decade research on MR has also revealed its important role in regulating functions of extrarenal tissues, including adipose tissue, where MR is involved in adipocyte fundamental processes such as differentiation, autophagy and adipokines secretion. MR expression is increased in adipose tissue of murine models of obesity and in obese human subjects, suggesting that over-activation of the mineralocorticoid signaling leads to dysfunctional adipocyte and associated metabolic disorders. Notably, pharmacological blockade of MR prevents metabolic dysfunctions observed in obese mice and suggests a potential therapeutic use of MR antagonists in the treatment of obesity and metabolic syndrome. In fact, disturbances in glucose metabolism due to inappropriate activation of MR are frequently observed in patients with primary aldosteronism as well as in obese people. Although MR antagonists have been shown as beneficial players in glucose tolerance and metabolic parameters in most studies performed in animals, their role in humans remains still unclear. Moreover, the molecular pathways affected by MR blockade have been poorly investigated. Aim of this talk is to discuss the pathophysiology of MR activation in preclinical experimental models, particularly at the level of the adipose organ, focusing on potential signaling pathways mediating MR action.

Breathing and Mechanisms of Exertional Dyspnoea: From Physiology to Clinical Applications

SYM.028

Back to basics: the fundamental principles of exercise physiology

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Exercise tolerance is determined by the ability to minimize the rate at which metabolic acidaemia and related contributors to fatigue develop. Minimizing the H^+ production rate depends on an aerobic energy-transfer system with rapid dynamics and high capacity; constraining the consequent degree of acidaemia, however, depends on the ventilatory (V'_E) system (with its associated perceptual sequellae) clearing the additional CO_2 released by bicarbonate-buffering mechanisms and effecting compensatory hypocapnia ('respiratory compensation'). The determinants of an 'appropriate' exercise V'_E response are pulmonary CO_2 output ($V'CO_2$) ('metabolic' component), arterial PCO_2 ($PaCO_2$) (control 'set-point'), and the dead space fraction of the breath (V_D/V_T) ('gas-exchange

inefficiency'): $V'_E = 863 \times V'CO_2/[PaCO_2 \ (1 - V_D/V_T)]$. The role of V'_E and therefore dyspnoea in limiting exercise tolerance requires consideration of: whether blood-gas and acid-base 'requirements' are met; the 'cost' of meeting these requirements; whether the V'_E system is 'constrained' or 'limited'; and the intensity with which the V'_E response perceived. Sources of exercise limitation may include: expiratory air-flow limitation (high demands for CO_2 clearance imposing V'_E requirements that approach, or even exceed, respiratory-mechanical limits); inadequate pulmonary capillary transit-time for oxygenation (high cardiac outputs; a compromised capillary bed); intolerable dyspnoea (low or absent 'breathing reserve'; exaggerated peripheral-chemoreceptor sensitization by arterial hypoxaemia); and interstitial pulmonary oedema (high intra-pulmonary vascular pressures). Therefore, in ventilatory-limited situations (e.g. lung disease; highly-fit endurance athletes), factors which reduce *ventilatory* demand (e.g. reducing arterial hypoxaemia and/or metabolic acidaemia; improving V_D/V_T; ameliorating 'dynamic hyperinflation' via altered breathing-pattern) have the potential to improve exercise tolerance.

SYM.029

The mechanics of breathing and exertional dyspnoea: from physiology to clinical applications

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Dyspnoea is a complex, multifactorial and highly personalised sensory experience, and its mechanisms are incompletely understood. Exertional dyspnoea can be easily defined as 'the perception of respiratory discomfort that occurs for an activity level that does not normally lead to breathing discomfort'. The intensity of dyspnoea can be determined by assessing activity level required to produce dyspnoea (i.e. dyspnoea at rest is more severe than dyspnoea while climbing stairs). Dyspnoea can be, therefore, evaluated during a physical task, such as cardiopulmonary exercise testing (CPET). For this purpose, the 10-point Borg scale can be used to rate a specific respiratory sensation (e.g., inspiration difficulty, breathing effort, expiration difficulty, air hunger, etc.) or a more general one (e.g., breathing difficulty, breathlessness). Though somewhat less popular, the visual analogue scale (VAS) is another dyspnoea measuring instrument with proven construct validity used during CPET. Both the VAS and Borg scale have been shown to provide similar scores during CPET, and to be reliable and reproducible over time in healthy subjects, and in patients with chronic respiratory diseases undergoing CPET. The advantage of using the Borg or VAS scales in individual patients is the possibility of reliably comparing 'intensity of exertional dyspnoea' at the same level of exercise activity (*standardised* work-rate or oxygen consumption or ventilation during CPET) between subjects, and before and after a pharmacological and/or non-pharmacological treatment. Studies in cardiopulmonary diseases have shown that during CPET, there exists a close correlationship between the magnitude (and duration) of respiratory effort (measured by tidal oesophageal pressure relative to maximum) and the intensity of dyspnoea (measured by the Borg scale). Hence, the pharmacological manipulations can reduce the magnitude (and duration) of respiratory effort which might be associated with reduced dyspnoea intensity.

SYM.030

The role of exercise physiology in cardiac diseases and pulmonary arterial hypertension

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Exercise Physiology, has assessed by cardiopulmonary exercise testing (CPET), has a pivotal role in the diagnosis, the clinical management and the prognosis of patients with cardiac diseases, and more precisely, in heart failure, and in pulmonary arterial hypertension. CPET is used to confirm the diagnosis of heart failure in presence of a low VO_2 at anaerobic threshold and at peak exercise, a low oxygen pulse and slope of VO_2/work, and an increased slope or ratio of VE/VCO_2 at the anaerobic threshold. CPET is useful to assess the presence of heart failure and COPD in a same patient, being both characterized by a low peak VO_2. However, the VE/VCO_2 relationship shows a high slope and a low intercept on the VE axis in heart failure while the latter index is high in presence of COPD, alone or combined with heart failure. CPET data associated with spirometry and measurements of lung diffusion can be used to choose the most appropriate beta-blocker and antagonists of the renin angiotensin system as well as to suggest if an anti-aldosterone treatment would improve exercise performance. For a prognostic point of view, peak VO_2 and VE/VCO_2 slope have a strong independent capacity when multiparametric prognostic approach of heart failure is considered. Consequently, CPET has a well-defined role in heart failure assessment as reported by several heart failure guidelines. The role of CPET in pulmonary artery hypertension has been recognized only more recently. However, nowadays, CPET should be regularly used in patients with pulmonary artery hypertension to define prognosis and, most importantly, to assess the efficiency of treatment.

SYM.031

Respiratory and locomotor muscles in cardio-respiratory disease: the role of exercise and rehabilitation

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Skeletal muscle is essential for movement and ventilation. Patients with chronic respiratory and heart diseases experience dysfunction of their muscles. Although both respiratory and lower limb muscles are usually affected in those patients, the latter are more severely affected than the former. Several studies have shown the existence of muscle dysfunction of the lower extremities in patients with heart failure. In addition, the contribution of biological mechanisms in the process of muscle dysfunction and atrophy has been previously shown in patients with chronic heart failure. The relationship between muscle atrophy and loss of exercise tolerance was also observed in patients with heart failure. In patients with respiratory disorders such as chronic obstructive pulmonary diseases (COPD), muscle weakness and atrophy were excellent predictors of disease progression and mortality. Several factors and biological mechanisms explain muscle dysfunction in COPD. On the basis of several studies that were published in the last two decades, including those of our team, mechanisms such as oxidative stress, systemic inflammation, epigenetics, metabolic alterations, cigarette smoking, ubiquitin-proteasome system, and stimulation of signaling pathways (NF-κB, FoxO) are considered as among the most relevant events implied in the dysfunction of the lower limb muscles (quadriceps) in COPD patients. Exercise training of high intensity is a key component of rehabilitation programs in patients with chronic respiratory and cardiac conditions. Exercise tolerance and muscle function are significantly improved following exercise training in those patients. Moreover, muscle structure and metabolism were also significantly ameliorated in response to exercise training programs of high intensity and long duration in patients with chronic respiratory and cardiac diseases. Additionally, several nutritional and pharmacological strategies have been proposed to exert beneficial effects on skeletal muscle dysfunction and atrophy of patients with chronic respiratory and heart failure. Nonetheless, until effective and safer pharmacological therapies emerge, exercise and muscle training modalities, alone or in combination with nutritional support, are undoubtedly the best treatment options to improve muscle mass and function, and quality of life in COPD patients.

Physiology and Inherited Rare Diseases: When Function Meets the Gene

SYM.032

The multifaceted effects of *Enpp1* deficiency

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The recent elucidation of rare human genetic disorders resulting from mutations in ectonucleotide pyrophosphotase/phosphodiesterase (*ENPP1*), also known as plasma cell membrane glycoprotein 1 (PC-1), has highlighted the vital importance of this molecule in human health and disease.

Generalised Arterial Calcification in Infants (GACI), a frequently lethal disease, has been reported in recessive inactivating mutations in *ENPP1*. Recent findings have also linked hypophosphatemia to a lack of NPP1 function. A number of human genetic studies have indicated that NPP1 is a vital regulator that influences a wide range of tissues through various signalling pathways and when disrupted can lead to significant pathology.

The function of *Enpp1* has been widely studied in rodent models, where both the naturally occurring tiptoe walking (*ttw/ttw*) mouse and genetically engineered $Enpp1^{-/-}$ mice show significant alterations in skeletal and soft tissue mineralization, calcium/phosphate balance and glucose homeostasis. These models therefore provide important tools with which to study the potential mechanisms underpinning the human diseases associated with altered NPP1.

A fuller understanding of the mechanisms through which NPP1 exerts it pathological effects may stimulate the development of novel therapeutic strategies for patients at risk from the devastating clinical outcomes associated with disrupted NPP1 function.

SYM.033

4-Phenylbutyrate reduces calcification in mice expressing human ABCC6 mutants: a preclinical *in vivo* model for allele-specific therapy of PXE and GACI

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A molecular pathway inhibiting ectopic calcification is initiated by ABCC6, a membrane transporter primarily expressed in liver. ABCC6 facilitates the efflux of ATP from hepatocytes, what is rapidly converted into pyrophosphate (PPi) in the liver vasculature. PPi is the major inhibitor of calcification. Mutations in *ABCC6* are responsible for the currently incurable calcification disorder pseudoxanthoma elasticum (PXE) and some cases of generalized arterial calcification of infancy (GACI). Most mutations in ABCC6 are missense, many of which have preserved transport activity but are retained intracellular. We have shown that the chemical chaperone 4-phenylbutyrate (4-PBA) can reorient ABCC6 mutants into the plasma membrane.

In a humanized mouse model transiently expressing human ABCC6 mutants in the liver, we addressed whether treatment with 4-PBA could rescue the physiological function of ABCC6 *in vivo* by restoring its potential to inhibit calcification. We used the dystrophic cardiac calcification phenotype as a marker in *Abcc6*$^{-/-}$ mice to determine the effect of 4-PBA on mineralization.

Introduction of human wtABCC6 to the livers of *Abcc6*$^{-/-}$ mice restored hepatic PPi release. Administration of 4-PBA to *Abcc6*-deficient mice expressing human ABCC6 mutants in their livers restored the calcification inhibitory capacity of the protein.

As 75% of patients are with at least one missense allele, the potential of 4-PBA (an FDA-approved drug) seems to be a promising strategy for allele-specific therapy of ABCC6-associated calcification disorders and plasma PPi as a useful indicator of ABCC6 activity.

Plenary Lecture

P.005

Sarcomeric signalling proteins: hubs for mechanosensation and hotspots for inherited myopathies

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Titin is the largest single polypeptide protein, linking myofilaments from Z-disc to M-band. It is composed of many domains of the Ig (Immunoglobulin like) and Fn3 (Fibronectin type 3) type and contains a signalling domain, an MLCK-like kinase domain. The giant size and modular structure of titin mediate multiple functions and enable it to perform several roles in the sarcomere, from development (sarcomerogenesis), to mechanics, maintenance and adaptation.

At the M-band, titin forms a ternary complex with obscurin (and its analogue obsl1) and myomesin that is indispensable for the function of the M-band as mechanical link of myosin filaments. Obscurin itself is a giant protein over up to 800 kDa with similar domain composition as titin that is expressed in isoforms containing GEF domains or protein kinase domains. The giant sarcomeric scaffolds titin and obscurin thus both combine architectural and signalling functions, and these are modulated by mechanical stresses on the muscle cytoskeleton.

Titin is emerging as a major disease gene in hereditary myopathies, with the titin M-band region, especially the last Ig domain (M10), being one of the hotspots of disease-related mutations causing tibial muscular dystrophy (TMD) and limb girdle muscular dystrophy 2J (LGMD2J), while several recessive truncation M-band mutations cause Salih myopathy. A growing number of disruptive, recessive or dominant missense mutations are being linked to a rapidly expanding spectrum of titinopathies, from early-onset paediatric disease to adult onset dilated and hypertrophic cardiomyopathies, that disrupt the mechanosignalling hubs formed by titin and obscurin.

25 Years of FEPS: Physiology & Pathophysiology – A European Perspective

P.006

Getting stoned: calcium in the heart from 1991 to 2016

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When FEPS was founded in 1991 the Berlin Wall had only recently come down and the European Union had only 12 member states. This has now more than doubled to 28. However, strains remain and by the time I present this talk at the FEPS Silver Anniversary meeting in Paris, the result of the referendum in the United Kingdom on whether to remain in the European Union will be known.

The political evolution in Europe has been paralleled by advances in our understanding of calcium signalling in the heart. Until the late 1980s, the only way to record Ca transients was to laboriously inject the photoprotein aequorin into the cells. The development of Ca dependent fluorescent proteins by Roger Tsien transformed the field such that calcium can now be measured in real time in isolated myocytes and whole hearts.

In the field of excitation-contraction coupling, by 1991 it was known that the bulk of the Ca that contributes to the Ca transient comes from the sarcoplasmic reticulum (SR) and that release occurs by the process of calcium induced calcium release (CICR). On this mechanism, Ca entering the cell on the L-type Ca current triggers release of a larger quantity from the SR. In 1991 the field was worried by an apparent paradox; CICR is a positive feedback process so how could the release of Ca be graded to control the force of contraction of the heart? This problem was solved with the discovery by Cheng, Cannell & Lederer of the calcium spark showing that Ca release is recruited independently from different regions of the SR.

Subsequent work (from my group and many others) has illuminated the factors regulating the size of the calcium transient and the changes that result in cardiac arrhythmias and heart failure. I will address some of these issues in the talk.

P.007

Vascular ultrasound – an interdisciplinary journey

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From the sixties of the past century on, measuring blood flow velocity with continuous wave Doppler, vascular ultrasound has expanded tremendously with the introduction of pulsed Doppler technologies. Sophisticated systems to simultaneously record artery lumen expansion and instantaneous velocity distributions during cardiac cycles were developed, allowing the assessment of artery wall distensibility, wall shear rate and stress and flow disturbances. It was shown that elastic arteries stiffen, not only in established hypertension, but also at young age in volunteers and borderline hypertensives and in older volunteers with insulin resistance without diabetes mellitus, changes independent of blood pressure. These findings indicate involvement of intrinsic artery wall properties, a possibility confirmed in spontaneously hypertensive rats. These observations changed patient management. Studies in humans revealed velocity profiles to be flattened rather than fully developed parabolas and wall shear stress to vary along the arterial tree and across species, findings deviating strongly from previous theories! Flow disturbances recorded in bifurcations exposed areas of reversed flow and low wall shear stress, increasing our insights into the relation between flow disturbances and atherogenesis. The progress in vascular ultrasound has only been possible by the interdisciplinary collaboration between basic scientists, as physiologists, electronic engineers and physicists, and a variety of clinical disciplines and epidemiologists, across the country and international. Interdisciplinary research is very fruitful, provided that the participating scientists are knowledgeable; it shall not be used to hide ignorance!

P.008

Twenty five years on: physiology returns to centre stage

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When FEPS was founded, physiology had been sidelined as irrelevant to the foundations of biology. The central theory of biology, i.e. the neo-Darwinist interpretation of evolution, had concluded that physiological adaptation to environmental challenges could not directly influence the germline or inheritance. I came to doubt this view through experiments and computational models on the cardiac pacemaker, showing that knock-out experiments could not accurately reveal gene function without reverse engineering the

physiological mechanisms (Noble 2010). Coincidentally, both genome sequencing and the growth of epigenetics were revealing that the germline is not isolated in the way supposed by neo-Darwinism, and that various forms of inheritance of acquired characteristics exist. By 2010, evolutionary biologists were already proposing replacement of neo-Darwinism by a more inclusive theory (Pigliucci & Müller, 2010). The full extent of the implications were explored in a Special Issue of the *Journal of Physiology* (Noble et al, 2014), and even further in Noble (2015). The consequence is that the physiological study of function becomes relevant to the processes by which inheritable changes occur. Physiology therefore returns to centre stage.

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P.009

Physiology and pathophysiology of pancreatic Ca^{2+} signalling: 25 years of progress

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In pancreatic acinar cells, acetylcholine and cholecystokinin evoke repetitive local cytosolic Ca^{2+} spikes in the secretory region. This causes uptake of Ca^{2+} into the mitochondria generating ATP and triggering the physiological secretion of digestive pro-enzymes. The human disease acute pancreatitis, due to excessive alcohol intake, gallstone complications or side effects of L-asparaginase treatment of acute lymphoblastic leukaemia, is in all cases triggered by excessive and sustained elevations of the global cytosolic Ca^{2+} concentration. This cellular Ca^{2+} overloading depends on Ca^{2+} entry via conventional CRAC (Ca^{2+} Release-Activated Ca^{2+}) channels and causes intracellular protease activation and auto-digestion. Due to opening of the mitochondrial permeability transition pore, ATP levels are severely reduced and the overall result is necrosis followed by inflammation. One of the activated proteases leaking out of the necrotic acinar cells is kallikrein, which can liberate bradykinin from plasma kininogen. The elevated plasma bradykinin level elicits Ca^{2+} signals in peri-acinar stellate cells. The initial Ca^{2+} rise is due to intracellular Ca^{2+} release, but is quickly followed by a plateau phase depending on Ca^{2+} entry via CRAC channels. In case of repeated attacks of acute pancreatitis, which can then become chronic, the stellate cells produce a fibrotic (and potentially cancer promoting) extracellular matrix. Blockade of CRAC channels prevents all the adverse effects in both acinar and stellate cells and is currently the most promising rational therapy for pancreatitis.

Plasticity of Adipose Tissue: Beneficial or Harmful?

SYM.036

Genetic determinants of human adipose tissue plasticity

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We are only beginning to understand the genetic control of human adipose tissue plasticity. The remodelling and build-up of adipose tissue may involve a hyperplastic (increase in adipocyte cell numbers) or hypertrophic (maintained cell numbers, but increase in cell size) responses. In turn, these responses may have implications for integrated responses such as angiogenesis, blood flow control and metabolic control. The hyperplastic response requires recruitment of new adipocytes from resident stem cells; inevitably this will involve expansion of the pre-adipocyte pool and control of differentiation. The hypertrophic response relies on allowing additional lipid storage in existing cells. Recent genome-wide association studies (GWAS) of human fat distribution have pointed in the direction of Wnt signalling genes as important of body shape. In addition, GWAS of type 2 diabetes have identified a minority of genes determining insulin resistance and some of these genes point in the direction of adipose tissue being the culprit tissue. Using whole body studies in combination with in vitro cellular physiology and functional genomics, I will give examples of genes from these areas demonstrating functions that explain some aspects of genetic regulation of human adipose tissue plasticity.

SYM.037

The microenvironment of human subcutaneous and visceral adipose tissues: changes with obesity and involvement in obesity-associated pathologies

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The human adipose tissue plays a key role in the energy homeostasis. Mature adipocytes exert metabolic function, that is lipolysis and lipogenesis, and produce the main adipokines, leptin and adiponectin controlling metabolic fluxes. The human adipose tissue, present in subcutaneous and visceral anatomical depots, exhibits distinct metabolic, endocrine and growth properties according to anatomical location. The risk to develop cardiovascular and metabolic diseases in obesity is clearly associated with the body fat mass repartition and deposition of fat mass in the central part of the body. Several studies, including ours, show that the adipose tissue microenvironment is distinct according to fat mass location and obesity. Progenitor cells, vascular cells and immune cells are present in adipose tissue and are key players in defining the fat mass function and growth capacity by impacting on adipogenesis, an event required for adipocyte renewal and hyperplasia, angiogenesis and fibrosis. Using flow cytometry approaches developed on the stroma-vascular fraction of human adipose tissues, we show that the native adipogenic progenitor cells, characterized by a specific profile of cell surface markers ($CD45^{-}/CD34^{+}/CD31$) are constituted by distinct cell subsets, identified by two additional cell surface markers, Msca1 and CD271, exhibiting distinct differentiation capacities including white and beige adipogenesis. The repartition of the progenitor cell subsets is different according to fat mass location and obesity. Flow cytometry analyses demonstrate that macrophages and lymphocytes are present in distinct proportions and phenotypes depending on fat mass and obesity. They impact, through their secretions, angiogenesis as well as adipogenesis and fibrosis. Finally, recent studies stress the role of aging-related event in the changes of adipose tissue microenvironment according to location and obesity.

Pathophysiology of the Microcirculation: The Eye, Kidney and Beyond

SYM.041

The protective effect of annexin A1-FPR2/ALX pathway in sickle cell disease

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Sickle cell disease (SCD), an inherited autosomal recessive disorder, resulting from a single amino acid substitution in the haemoglobin β chain. SCD patients are more susceptible to thrombotic events, in which pathogenesis leukocytes, platelets and erythrocytes have all been implicated. It is known that anti-inflammatory therapies limit thrombosis and vice versa. One such anti-inflammatory target is the interaction of annexin A1 (AnxA1) with its formyl peptide receptor 2 (FPR2/ALX). Therefore, we tested whether Annexin A1 N-terminal derived peptide (Ac2–26) is effective in mediating thrombus formation in the brain of mice with SCD and if so, whether this effect is via the FPR2/ALX pathway.

Intravital microscopy was performed in the cerebral microcirculation (both venules and arterioles) of wild-type (WT) and sickle cell transgenic (SC) mice. Thrombosis was induced using the light/dye method1. Mice were treated with Ac2–26 with and without antagonists of the FPR pathway, for example Boc2 (pan-FPR antagonist) and WRW4 (Fpr2 antagonist).

SC mice had heightened thrombosis compared with their WT counterparts. Treatment with AnxA1Ac2–26 produced a significant dose dependent increase in flow cessation time in SC mice. These effects were abrogated following co-administration of Boc2 + Ac2–26 or WRW4 + Ac2–26. We also identified a novel role for Ac2–26 in the induction of neutrophil extracellular trap formation in SCD.

Our results demonstrate the importance of the AnxA1/FPR2 pathway in cerebral thrombosis in experimental SCD, and point to a potential therapeutic target for the treatment of this life-threatening disease.

SYM.042

Optical coherence tomography in chronic kidney disease: vasculopathy in the eye linked to kidney injury, inflammation & endothelial dysfunction

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Chronic kidney disease (CKD) is strongly associated with cardiovascular disease (CVD) and there is an established association between vasculopathy affecting the kidney and eye. Optical coherence tomography (OCT) is a novel, rapid method for high-definition imaging of the retina and choroid. Its use in patients at high CVD risk remains unexplored.

We used the new SPECTRALIS OCT machine to examine retinal and retinal nerve fibre layer (RNFL) thickness, macular volume and choroidal thickness in a prospective cross-sectional study in 50 patients with hypertension, 50 with CKD and 50 matched healthy controls. The same, masked ophthalmologist carried out each study. Plasma IL-6, TNFα, ADMA, and ET-1, as measures of inflammation and endothelial function, were also assessed.

Retinal thickness was reduced in CKD compared to hypertension ($P < 0.01$) and health ($P < 0.05$). RNFL thickness did not differ between groups. Macular volume was lower in CKD compared to both hypertension and health ($P < 0.001$ for both). Similarly, CKD was associated with a reduced choroidal thickness compared to hypertension ($P < 0.001$) and health ($P < 0.01$). Interestingly, in CKD, a thinner choroid was associated with a lower eGFR and greater proteinuria as well as increased plasma concentrations of CRP, IL-6, ADMA and ET-1 (all $P < 0.05$). Finally, both retinal and choroidal thicknesses associated inversely with tubulointerstitial damage whereas only choroidal thickness related to glomerulosclerosis.

The decreases in retinal and choroidal thickness in CKD are associated with lower eGFR and higher proteinuria but not blood pressure. Larger studies, in more diverse groups of CKD patients, are now warranted to clarify whether these eye changes reflect kidney pathology. Similarly, the associations with measures of inflammation and endothelial dysfunction should be examined further.

The Vestibular System a Key Sensory Organ for Homeostasis and Cognition in Mammals

SYM.043

The vestibular system as a key organ for cardiovascular homeostasis

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Over the last 40 years, it has been demonstrated that inputs from the vestibular organs affect blood flow and blood pressure control in Animals and Humans. Most studies showed that the otolithic organs and the sympathetic system were mainly involved and the term 'vestibulosympathetic reflex' has been extensively used to describe the effects of head orientation on sympathetic activity. It has been postulated that the vestibular influence on the cardiovascular system could serve as an anticipatory mechanism for blood pressure control during changes in body position. Animal studies support this hypothesis and recently, a correlation has been demonstrated between otolithic deactivation during spaceflight and post-flight orthostatic intolerance.

However, recent evidences suggest that sympathetic responses to vestibular stimulation are highly integrative. The brainstem areas that control sympathetic activity receive inputs from the vestibular nuclei. Integration of numerous signals related to body position to produce stable blood pressure is likely as for instance the effect of labyrinthectomy on blood pressure control is temporary whereas the effect of the lesions of the vestibular nuclei is permanent. The vestibular signals are also largely modulated by the cerebellum and higher brain centers.

To determine the spatial position of the body, the central nervous system elaborates an internal representation of gravity. This representation is multimodal supported with several sensory inputs with a high level of plasticity (adaptation to the loss of a sensory input) and subject-dependent. A seducing hypothesis could be that the sympathetic activation during orthostatism calls on such an internal representation of gravity.

SYM.044

Role of vestibular system on muscle and bone structure and function

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The molecular mechanisms regulating muscle and bone plasticity under unloading conditions such as extended period of disuse or exposure to real microgravity (μG) are not yet fully understood. By using the Heat Tilt (*het*) mouse as a model, we investigated the effect of vestibular dysfunction combined with 2G hyper-gravity on muscle and bone structure and function.

Vestibular dysfunction affects specific cell signaling pathways responsible for the altered muscle and bone structure and function following chronic exposure to μG and the application of hyper-gravity as countermeasure protocol might be able to counteract such changes. Four groups of mice were used: $Het^{+/-}$ control (CTR), $Het^{-/-}$ mutant (KO), $Het^{+/-}$ CTR submitted to intermittent centrifugation during 1 month (hyper-gravity by 2G), and $Het^{-/-}$ KO plus hyper-gravity by 2G.

Overall vestibular dysfunction has major impact on muscle myofiber phenotype composition and cross-sectional area compared to CTR mice that was in part rescued by chronic exposure to 2G hyper-gravity. Similarly, in the bone vestibular dysfunction induced lower limbs bone loss in adult mice which was reversed by beta-blocker treatment.

Discussion: The vestibular system through the sympathetic nerve system is able to influence muscle plasticity and these effects can be, at least in part, reversed by chronic exposure to 2G hyper-gravity. Combined with recent results on vestibulo-lesioned beta2-adrenergic KO mice, bone density regulation is similarly under the influence of vestibular graviceptors through a sympathetic pathway mediated by specific beta2-adrenergic bone receptors.

SYM.045

Motion sickness: physiopathology, therapeutic and rehabilitation

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Technological evolution of modern transports significantly increases motion sickness symptoms occurrence. Motion sickness is defined by a set of four main symptoms that regularly appear: facial pallor, cold sweats, nausea and vomiting. Other additional signs such as dizziness, headache, fatigue and postural instability can be also observed. One of the most established theories to explain in which circumstances motion sickness arises is the 'sensory conflict' theory. This theory postulates that motion sickness originates from a sensory mismatch between actual versus expected invariant patterns of vestibular, visual and somatosensory inputs. It has been accepted that the vestibular system influences individual motion sickness susceptibility since patients with bilateral vestibular deficit have greatly reduced susceptibility or do not become motion sick at all. This sensory mismatch leads to an activation of vestibule-autonomic pathways, which have been shown to be also involved in producing nausea and vomiting during motion sickness and those that generate illness after ingestion of toxins. The characteristics of movements that induce motion sickness have been defined: otolith stimulations around 0.2 Hz are involved probably because the signals at this frequency are ambiguous and difficult to interpret by the brain in terms of inclination or translational movements perception. Other factors such as vestibular reflex characteristics, gender, age are involved; strong smells, temperature are often reported. Preventive and curative pharmacological treatments are the same as 30 years ago. New pharmacological classes without undesirable side effects have to be tested on humans. Behavioral measures can be useful.

Cerebral Microcirculation and Regulation

SYM.053

The role of pericytes at the neurovascular unit

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The CNS vasculature is largely impermeable to blood-born molecules due to specific characteristics of endothelial cells and is referred to as the blood-brain barrier (BBB). The BBB is a collective term for several brain endothelial cell characteristics that render these cells impermeable to blood-borne molecules (i.e., closed endothelial cell-cell junctions, low rate of vesicular transport, expression of ATP-binding cassette efflux transporters), but allows the entry of essential nutrients via facilitated influx (SLC transporters). In addition, leukocyte entry into the brain parenchyma is tightly regulated. The development and maintenance of BBB characteristics is induced by the developing

neural tissue and cell types that form the neurovascular unit (NVU), a term that is used to describe the spatial orientation of neurons, astrocytes, and brain vascular cells (endothelial cells and pericytes) that underlie the integrated control of neural tissue and blood vessels in brain homeostasis and function. While the importance of the NVU as a modulator of brain homeostasis is still emerging as an accepted concept, there is only limited understanding of the interactions at the molecular level between the acellular and cellular components of the NVU in healthy brain, and the mechanisms underlying deregulation in the diseased state. The CNS vasculature has the highest longitudinal pericyte coverage, which reaches almost 100%, meaning that all endothelial cells and astrocyte end-feet have a cellular surface faced by a pericyte. Since pericytes are strategically positioned between endothelial cells and astrocytes, they may coordinate signalling at the NVU. I will present our work on pericyte-regulated characteristics of the BBB with a focus on the leukocyte trafficking into the CNS parenchyma. In addition, I will discuss the pathogenic role of pericytes in brain capillary calcification.

SYM.054

Cerebrovascular dysfunction in cadasil: linking a pathological change of the microvascular extracellular matrix with a channelopathy-like defect

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Cerebral small vessel diseases (SVD) are a leading and incurable cause of stroke and age-related cognitive decline. CADASIL, the most common hereditary SVDs, is caused by NOTCH3 mutations that stereotypically lead to the extracellular deposition of NOTCH3 ectodomain ($Notch3^{ECD}$) in the vessels. The disease is characterized by early cerebrovascular dysfunction with diminished pressure-induced constriction (myogenic tone) of cerebral arteries and arterioles and attenuated cerebral blood flow (CBF) responses to neural activity (functional hyperemia) and decreases in blood pressure (CBF autoregulation). Recently, we found that tissue inhibitor of metalloproteinases-3 (TIMP3) complexes with $Notch3^{ECD}$ and abnormally accumulates in the extracellular matrix of brain vessels of patients and mice with CADASIL. Here, we show that genetic overexpression of TIMP3 recapitulates both CBF and myogenic response deficits of the $TgNotch3^{R169C}$ CADASIL mouse model; conversely genetic reduction of TIMP3 in $TgNotch3^{R169C}$ mice restores normal cerebrovascular function. Further, we uncover a key TIMP3-sensitive signaling module, involving the disintegrin metalloprotease ADAM17, Heparin-binding EGF-like growth factor (HB-EGF) and epidermal growth factor receptors ErbB1/ErbB4 in the tonic regulation of cerebral arterial tone and evoked CBF responses. In the $TgNotch3^{R169C}$ model, we show that exogenous soluble ADAM17 or HB-EGF restores cerebral arterial tone and evoked CBF responses, and identify upregulated voltage-dependent potassium channel (K_V) number in cerebral arterial myocytes as a heretofore-unrecognized downstream effector of TIMP3-induced cerebrovascular deficits. Our data, collectively, suggest that elevated TIMP3 blunts the ADAM17 – HB-EGF – ErbB1/ErbB4 – K_V pathway in cerebral arterial myocytes to attenuate myogenic tone of cerebral arteries and compromise CBF regulation in CADASIL.

SYM.055

Neurovascular coupling in health and disease

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Focal increase in neuronal activity is associated with an enhanced blood flow to the active brain area. This functional neurovascular coupling is regulated by multiple mechanisms including the vasodilation induced by end-products of increased metabolism (e.g. adenosine and lactate) like in other organs. As a specific mechanism to the brain, synaptic activity-driven Ca^{2+} increases in neurons, interneurons and astrocytes translate the intensity of neuronal activity to vasodilatory signals such as arachidonic acid metabolites and nitric oxide. Enhanced K^+ release onto vascular smooth muscle cells through Ca^{2+}-activated K^+ channels on perivascular end-feet may also contribute to functional vasodilation. Penetrating cortical arterioles supply O_2 to capillary-free zone around them, whereas majority of the remaining O_2 is extracted from the first few order capillaries during rest and also from high-branching-order capillaries during activation. This requires a final step of regulation after the penetrating arterioles in the brain to match the focal demand, which appears to be mediated by microvascular pericytes. The neurovascular coupling requires a close communication between the endothelia, pericytes, astrocytes and perivascular nerves. The neurovascular unit and microvessels are vulnerable to

injury; for example, the microvascular injury induced by ischemia plays a critical role in tissue survival after recanalization by inducing sustained pericyte contraction and microcirculatory clogging (no-reflow). Suppression of oxidative/nitrative stress or sustained adenosine delivery during recanalization improves the outcome by promoting microcirculatory reperfusion. Microvascular dysfunction also contributes to the pathophysiology of dementia, small vessel disease, adverse effects of diabetes and hypertension on brain, and diabetic retinopathy.

Preterm Delivery, Difficult Labours and Infertility: Translating Reproductive Physiological Research into Clinical Benefit

SYM.056

From rotten egg to control of uterine contractility: H_2S

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Although significant progress has been made in the understanding how uterine contractility is controlled, still there is a pressing need to develop better tocolytics. Hydrogen sulphide (H_2S), an endogenous signalling molecule, plays a prominent role in reproductive physiology and appears to be an important regulator of uterine contractility. The production of H_2S and the presence of enzymes responsible for its production have been demonstrated in rat uterus. It relaxes non-pregnant (and pregnant myometrium. Mechanistical understanding of how H_2S reduces uterine contractility are still limited. Role of ion channels (bestrophins) as mediators of H_2S-induced relaxation will be disscused. In addition, despite the profound effects elicited by H_2S the chemical species involved in its signaling remain elusive. H_2S exerts a wide range of anti-inflammatory, anti-oxidant and cytoprotective actions. Interplay between redox sensitivity of uteri and H_2S might be beneficial in conditions with impaired antioxidant defence. These effects of H_2S have been exploited in the development of several novel drugs. H_2S-releasing derivative of the NSAID naproxen (ATB-346) has been developed with a primary aim of producing anti-inflammatory drug that will reduce GI toxicity. On the other hand, NSAIDs have proven to be effective agents in attenuating the human uterine contractility. Therefore, the effects of ATB-346 have been studied on human myometrium study. Combination of COX inhibition together with release of H_2S resulted in significant reduce of human myometrial contractility. More profound relaxatory effect of ATB-346 compared to the effect of naproxen itself, are most likely due to H_2S release.

This work enables better understanding of hydrogen sulphide effects which may help to design an attractive tool for management of dysfunctional uteri.

SYM.057

Labor dystocia

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Objectives: Labor dystocia is one of the largest and most high-profile issues in obstetric care. Labor dystocia affect about 20% of all deliveries. At the moment, oxytocin is the only available treatment of this condition. Studies have shown that the AFL value (amniotic fluid lactate) sampled and analyzed at the bedside in the delivery room, gives important information about the metabolic status of the uterus. Low levels of AFL have an association to a normal delivery, high AFL levels have a strong association to arrested labour progress and an operative intervention with associated complications.

In sports medicine, it is known that the levels of lactic acid can be affected and decreased by bicarbonate given orally before vigorous physical activity. Aims of this talk are to discuss if the use of AFL in obstetric care will be a new way of handling dystocic deliveries. A second aim to discuss is if an oral intake of bicarbonate improves condition of stimulation and enhance delivery outcome in dystocic deliveries.

SYM.058

Expression and function of vasopressin receptor subtypes in human myometrium: implications for effective tocolysis

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Oxytocin receptor (OTR) antagonists (ORAs) are used as tocolytic agents for women in preterm labour (PTL). However, OT can also activate the three vasopressin receptors (AVPRs) subtypes, including AVPR1a, which is highly expressed in myometrium throughout gestation. It is not clear if AVPR antagonism is also required for effective tocolysis. We used OTR- and AVPR subtype-selective peptides and expression studies to determine the importance of AVPR subtypes in human myometrium.

Peptides were synthesised by manual Boc-SPPS and purified by RP-HPLC. Ligand selectivity was assessed by Ca or cAMP responses in CHO cell lines heterologously expressing the different receptor subtypes. Isometric tension recordings were performed on myometrial strips ($1 \times 5 \times 2$ mm) obtained from women undergoing pre-labour Caesarean section at term with informed consent ($n \geq 5$/ligand). Receptor subtype expression was examined by RT-PCR and immunohistochemistry.

AVPR-selective ligands increased myometrial contraction. Selective inhibition of OTRs completely inhibited OTR-selective ligand activity but did not completely abolish OT action, indicating some involvement of the AVPRs. RT-PCR identified expression of V2R in all myometrial samples and V1bR in some samples. Immunohistochemistry findings will be discussed.

Data confirm the presence and activity of AVPRs in human myometrium but suggests that contraction is mainly driven by the OTR. The involvement of AVPR1a in the response to OT and the confirmed presence of V2R warrant further investigation. Ligand functional selectivity studies to determine the G-proteins and effectors activated by these peptides will be discussed, as will the development of better and safer tocolytic agents for PTL.

SYM.059

PLC-Zeta induced Ca^{2+} oscillations in mammalian eggs and its potential use in improving the success rates of male factor fertility treatment

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At fertilization in mammals the sperm activates embryo development by triggering a series of intracellular Ca^{2+} oscillations. We have discovered a sperm specific isoform of phospholipase C (PLCzeta) which triggers Ca^{2+} oscillations in eggs and embryo development. PLCzeta appears to be the physiological agent in sperm that activates the egg after sperm egg fusion in mammals. PLCzeta-induced Ca^{2+} oscillations can also account for Ca^{2+} oscillations after the intracytoplasmic sperm injection (ICSI). ICSI is widely used as a means of fertilizing human eggs when sperm quality is poor. However, there are a number of scenarios where ICSI is not successful. Such clinical ICSI failure has been associated with a lack of Ca^{2+} oscillations in eggs and a lack of PLCzeta sperm. We have shown that recombinant human PLCzeta can cause Ca^{2+} oscillations in mouse and human oocytes. Data will be presented where human PLCzeta protein is used to rescue failed fertilization after ICSI in a mouse model. Human PLCzeta protein may prove useful in ensuring high rates of egg activation after ICSI. Our work represents an example where the knowledge of the mechanism of Ca^{2+} oscillations has direct implications for clinical diagnosis and treatment.

The Frontier of Astroglial Physiology: The Gliocrine System

SYM.060

Ionic signalling in astroglia

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Activation of glial receptors conveys chemical transmission from neurones to astrocytes, and initiates a specific form of astroglial excitability that is mediated by spatio-temporal cytosolic changes in the concentration of several ions, most notably Ca^{2+}, Na^+ and K^+. Astroglial calcium signalling can be global (mainly due to Ca^{2+} release from the endoplasmic reticulum Ca^{2+} store) or local (mainly mediated by Ca^{2+} entry through plasmalemmal channels and Na^+/Ca^{2+} exchanger); these Ca^{2+} signals regulate cellular metabolism, gene expression as well as trafficking and release of secretory vesicles. Dynamic changes in cytosolic Na^+ concentration, which occur in response to neuronal activity, specifically regulate numerous homeostatic molecular cascades expressed in astrocytes. Coordinated ionic signals in astrocytes ensure complexity and versatility of bidirectional astrocyte-neuron communication at the multipartite synapse.

SYM.061

Metabolic regulation of vesicular glutamate release from cultured astrocytes

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Astrocytes have a prominent role in brain (patho)-physiology. They can signal to adjacent neurons by releasing glutamate via process of regulated

exocytosis. Astrocytes synthesize glutamate *de novo* owing to pyruvate entry to the citric acid cycle via pyruvate carboxylase. Pyruvate is sourced from the utilization of two metabolic fuels, glucose and lactate. Glucose can be polymerized to glycogen and stored as fuel within astrocytes and/or lysed to pyruvate, while lactate can be converted to pyruvate. To that end, we investigated the role of the above energy sources, glycogen, glucose and lactate, in exocytotic glutamate release from astrocytes. We used purified primary astrocyte cultures acutely incubated (1 h) in glucose and/or lactate-containing media. We used mechanical stimulation, known to increase intracellular calcium levels and cause exocytotic glutamate release. Using single cell fluorescence microscopy, we monitored stimulus-induced intracellular calcium responses as well as glutamate release to the extracellular space. Our data indicate that glucose, either taken-up from media or mobilized from the glycogen storage, sustained glutamate release, while the availability of lactate significantly reduced the release of glutamate from astrocytes. Based on further pharmacological manipulation, it appears that lactate caused metabolic changes consistent with an increased synthesis of fatty acids. The above metabolic and functional changes were corroborated by tandem mass spectrometry proteomics analysis which confirmed appropriate altered protein expression. These findings support the notion that the availability of energy sources and metabolic milieu play a role in glial-neuronal interactions and modulation of synaptic activity in health and disease.

SYM.062

Excitation-energy coupling and vesicle-based signaling in astrocytes

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Introduction: Astrocytes, a heterogeneous glial cell type, get excited when neurotransmitters, such as noradrenaline (NA) and ATP bind to their membrane receptors and respond back by releasing their own signals. This involves vesicles, which store chemicals termed gliotransmitters or more generally gliosignaling molecules. In the former case chemical messengers get released from astrocytic sites proximal to the synapse, which defines communication to occur in the microspace of contact between the synapse and the astrocyte. In contrast gliosignaling molecules may also be released into the extracellular space and get transported to locations far away from the active astrocyte. This mode of release resembles the endocrine system. Hence astrocytes are considered to be part of the gliocrine system in the brain, where the glymphatic system mediates the convection of released molecules. This complex system not only plays a role in cell-to-cell communication but also synchronizes the provision of energy for neural networks. Astrocytes contain glycogen, a form of energy store. Excitation of astrocytes by volume transmitters, such as NA, released by *locus coeruleus* neurons, activates adrenergic receptors and stimulates glycogenolysis, providing lactate. This lecture will discuss how astrocytes operate to synchronize excitation and energy provision. Moreover, Ca^{2+}-dependent fusion of the vesicle membrane with the plasma membrane in astrocytes will be presented.

Materials and methods: Using an approach to study single astrocytes by quantitative imaging confocal microscopy, we studied how stimuli like noradrenaline or ATP activate cytosolic calcium signals and how the mobility of fluorescently labelled secretory vesicles is affected by physiological states of astrocytes. By fluorescence resonance energy transfer (FRET) nanosensors we also measured second messenger cAMP and metabolites, such as D-glucose and L-lactate.

Results: Stimulation of astrocytes by noradrenaline increases cytosolic calcium and cAMP in distinct time-domains. Vesicle mobility was differentially modulated, depending of the vesicle cargo, by elevations in cytosolic calcium levels. NA also stimulated glycolysis monitored as an increase in FRET-based cAMP and cytosolic L-lactate increase, while cytosolic D-glucose levels were decreased due to facilitated consumption in glycolysis.

Discussion: It is proposed that excited astrocytes liberate energy by enhanced glycolysis, while a complex vesicle -ased signalling response is taking place in the same time domain. Hence, excitation-energy coupling is time-associated with alterations in astrocytic vesicle-based communication capacity.

SYM.063

Role of astrocytes in bi-directional regulation of vascular tone

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Considerable efforts have been devoted to understanding the cellular events underlying changes in cerebral blood flow (CBF) driven by increases in neuronal activity, that is, neurovascular coupling (NVC). However, less is understood about the mechanisms defining basal CBF and resting neuronal brain activity, and how these factors influence NVC. Here we addressed the cellular mechanisms by which changes in steady-state vascular tone, and thus perfusion, affect

astrocyte and resting pyramidal neuron activity. We hypothesize astrocytes tonically sense brain perfusion levels and adjust resting neuronal activity accordingly.

Using an advanced *in vitro* approach, astrocyte Ca^{2+} imaging and electrophysiological recordings we present evidence for a novel form of intercellular communication in the brain involving reverse flow of information at the neurovascular unit; referred here as vasculo-neuronal coupling (VNC).

Simultaneous astrocyte Ca^{2+} activity and pyramidal neuron electrophysiological recordings revealed that perivascular astrocytes respond (increased Ca^{2+}) to myogenic-evoked parenchymal arteriole vasoconstriction. Astrocyte Ca^{2+} activity changes preceded pyramidal neuron membrane hyperpolarization and decreased firing activity. VNC was unaffected by the presence of nitric oxide, GABA, glutamate and an Ecto ATPase blocker. Conversely, VNC was abrogated when the astrocytic syncytium was loaded with high BAPTA or in the presence of the adenosine A1 receptor blocker, DPCPX.

We propose VNC stands as a novel communication modality in the neurovascular unit enabling adjustments in neuronal activity according to brain perfusion levels safeguarding cellular homeostasis by preventing mismatches in energy supply and demand. We propose adenosine as the main astrocyte-derived signal underlying VNC.

Plenary Lecture

P.O11

Cytoskeletal plasticity and dynamic remodeling are fundamental processes coupled to vascular smooth muscle contraction

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As in all muscle, the actin cytoskeleton is fundamental to the structure and contraction of vascular smooth muscle cells (VSMC). Increasing evidence demonstrates coordination of actin-myosin based contraction with rapid remodeling and reorganization of the actin cytoskeleton. The process of remodeling in VSMC is a dynamic event occurring in parallel with contraction and relaxation, which involves rapid cytoskeletal F-actin polymerization and/or depolymerization that is triggered by mechanical forces and agonists, which induce contraction or relaxation, respectively. This remodeling is particularly evident in the cytoskeletal compartment containing cortical actin stress fibers. Coupled with this cytoskeletal remodeling, we have found that integrin-dependent VSMC-extracellular matrix adhesions, in addition to N-cadherin based cell-cell adhesions, are also rapidly up-regulated with contraction and down-regulated during relaxation. Taken together, this demonstrates that VSMC contractility, and the force transmission axis of the cell, are highly flexible and responsive to the state of vascular contractile tone. The definitive mechanisms that regulate the ability of the actin cytoskeleton and cell adhesion to rapidly reorganize are beginning to emerge, and appear to support potential roles for Rho-kinase, Lim-kinase, transglutaminase, cofilin and Ca^{2+} dependent processes as well as other focal adhesion and adherens junction-associated proteins. The remodeling of the actin cytoskeleton appears linked to the cellular and vascular remodeling that occurs in the vasculature during aging and disease, for example hypertension. Collectively, the dynamic behavior of the cytoskeleton with adhesion supports an active view of VSMC contraction involving a range of cellular processes.

Oral Presentations

Cardiovascular Physiology

CO.001

Aldosterone induction alters micrornas profile in rat heart

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Small, smart interfering agents, miRNAs regulate expression of many genes those have roles in formation of cancer and cardiovascular diseases.

Aldosterone, steroid hormone, has a crucial importance on the blood pressure and like many hormones its expression is also regulated by miRNAs. The aim of this study to clarify which miRNAs have been altered after treatment with aldosterone in rats. In order to investigate the relation between miRNAs and aldosterone regulation, aldosterone induced rat models were generated. Quantitative amount of aldosterone was measured by Aldosterone ELISA Kit. Systolic blood pressure of rat was measured by non-invasive 'Tail-cuff' method. RNA samples from heart tissue were isolated using TRIZOL, quality and quantity of RNA samples were calculated using Bioanalyzer using Agilent RNA 6000 Nano Kit. Alteration in miRNA expression has been detected via customized miRNA array and selected aldosterone related gene expressions subsequently confirmed by qRT-PCR. Serum samples of rat were analyzed for aldosterone

concentration and aldosterone concentration increased from 288.1 to 623 pg/mL when compared with control group ($P = 0.0008$). Blood pressure measurement studies showed that aldosterone induction causes formation of hypertension in rats (control group is 118 ± 9 mmHg while ALDO group has blood pressure as 164 ± 2 mmHg ($P < 0.0001$). miRNA array studies showed that 68 miRNAs have been elevated (twofold, $P < 0.05$) and two of which showed fivefold up-regulated after treatment with 75 μg/kg aldosterone in heart tissues. These results indicate that elevation in miRNA expression may modulate aldosterone level and causes formation of hypertension.

CO.002

Finerenone improves left ventricular diastolic, coronary and cardiac mitochondrial function in post-menopausal mice

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After menopause, women no longer have lower risk of developing heart failure with preserved ejection fraction. The involvement of mineralocorticoid receptor (MR) in diastolic dysfunction development remains unknown.

Mice aged 4 months were ovariectomized (Ovx) and at 7 months, treated or not for 1 month with the non-steroidal MR antagonist finerenone or its precursor BR-4628 (1 mg/kg/day in food). We assessed (i) invasive hemodynamic; (ii) acetylcholine-induced NO-mediated relaxation of isolated coronary arteries; (iii) reactive oxygen species production (ROS), ATP production, and respiratory response in isolated myocardial mitochondria.

In Ovx mice, hemodynamic showed impaired left ventricular (LV) compliance whereas it was normalized after BR4628 treatment (*LVEDPVR*: Ctl, 2.75 ± 0.58; Ovx, 4.38 ± 0.38; Ovx + BR, 2.70 ± 0.32 mmHg/RVU; $P < 0.05$, $n = 8$–9). In coronary arteries, NO-mediated relaxation was impaired in Ovx mice and normalized by finerenone treatment (% relaxation: Ctl 86.84 ± 6.47; Ovx, 38.31 ± 3.52; Ovx + Fine, 83.70 ± 3.45; $P < 0.01$, $n = 5$–6). Moreover, addition of NADPH oxidase inhibitor apocynin restored relaxation of arteries from Ovx mice, suggesting that finerenone lowered vascular oxidative stress. There was no difference in mitochondrial ROS production among groups. However, the respiratory response of mitochondria from Ovx mice indicated disrupted O_2 consumption that was corrected by finerenone (O_2: Ctl, 19.8 ± 1.4; Ovx, 26.9 ± 2.7; Ovx + Fine, 19.5 ± 2.7 mM/min/mg proteins; $P < 0.05$, $n = 8$–9). Moreover, a decrease in ATP production in mitochondria from Ovx mice was normalized by finerenone (ATP: Ctl, 13.3 ± 1.0; Ovx, 9.4 ± 0.6; Ovx + Fine, 12.8 ± 1.5 nM/mg of protein after 3 min; $P < 0.05$, $n = 9$–10).

MR is involved in post-menopausal coronary and diastolic dysfunctions in mice, with clear benefits provided by MR antagonist.

Respiratory Physiology

CO.003

Jund protects mice from age-related lung alterations consisting of emphysema, lymphoid hyperplasia, and adenocarcinoma

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Cellular senescence is now recognised as a critical player in the pathogenesis of chronic lung diseases such as COPD. One mechanism underlying premature cell senescence in COPD is oxidative stress. JunD, broadly expressed member of the AP-1 family of transcription factor, protects cells from oxidative stress by up-regulating the expression of antioxidant enzymes. The aim of the study was to investigate whether JunD deletion would induce lung alterations caused by oxidative stress and whether this effect would be related to cellular senescence.

$JunD^{-/-}$ were studied at various ages, during continuous treatment with vehicle or antioxidant NAC.

Lung junD expression increased with age in wild-type (WT) and was undetectable in $JunD^{-/-}$ mice. Compared with WT, $JunD^{-/-}$ mice presented with increased lung oxidative stress with a twofold elevation in lung pH2AX protein; this effect was suppressed in NAC-treated mice. Results were similar in cultured pulmonary artery smooth muscle cells (PA-SMC) derived from $JunD^{-/-}$ *versus* WT mice.

In aged $JunD^{-/-}$ mice, lung sections showed lymphoid infiltration ranging from small lymphoid aggregates to numerous, large clusters of lymphocytes, some mice exhibiting multiple sites of lymphoid hyperplasia. These mice developed lung emphysema and exhibited large lymphoid areas resembling to

tumor-like lymphoid structures. NAC provoked development of lung adenocarcinoma rather than protecting from the latter. Cancer development in NAC treated JunD$^{-/-}$ mice could be related to protection of cells from ROS-induced cellular senescence, as p16 and p21 positive cells were found decreased.

These results indicate that JunD protects against age-related emphysema, lymphoid hyperplasia, and adenocarcinoma in mice. They are consistent with tumor development promoted by antioxidant treatment.

Endocrinology

CO.004

Repeated low-grade bacterial challenges early in life affect cytokine levels in rats fed long-term high-fat diet

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Bacterial infections early in life cause permanent reorganization of the immune mechanisms. High-fat diets (HF) are reported to increase leakage of the intestinal microbial motifs into the circulation and to cause low grade inflammation. We hypothesized that earlier familiarization to bacterial motifs could modify cytokine responses to long-term high-fat diets.

Rat pups (female, $n = 32$; male, $n = 32$) were challenged (i.p.) seven times in the neonatal period (on days 7, 9, 11, 13, 15, 17 and 19) with *Escherichia coli* cell wall constituent (lipopolysaccharide, LPS, 15 μg/kg) or saline. Following weaning, they were divided into two subgroups and were either offered standard or high fat diet until day 150. Blood samples were analyzed for total cholesterol, triglyceride, TNF-α, IL-1β, IL-4, CRP, MCP-1 and IFN-γ.

Cholesterol and triglyceride levels were higher in HF and in female groups ($P < 0.01$). TNF-α was higher in HF groups ($P < 0.01$). MCP-1 was higher in males ($P < 0.001$) and in saline ($P < 0.05$) groups. CRP was lower in HF groups ($P < 0.001$), but higher saline groups ($P < 0.05$). IL-1β, IL-4 and IFN-γ levels were unchanged ($P > 0.05$).

Results indicate that (i) high-fat diet for 5 months influences TNF-α, CRP, cholesterol and triglyceride levels, that (ii) early life bacterial immune challenge effects CRP and MCP-1, and that (iii) sexually dimorphic effect is observed on MCP-1, cholesterol and triglyceride.

Integrative Biology

CO.005

The age–performance relationship

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Introduction: The physiological traits characterizing human capacities (the ability to move, reproduce or perform tasks) change with age: performance is limited at birth, increases to a maximum, then decrease back to zero at death. Both physical and intellectual skills follow similar ontogenies. For all data sets, a biphasic pattern of growth and decline is described by a simple equation. Here we aim to demonstrate that this biphasic behaviour is probably widespread among biological phenomena and compare the characteristics of the biphasic patterns such as the age of peak performance.

Performances data were gathered for human, greyhound, mice and *Caenorhabditis elegans* (using an experimental eletrotaxis device). Other data-sets included performance in different human related-tasks plus physical performance in greyhounds and mice, and in plants systems.

A U-inversed biphasic pattern is found in all the studied processes, in both the athletic and non-athletic species. The pattern is always asymmetrical and we found that the estimated ages of peak performance always occur in the early part of life: 21 $\pm$ 6.6% of estimated lifespan.

Our results suggest a similar age-related pattern in very different species. The description of the physiological limits shows that there is no brutal transition between the developmental and senescent periods.

CO.006

Targeting brain network plasticity by chronic vagus nerve stimulation associated with weight loss

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Obesity alters the behavior of the meso-limbic reward network that ultimately impacts on the activity of the

prefrontal cortex. Chronic vagal stimulation (VNS) has the capability to change food intake pattern and facilitate plasticity. The aim of this study was to correlate these plastic neuronal changes with the weight loss.

Ten adult miniature minipigs were given 3 months of obesogenic diet. Once obese, they were fitted with cuff electrodes around the abdominal vagi using laparoscopy and VNS was applied immediately in half of them (VNS group) while the remaining were fitted with non-functional stimulators (Sham group). Brain metabolism maps were obtained 10 days (Early) and 90 days (Late) after surgery. These maps were derived from FDG PET imaging coupled with arterial input measurement during euglycemic hyperinsulinic clamp using pixel-wise modeling. The activation maps were analyzed using statistical parameter mapping.

Body weight was reduced in VNS compared to sham group, 75 and 90 days after the onset of VNS. Ten days after the onset of stimulation, activations were detectable in the substance nigra, putamen, cingular and pyriform cortices. The same areas remained activated 90 days after the onset of stimulation. Several activation blots were detectable in the prefrontal cortex in the late condition.

Conclusion: Irrespective of the duration of the stimulation, VNS activates the nigrostriatal network but this activation diminished with time. On the contrary, prefrontal activation was present in late scans only suggesting that it might be causative for the reduced body weight.

Neurophysiology

CO.007

Endothelial S1P Receptor-1 promotes collateral blood supply in the early phase of stroke

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Introduction: Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator that is critical for vascular development and homeostasis. S1P has been shown to modulate endothelial function through the $S1P_1$ and $S1P_3$ receptor subtypes and contributes to the endothelial protective functions of high-density lipoprotein. We addressed the impact of endothelial $S1P_1$ deficiency on cerebral vascular reactivity and outcome of experimental stroke in mice.

Material and methods: Cerebral vasoreactivity was evaluated by ultrasound recordings of blood flow velocity changes in the basilar trunk (BT) under CO_2 (5%) gas mixture compared to air. Stroke was induced by permanent occlusion of the left middle cerebral artery (MCAo). Cortical anastomoses and microvascular perfusion were observed between middle and anterior arterial territories by Microscan.

Results: Cerebral blood flow responses to hypercapnia were profoundly reduced and stroke volumes increased in mice with endothelial specific $S1P_1$ deficiency. Measurements of blood flow velocities in internal carotid arteries and BT before, 50 min and 2 h after stroke initiation revealed a reduction followed by a recovery of blood flow in the ipsilateral internal carotid artery in control animals. The recovery was not present in $S1P_1$ deficient animals. Microvascular perfusion in the peri-infarct region was also reduced in $S1P_1$ deficient animals relative to controls.

Discussion: These observations suggest that endothelial $S1P_1$ regulates cerebral vasoreactivity and thereby promotes the early establishment of collateral supply through cortical anastomoses after stroke initiation. Loss of this protective mechanism reduces the perfusion of the peri-infarct area after vascular occlusion, thus exacerbating irreversible brain damage.

CO.008

Sildenafil, a cyclic GMP phosphodiesterase inhibitor, induces microglial modulation after focal ischemia in the neonatal mouse brain

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Perinatal ischemic stroke is the most frequent form of cerebral infarction in neonates with no currently evidence-based treatment. The present study investigated the effects of sildenafil in a neonatal mouse stroke model on neuroinflammation. Because microglia is reported with two opposing activated phenotypes, we have focused on M1 and M2 markers in the penumbra.

Ischemia was induced in C57Bl/6 P9 mice by permanent middle cerebral artery (pMCAo), and followed by either PBS or sildenafil. Blood-flow (BF) velocities were measured by ultrasound imaging with sequential Doppler recordings and Laser Speckle contrast imaging. Animals were euthanized at 3–8 days of reperfusion. Reverse-transcriptase polymerase chain reaction and immunohistochemical staining for M1 and M2 markers were performed to characterize phenotypic changes in brain cells, including microglia.

Sildenafil (10 mg/kg) did not induce early collateral recruitment in mice, and did not reduce infarct volume 3 days after pMCAo. In contrast, sildenafil (0.5 and 10 mg/kg) induced a significant dose-dependence reduction of the extent of the lesion 8 days after pMCAo. Sildenafil (10 mg/kg) significantly decreased microglial density at 3 and 8 days after pMCAo, and significantly increased M2a genes transcription (CD206, Arg-1, Lgals3). The number of Cox-2-positive cells significantly increased in the penumbra at 3 days after pMCAo but significantly decreased 8 days.

Conclusions: Our findings strongly indicate that sildenafil has anti-inflammatory effects in MCAo neonatal mice, which are modulated in a divergent way between 3 and 8 days after pMCAo, leading to a reduced damage.

CO.009

Hrvanalysis: a free software for analyzing cardiac autonomic activity

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Since the pioneering studies of the 1960s, heart rate variability (HRV) has become an increasingly used non-invasive tool for examining cardiac autonomic functions and dysfunctions in various populations and conditions, including death and health prediction, training and overtraining, cardiac and respiratory rehabilitation, sleep-disordered breathing, large cohort follow-ups, children's autonomic status, anesthesia, or neurophysiological studies.

We developed *HRVanalysis,* a software to analyse HRV, used and improved for over 15 years and, thus, designed to meet laboratory requirements. The main strength of *HRVanalysis* is its wide application scope. In addition to standard analysis over short and long periods of RR intervals, the software allows time-frequency analysis using wavelet transform as well as analysis of autonomic nervous system status surrounding scored events and on preselected labeled areas. Moreover, the interface is designed for easy study of large cohorts, including batch mode signal processing to avoid running repetitive operations. Results are displayed as figures or saved in TXT files directly employable in statistical software. Recordings can arise from RR or EKG files of different types such as cardiofrequencemeters, holters EKG, polygraphs, and data acquisition systems.

HRVanalysis is meticulously maintained and developed for in-house laboratory use, and in response to users' comments and needs. *HRVanalysis* was developed using MATLAB® 2015. HRVanalysis works with Windows 64-bit operating systems and it is not necessary to have MATLAB® installed on the computer, because MATLAB® Runtime is packaged with the software and automatically installed. *HRVanalysis* can be downloaded freely from the Web page at: anslabtools.univ-st-etienne.fr.

CO.010

M4 muscarinic receptors regulate locomotor activity biorhythm but not temperature biorhythm

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We have reported recently[1] differences between female mice lacking (KO) M_4 muscarinic receptor (MR) and their control counterparts (WT). Here we show sex differences in locomotor regulation and MR changes in different KO brain regions.

Total amount of 60 mice was used (30 males and 30 females). Mice were anesthetized; the telemetric probes (Vital View, Starr Life, USA) were implanted into peritoneal cavity. Mice were left at least for 2 weeks to recover and then the spontaneous motor activity and temperature were analyzed using Chronos-Fit software. Autoradiography was used for MR identification in motor, somatosensory cortex, striatum, thalamus. Statistical differences among WT and KO animals were determined using Student *t*-test, $P < 0.05$ was considered as statistically significant.

We have found biorhythm differences comparing males and females. While in males, there was almost no effect of M_4 MR absence the females revealed strong increase in activity mainly during dark phase. Temperature biorhythm was not changed substantially in both sexes (WT vs. KO), however sex difference was present. KO animals showed decrease in MR in motor (−24.2%), somatosensory (−28.8%) cortex, striatum (−50%), thalamus (−68.9%).

Discussion: Our results show that M_4 MR regulate locomotor activity biorhythm but not temperature biorhythm. These effects were sexually dependent and have been accompanied by MR decrease in multiple brain region.

Nutritional Physiology

CO.011

Association of genetic polymorphism with fat and bitter taste modalities in obese participants

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Orosensory perception of food strongly affects the ingestive behavior and intake of nutrients. The genetic variation in the genes encoding taste receptors also modulate the orosensory properties in human beings. The aim of this study was to evaluate the polymorphism in genes encoding bitter and lipid taste receptor in lean and obese subjects.

We conducted our study on lean (n = 53) and obese (n = 53) participants that were recruited in National Institute of Nutrition (Tunis). We isolated the genomic DNA from the saliva swabs and, by employing restriction-fragment polymorphism (RFLP), we looked for the single nucleotide polymorphism (SNP) of two genes, CD36 (fat taste) and TAS2R38 (bitter taste). The CD36 gene variant was rs1761667 and TAS2R38 gene variants were rs1726866 and rs10246939.

We noticed that A-genotype of rs1761667 was positively associated with obesity. Similarly, A-genotype and C-genotype, respectively of two rs1726866 and rs10246939 were associated with obesity.

Our study shows that fat taste and bitter taste modalities play an important role in obesity.

CO.012

Autonomic nervous imbalance induced by obesity is directly related to altered brain glucose metabolism

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Morbidly obese patients display a peripheral autonomic nervous imbalance (Windham et al, 2012) and an impaired glucose metabolism (Iozzo, 2015). The aim of this study is to evaluate the relationship between these parameters on an animal model of acquired obesity.

Fifteen adults age-matched miniature pigs were tested for autonomic balance and glucose metabolism before (32 ± 2.5 kg) and after 3 months of an obesogenic diet (48 ± 3.6 kg). Autonomic balance was obtained through the measurement of heart rate variability. ECG was recorded for 24 h using a portable digital recorder. HRV was extracted using frequency domain analysis. Brain, hepatic and skeletal muscle metabolisms were quantified as insulin-mediated glucose uptake using FDG PET imaging coupled with arterial input measurement.

LF/HF ratio was significantly increased in obese condition (0.55 ± 0.074 versus 3.1 ± 0.301 lean and obese conditions, P = 0.004). Insulin-mediated glucose uptake was significantly reduced at the brain, liver and skeletal muscle level (reduction by 36.8, 46.1 and 22.7% respectively lean and obese conditions). Correlation between LF/HF and brain glucose uptake was negative (R^2 = 0.87, r = −0.811, P = 0.0014). Similar correlations were found for the frontal, temporal, parietal, insular and occipital cortices as well as the striatal and thalamic areas. On the contrary, the correlation between LF/HF and hepatic or skeletal muscle glucose uptake was poor.

Peripheral autonomic nervous imbalance induced by obesity is directly related to altered brain glucose metabolism. This relationship did not exist for the liver and the skeletal muscle for which the decreased glucose uptake cannot be related to changes in HRV.

Renal Physiology

CO.013

The skipping of exon 9 in cullin-3 causes a severe form of familial hyperkalemic hypertension in mice

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Familial Hyperkalemic Hypertension (FHHt) is caused by mutations in WNK1, WNK4, KLHL3 or CUL3 (cullin-3). Patients with *CUL3* mutation display a more severe phenotype. The mechanisms associated with this severity remain unclear.

Material and methods: All *CUL3* mutations result in the skipping of exon 9. We have generated a mouse model of 'Cul3-FHHt' by deleting *Cul3* exon 9.

Results: RT-PCR proved that the exon skipping occurred as expected in the kidney of $Cul3^{+/\Delta 9}$ mice. They developed the classical FHHt features, which were all corrected by hydrochlorothiazide administration, a blocker of the Na^+–Cl^- cotransporter NCC.

Accordingly, NCC expression and phosphorylation were increased in *Cul3*$^{+/\Delta 9}$ mice. *Cul3*$^{+/\Delta 9}$ mice are smaller and lighter than control littermates. Preliminary results suggest that this could be due to the hyperkalemia and/or metabolic acidosis. Cul3 serves as a scaffold for RING-type E3 ubiquitin-ligase complexes. It interacts with KLHL3, which recruits the substrates (such as WNK1/4) for ubiquitination. The consequences of exon 9 skipping on Cul3 activity are still debated. It could result in an increased degradation of KLHL3 and thus decreased recruitment and degradation of the substrates. However, the expression level of KLHL3 was similar in *Cul3*$^{+/\Delta 9}$ and control mice.

As in humans, the phenotype of Cul3-FHHt mice is more severe than that of the WNK1-FHHt mice we previously described. Two hypotheses have been proposed: a broader dysfunction of the distal nephron or an increased vascular reactivity. Further studies of the *Cul3*$^{+/\Delta 9}$ mice are required to define the causes of this severity.

CO.014

Hypovolemic renin-aldosterone axis deficiency without hyperkalemia following unilateral adrenalectomy for primary aldosteronism

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Aldosterone-producing adenoma is classically treated by unilateral adrenalectomy. Severe hyperkalemia, related to hypoaldosteronism, have been already reported after surgery. We hereby describe six male patients exhibiting prolonged failure of the renin-aldosterone (RA) axis, in association with normal-to-high kalemia or labile blood pressure and, most significantly, decrease in extracellular fluid volume (ECFV).

Primary aldosteronism diagnosis was established according to French recommendations. Unilateral adrenalectomy was performed in all. Postoperative explorations included ECFV measurement using inulin, and RA axis functionally tests by orthostatic and ACTH stimulation.

A decrease in ECFV with inappropriately low renin level, and insufficient orthostatism-induced aldosterone production were depicted. The ACTH test demonstrated no glucocorticoid deficiency, along with responsive aldosterone secretion. The discrepancy in aldosterone response in orthostatic position *versus* ACTH stimulation test suggested that hypoaldosteronism primarily results from the lack of angiotensin 2 stimulation as a result of hyporeninism.

Following unilateral adrenalectomy for primary aldosteronism, the occurrence of normal-to-high kalemia prompted an evaluation of the RA system using orthostatic stimulation test rather than simply measuring baseline values and evaluating the glucocorticoid axis. When confirmed, RA axis depression causes latent hypovolemia, meaning that all treatment likely to further decrease plasma volume should be avoided, while this may at times require mineralocorticoid substitution.

Cardiovascular Physiology

PO.001

Crosstalk between neuropeptide Y and endothelin-1 induces secretion of right human ventricular endocardial endothelial cells

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The aim of this study was to test the hypothesis that a crosstalk may exist between the neuropeptide Y (NPY) and the ET-1 systems. In order to verify this hypothesis, we used human left and right ventricular endocardial endothelial cells (hLEECs or hREECs, respectively) and verified whether the NPY-induced secretion of ET-1 is mediated at least in part through stimulation of ETA and/or ETB receptors by their ligand, ET-1. Using the technique of indirect immunofluorescence coupled to quantitative 3-D confocal microscopy, as well as ELISA, our results showed that in hREECs, the NPY-induced release of ET-1 seems to be partly due to the activation of both ETA and ETB receptors. However, in hLEECs, this crosstalk either did not take place or contributed slightly to ET-1 secretion. Thus, our work highly suggests that the NPY-induced release of ET-1 in EECRs is partly due to NPY receptor activation as well as subsequent stimulation of the ETA and ETB receptors by the released ET-1. In contrast, the release of ET-1 by NPY in hLEECs is primarily due to NPY receptor activation and more specifically Y2 and Y5 receptors. These results support the concept that secretion of ET-1 could depend on the endothelial cell type and that right ventricular EECs play a role in releasing ET-1 into the right ventricle, whereas left ventricular EECs tune the level of ET-1 prior to its release into the arterial circulation. This work also highlights the importance of the secretory process of EECs.

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PO.002

Endoplasmic reticulum stress as a novel inducer of hypoxia inducible factor-1 activity: its role in the susceptibility to myocardial ischemia-reperfusion induced by chronic intermittent hypoxia

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Introduction: Obstructive sleep apnea (OSA) is a highly prevalent disease and a risk factor for myocardial infarction expansion in humans. Intermittent hypoxia (IH) is known to be the most important OSA feature in terms of cardiovascular mortality.

Since ER stress and HIF-1 are known to be involved in cardiomyocyte life or death, this study investigates the role of ER stress on HIF-1 activation in myocardial susceptibility to ischemia-reperfusion (I/R) induced by IH.

Methods: C57Bl6J, HIF-1$\alpha^{+/-}$ and their control mice were exposed to 14 days of IH (21–5% FiO_2, 60 s cycle, 8 h/day). Myocardial inter-organelle calcium exchanges, ER stress and HIF-1 activity were investigated and *in vivo* I/R was performed to measure infarct size. In additional groups, tauroursodeoxycholic acid (TUDCA, 75 mg/kg), an ER stress inhibitor, was administered daily during exposure.

Results: In C57Bl6J mice, chronic IH induced an increase in ER-Ca^{2+} content, ER stress markers and HIF-1 activity, associated with an enhanced infarct size (33.7 ± 9.4 *versus* $61.0 \pm 5.6\%$ in N and IH, respectively, $P < 0.05$). IH failed to increase infarct size in HIF-1α deficient mice (42.4 ± 2.7 and $24.7 \pm 3.4\%$ N and IH, respectively). Finally, TUDCA abolished the IH-induced increase in HIF-1 activity (1.3 ± 0.04 *versus* 0.14 ± 0.02 fold increase in IH *versus* IH-TUDCA respectively, $P < 0.0001$) and in infarct size (55.5 ± 7.6 *versus* 49.9 ± 3.0 in N-TUDCA and IH-TUDCA, respectively).

Discussion: This novel regulatory mechanism of HIF-1 activity by ER stress should be considered as a potential diagnostic tool for cardiovascular complications in OSA as well as a therapeutic target to limit myocardial ischemic damage.

PO.003

Valvular heart disease and pulmonary hypertension in fawn-hooded rats: the role of 5HT2B receptors

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Background: Patients elevated serotonin levels are prone to develop valvular heart disease and also pulmonary hypertension (PH) via induction of pulmonary vasoconstriction and proliferation of pulmonary artery smooth muscle cells. In order to explore the role of 5HT2B receptors, we treated Fawn Hooded rats by terguride, a 5-HT2B inhibitor.

Methods: Fawn Hooded rats (FH of age 1, 3, 6 and 9 months) which genetically exhibit a platelet storage pool disease, hence are unable to store serotonin in platelets were compared with Brown Norway rats (BN of age 1, 3, 6 and 9 months). Pulmonary artery systolic pressure (PAPs) was assessed by *in vivo* hemodynamic measurements (Millar catheter) along with echocardiography. Cardiac fibrosis was evaluated by Masson's trichrome staining and aortic valve calcification by Von Kossa staining.

Results: FH rats presented with elevated PAPs starting at 3 months of age compared to age-matched BN rats. PAPs were reduced by treatment with terguride, evidenced by decreased RV hypertrophy and increased pulmonary acceleration time.

Aortic and mitral valvular abnormalities progressively developed in FH but not BN rats as demonstrated by increased transaortic gradient and inappropriate presence of chondrocytes and fibrosis in the valves. Calcification in aortic valves of FH rats was completely rescued by terguride.

Progressive fibrosis did not limit to the valves, but pervaded LV myocardial interstitium. These findings were associated with a progressive LV remodeling, at echocardiography.

Conclusions: FH rats developed both PH and valvular heart disease. Both PH and cardiac valvulopathy were attenuated by treatment with the 5-HT2B antagonist terguride.

PO.004

Age-dependent changes in structure, functions and IGF-1/miRNA-1 regulation in hearts of spontaneously hypertensive and wistar rats

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Purpose: The miRNA-1 has inhibitory effect on IGF-1, which is known as a potent stimulator of cardiac hypertrophy. The aim of the study was to determine the features of IGF-1/miRNA-1 regulation of the cardiac hypertrophy development under high arterial pressure and in ageing.
Methods: The experiments were performed in male Wistar and SHR 18 months in age, parameters of heart function were investigated using the ultra-small catheter2F 'Millar Instruments'. The samples of LV tissue were collected from narcotized rats, and were assayed using morphological methods, levels of mRNA, miRNA, protein expression were estimated by RTPCR and WestBlot.
Results: The decrease of cardiohemodynamic parameters in SHR was shown: stroke volume 2 times, ejection fraction 1.8 times, stroke work 5 times, end-diastolic volume on 17%, end-systolic volume 1.3 times, increase of arterial rigidity 1.4 times was observed. Morphological changes were revealed: fibrosis compiled 18.1% in the left ventricle area (comparing with 1.8% in Wistar rats). It was found that initial IGF-1 protein expression was higher in SHR than in Wistar rats. In opposite, IGF-1 mRNA expression was prevailing in Wistar rats. The expression of miRNA-1 was also changed showing the dynamic of its reciprocal regulation with IGF-1.
Conclusions: The data obtained indicate that changes in miRNA-1 expression occur in heart due to pressure afterloading and senescence. Thus presenile SHR show first stages of pathological heart remodeling with pump dysfunction. In ageing, reduction of miRNA-1 level and its inhibitory effect on IGF-1 may be a factor of the progression of heart failure, especially in SHR.

PO.005

Susceptibility to develop pulmonary hypertension in SAD mice: effects of no donors

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Nitric Oxide (NO) protects against pulmonary vasoconstriction and pulmonary vascular remodeling mainly through its ability to induce cyclic guanosine monophosphate (cGMP) accumulation. Here we investigated pulmonary hypertension (PH) in wild-type (C57BL/6J) and transgenic SAD mice, a mouse model of sickle cell disease (SCD), and examined the effects of treatment with NCX1443, a new generation of NO donors.

One hour after a single oral administration of NCX1443 (100 mg/kg), the pulmonary pressure response to acute hypoxia in WT mice and SAD mice was completely suppressed, which remained significant after 6 h. Transgenic SAD mice exposed to chronic hypoxia or subjected to combined SU5416 treatment and hypoxia developed similar degree of PH as WT mice. Daily NCX1443 administration attenuated PH development, in association with an increase in lung cGMP levels, these effects were similar in SAD and WT mice. SAD mice exhibited higher lung protein levels of heme-oxygenase-1 and eNOS but similar levels of lung cGMP, and increased levels of PDE-5 protein than WT mice. PA-SMCs from SAD mice proliferated faster than those of WT mice, which was suppressed in the presence of NCX1443 combined to PDE-5 inhibitor.

In conclusion, SAD mice do not develop more severe PH than WT mice despite increased growth rate of PASMC measured *in vitro*. An increased expression of HO-1 and eNOS may be responsible for protection against excessive PA-SMC proliferation in SAD mice. Exogenous NO, given via NO donors, potentiates this endogenous protective mechanism. NCX1443 may represent a therapeutic approach for PH complicating sickle cell disease.

PO.006

Voltage gated sodium channel expression in muscles during sepsis

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Voltage gated sodium channels (Na_V) are responsible of membrane excitability, and are involved in triggering and propagation of heart and muscle action potential. $Na_V1.4$ and $Na_V1.5$ are the mains α-isoforms in cardiomyocytes, with a greater number of $Na_V1.5$ in heart, regulated by β subunits. Muscle excitability is decreased during sepsis by inflammation, but the impact of sepsis on heart channel expression is still not clear.

After 7 days of chronic sepsis induced by caecal ligature and perforation (CLP) in rat, heart was removed for contraction recording, then was dissected for mRNA and protein extraction. The quantification of $Na_V1.4$/$Na_V1.5$ and $\beta1$–$\beta4$ was done by RT-qPCR, and western blotting on membrane protein extracts. Sodium currents were recorded by patch-clamp.

Chronic CLP had no significant effect on heart frequency or on force compared to control heart. $Na_V1.4$ and $\beta4$ are less expressed during CLP, but are already poorly expressed in control cardiomyocytes. RT-qPCR shown no difference for both. This is in agreement with patch-clamp results on cardiomyocytes, because chronic CLP seemed to have no effect on sodium current and hence on membrane excitability.

In conclusion, we demonstrate that chronic sepsis had a negative effect on $Na_V1.4/\beta4$ expression, but as they are poorly expressed in cardiomyocytes the result on the total sodium current in negligible. Then we had no effect on cardiac function and membrane excitability suggesting a recovery of cardiac function during chronic sepsis contrary to skeletal muscle.

PO.007

$CD4^+$ $CD25^+$ Treg cell Kv1.3 potassium channel elevates in the state of CHF

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Introduction: Imbalance of immune activation and inflammatory plays an important role in the occurrence and development of chronic heart failure (CHF). $CD4^+$ $CD25^+$ regulatory T (Treg) cells maintain immune tolerance and response. Moreover, Kv1.3 potassium channels are mainly distributed in T cell membranes to regulate T lymphocyte activation, proliferation, differentiation and secretion of cytokines of important channel, and as the target of immune regulation.

Methods: Left anterior descending coronary artery was ligated to induce CHF in SD rats. $CD4^+$ $CD25^+$ Treg cells were isolated from spleen of CHF rat by magnetic-activated cell sorting. Whole-cell patch clamp technique was employed to quantify the current changes of Kvl.3 channel between CHF and control rats.

Results: Compared with Control group, LVEF in CHF group was decreased as same as that of LVEDP, while plasma BNP level of CHF group was increased ($P < 0.01$). And compared with control group, the $CD4^+$ $CD25^+$ Treg cell Kv1.3 potassium channel peak current density of CHF group was significantly increased ($P < 0.01$), while the membrane capacitance had no significant difference ($P > 0.05$).

Discussion: The occurrence and development of CHF has been accompanied by the excessive activation of the inflammatory response. In our study, $CD4^+$ $CD25^+$ Treg cell Kv1.3 channel peak current density of CHF group increased, suggested high Kv1.3 channel current density may adjust the function of $CD4^+$ $CD25^+$ Treg cells activity in CHF by increasing Kv1.3 current density. Therefore, enhance Treg cells Kv1.3 current density may be as a new target for the treatment of CHF.

PO.008

Hydrogen peroxide derived from the NADPH oxidase NOX4 is required for endothelial cells differentiation

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NADPH oxidase Nox4 is a constitutive source of hydrogen peroxide. Compared to other cells of the cardiovascular system, endothelial cells express high levels of Nox4. Nox4 maintains endothelial cell quiescence and prevents inflammation. We hypothesize that Nox4 contributes to differentiation of endothelial cells and that loss of Nox4 promotes de-differentiation.

Induced pluripotent stem cells (iPSCs) were generated from mouse embryonic fibroblasts. Differentiation of iPSCs into endothelial cells was induced by VEGF and BMP4. Endothelial marker expression was demonstrated by staining for isolectin B4, VEGFR2 and CD31 at the surface of the cells as well as by analyzing VEGFR2 mRNA level.

Nox4 deficiency results in genomic instability, which is a prerequisite for de-differentiation. Accordingly, in the course of de-differentiation the number of colonies formed from Nox4 deficient MEFs was higher than from MEFs of wildtype littermates, while the size of the iPSC colonies was smaller in the absence of Nox4. Therefore, it appears that Nox4 deficiency promotes the formation of iPSC colonies but not the proliferation of colony cells. In the course of differentiation Nox4 expression in wildtype cells increased and Nox4-deficient cells were less endothelial like than wildtype cells when differentiated. Additionally, tube formation of wildtype iPSCs derived endothelial cells was much higher than that of Nox4 deficient cells.

Together out data provide evidence that Nox4 deficiency promotes de-differentiation of MEFs into iPSCs. In contrast Nox4 promotes differentiation of endothelial cells out of iPSCs. We conclude that Nox4 maintains cellular differentiation state and supports the process of endothelial differentiation.

PO.009

Role of neutrophil gelatinase–associated lipocalin in the profibrotic effects of aldosterone in human cardiac fibroblasts

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Aldosterone (Aldo) plays an important pathophysiological role in cardiovascular remodeling and diseases. Cardiac remodeling is characterized by changes in the extracellular matrix (ECM), which favors the development of myocardial fibrosis, and finally could compromises cardiac function. Neutrophil gelatinase-associated lipocalin (NGAL) is a primary target of Aldosterone (Aldo)/MR in the cardiovascular system. However, the role of NGAL in cardiac remodeling is still unclear. We investigated the effects of NGAL on the production of ECM components in human cardiac fibroblasts (HCF) and whether NGAL could be a mediator of Aldo-induced ECM components in HCF.

Methods: Collagen I protein levels were measured in response to Aldo and NGAL in HCF for 12–24 h. Protein and gene expression of different profibrotic mediators in response to NGAL and Aldo. Finally, in order to study the role of NGAL on the profibrotic actions of Aldo, NGAL-silenced HCF were treated with Aldo.

Results: In HCF, Aldo and NGAL increased collagen I protein expression in a dose-dependent manner, reaching maximal values at 24 h. At this time, Aldo increased NGAL protein expression. NGAL and Aldo enhanced profibrotic mediators such as Gal-3, CT-1 and TGF-β, as well as MMP-1, MMP-2 and MMP-9 activities. In NGAL silenced cells, Aldo was not able to modify any of the parameters studied.

Conclusions: Our results indicated that NGAL exerts profibrotic effects in HCF. Furthermore, NGAL emerges as a potential mediator of Aldo-induced cardiac fibrosis and could be consider as a biotarget for novel pharmacological approaches, especially in diseases where Aldo/MR pathway is involved.

PO.010

Cardiac mitochondrial function is time-of-day dependent

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Introduction: Many cardiovascular (patho)physiological processes follow a circadian pattern. Liver mitochondria function has recently been shown to be paced by circadian rhythms. Mitochondria play a key role in cardiomyocyte functions. The aim of this study was to determine whether mitochondrial oxidative phosphorylation, ROS production and calcium retention capacity (mCRC) are time-of-day dependent.

Materiel and methods: Wild-type mice were sacrificed at the wake-to-sleep or sleep-to-wake transition period. Cardiac mitochondrial function was explored using heart homogenate preparations. Oxidative phosphorylation coupling in presence of pyruvate and malate was estimated by the respiratory control ratios (RCR), calculated as the ratio between ADP-stimulated (state 3) and ADP-free (state 2) respiration. H_2O_2 production relative to oxygen consumption (H_2O_2/O_2) and mCRC were measured under conditions mimicking a pathological substrate environment, such as the one encountered during reperfusion, that is high level of succinate, presence of oxygen, absence of ADP and energy substrates.

Results: Despite the absence of differences in state 3 and state 2 respiration, RCR was significantly lower ($P = 0.04$), H_2O_2/O_2 was 18% higher ($P = 0.05$) and mCRC was significantly reduced ($P = 0.01$) at the sleep-to-wake compared to the wake-to-sleep transition period.

Discussion: Mitochondrial function exhibits a time-of-day dependence in mouse cardiomyocytes with poorer mitochondrial coupling, higher ROS production and reduced mCRC at the sleep-to-wake transition period. Cardiac mitochondria could therefore represent a relevant target to impact on the circadian pattern of cardiovascular diseases.

PO.011

Cardiac specific mirnas expression levels in rat myocardium after 25 Gy irradiation

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Radiotherapy applied to the mediastinum area can cause significant damage also to the surrounding healthy tissues including heart and vessels. Recent studies suggest that miRNAs are involved in many cardiac diseases. They can be an important prognostic factor and therapeutic target also in radiation-induced heart disease.

The aim of this study was to measure the expression levels of cardiac specific miRNAs (miR-1, miR-15b and miR-21) in the rat myocardium after a single dose of ionizing radiation (25 Gy in radiation rate 6–7 Gy/min). The rats were treated with selected drugs [Atorvastatin, acetylsalicylic acid (ASA), Tadalafil and Enbrel] for 6 weeks after irradiation to reduce a negative impact of gamma rays. miRNA levels were measured by quantitative real-time PCR.

Irradiation downregulated miR-1 in irradiated hearts. In Tadalafil and Atorvastatin groups, miR-1 expression levels were decreased compared with control non-irradiated rats. ASA treatment decreased values of miR-1 in both control and irradiated groups. Irradiation caused downregulation of miR-15b by more than 26%. In the treated groups, no significant changes in miR-15b expression were seen. On the other hand, miR-21 was increased nearly twofold compared to its levels in non-treated irradiated hearts after treatment with Atorvastatin, Enbrel and ASA, whereas Tadalafil reduced miR-21 levels (about 40%).

Our study suggests that Enbrel and Tadalafil might have a protective effect on the heart damaged by irradiation. Modulation of miRNA levels with selected drugs may have implication on mitigation of radiation induced toxicity.

PO.012

Positive inotropic effect of IL-13 on heart is AMPc-PKA-dependent

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IL-13 is a cytokine produced during sepsis, but its pro- or anti-inflammatory effects on the heart are still not clear. The aim of this study was to clarify the impact of IL-13 on heart contraction, and on voltage-dependent Na^+ channels NaV1.4 and NaV1.5 which are responsible of the membrane excitability and essential for excitation/contraction coupling.

For this study, rat hearts were perfused *ex vivo* in a Langendorff system with IL-13 with or without inhibitors. Contractile force, heart frequency and coronary flow were recorded. Expression of $Na_V1.4$ and $Na_V1.5$ was analysed by western blot after protein extraction from ventricular cells.

IL-13 induced an increase of the contractile force (+28.3%), maximal speeds of contraction (+35.5%) and relaxation (+38.9%), but it had no effect on heart frequency and coronary flow. By using PKA or Adenyl cyclase (Ac) inhibitor we have shown that IL-13 acted by Ac-AMPc-PKA pathway activation.The hearts perfused with IL-13 had more NaV1.4 (+37.4%) and NaV1.5 (+52.2%) at the membrane level. In addition, the ratios of membrane/cytosol proteins were also increased too after IL-13 perfusion for NaV1.4 (+281.4%) and NaV1.5 (+214.4%) compared to hearts perfused without the cytokine. Moreover membrane targeting was abolished with Ac inhibition for both channels.

Here we demonstrate that IL-13 has a positive inotropic effect on perfused heart. This cytokine can increase NaV1.4/NaV1.5 membrane targeting by Ac-PKA pathway, and then increase membrane excitability. Activation of the Ac-PKA pathway can also have a stimulating effect on the calcium channels involved in excitation/contraction coupling mechanism.

PO.013

Modulations of adiponectin pathway induced by hypoxia in murin and cellular models

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Hypoxaemia is a component of respiratory diseases frequently observed in severe COPD patients (Chronic Obstructive Pulmonary Diseases). It initiates compensatory mechanisms mainly mediated by a family of transcription factors (Hypoxia Inducible Factors HIFs). Hypoxemia was suggested to modulate Adiponectin (Ad) pathway. Due to its anti-diabetic, anti-inflammatory and anti-atherosclerotic properties, we postulate that alteration of Ad pathway could participate to metabolic troubles and cardiovascular co-morbidities in COPD patients.

To better understand the specific impact of hypoxaemia on Ad pathway, we studied Ad plasmatic level, Ad multimeric forms and AdipoR1/AdipoR2 receptor abundance in a mouse model of chronic hypoxemia (FiO_2 10%, 8 h/day). After 35 days, hypoxaemia induced a decreased level of low molecular weight (MW) forms in favour of higher MW multimers as well as a tissue-specific modulation of AdipoR abundance.

As these alterations could impact the atherogenic risk, we evaluated AdipoR abundance in RAW murine macrophages exposed to low oxygen level (1%, 24 h). We first verified the induction of HIF 1α on nuclear protein extracts. We then demonstrated that exposure to hypoxia induced a reduced AdipoR2 level. Such deregulation could modify intra-cellular lipid accumulation and pro-inflammatory cytokine production. Investigations on these two hallmarks of cardiovascular risk are ongoing.

Therefore, we demonstrate that hypoxaemia modifies the distribution of Ad forms and causes AdipoR modulation both *in vivo* and *in vitro*. These troubles could participate to pathophysiological mechanisms linked to COPD co-morbidities.

PO.014

Implication of the neutrophil gelatinase-associated lipocalin (NGAL) from immune cells in aldosterone induced cardiovascular remodeling

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Background: Inadequate activation of the Mineralocorticoid Receptor (MR) promotes hypertension, inflammation and fibrosis. Neutrophil Gelatinase-Associated Lipocalin (NGAL), a pro-inflammatory/fibrotic glycoprotein, is a direct target of the MR in cardiovascular cells, and is increased in immune cells during inflammation. Recently, we demonstrated that NGAL is crucial for the hypertensive and pro-fibrotic effects of the nephrectomy-aldosterone-salt (NAS) challenge in mice. However, the specific cell types that modulate NGAL production during aldosterone-dependent hypertension are still unknown. The aim of this study was to characterize the implication of NGAL produced by immune cells in the pathophysiological effects of MR activation by aldosterone.

Methods: *In vivo*: Mâle C57Bl6 mice were submitted to 28 days of NAS challenge (200 μg/kg/day) and immune cells were sorted from the spleen and the lymph nodes. *In vitro:* DCs and Mø were cultured from WT and NGAL-KO mice and treated with aldosterone (100 nM) for 24 h.

Results: NGAL abundance was higher in PBMC, DCs and Mø, and further increased in NAS mice. *In vitro* MR activation by aldosterone in DCs induced an upregulation of NGAL and cytokines involved in the adaptive immune response (TGF-β1, IL-23). Interestingly, the absence of NGAL in DCs prevented this increase.

Conclusion: MR activation and subsequent NGAL induction in DCs could play a pivotal role in the inflammation observed during aldosterone-dependent hypertension.

PO.015

Quercetin prevents cardiac hypertrophy, fibrosis and lipidosis in spontaneously hypertensive rats and inhibits proteasomal activity

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Background: Quercetin is flavonoid-based preparate with hypotensive and anti-inflammatory effect, however, the molecular mechanisms are poorly studied.

Methods: We used Wistar and SHR. We monitored hemodynamic parameters (end-systolic pressure, end-diastolic pressure, stroke volume, ejection fraction, cardiac output) with 'Millar Instruments' equipment. We evaluated aortic lipidosis, cardiac fibrosis and hypertrophy. Caspase-, trypsin- and chymotrypsin-like activities of proteasome were measured. Quercetin (BCPP, Ukraine) was added to standard diet of SHR for 8 weeks in dose of 15 mg/kg.

Results: Cardiac output was decreased in 3.5 times ($P < 0.0001$), stroke volume in 3 times ($P < 0.0001$), ejection fraction in 2.5 times ($P < 0.0001$) in SHR. Quercetin application normalized disturbed cardiodynamic parameters: ejection fraction increased in 1.7 times ($P < 0.0001$), end-systolic pressure was decreased on 15.7% ($P < 0.0001$). Quecitin decreased heart-to-body weight ratio from 3.7 to 3.4 mg/g, decreased percentage of fibrotic tissue of left ventricle and aortic lipidosis in 2.9 and 2 times correspondingly ($P < 0.05$). Trypsin- and chymotrypsin-like activities of proteasome were lower in 1.6 times of SHR cardiac tissue comparing to Wistar rats. Quercetin decreased trypsin- and caspase- like activities in SHR heart in 2.4 and 9.3 times correspondingly ($P < 0.05$). Proteasome activities did not differ between Wistar and SHR aorta, but quercetin decreased trypsin- and chymotrypsin-like activities of proteasome in SHR ($P < 0.05$).

Discusion: Quercetin inhibits the proteasomal activity, thus, preventing remodeling of the myocardium and aortic lypidosis. Our results suggest the importance of proteasomal proteolysis in pathogenesis of

arterial hypertension. Quercetin can be recommended as promising antihypertensive approach.

PO.016

Modification of vascular tone in conductance and resistance arteries in response to cardiac hypertrophy

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Left ventricular hypertrophy (LVH) is a pathophysiological adaptive response associated with coronary endothelial dysfunction. This work aimed to evaluate if LVH causes vascular modifications outside of coronary circulation.

Vascular tone was assessed by wire myography in aorta and left carotid artery (LCA), and by pressure myography in small mesenteric resistance arteries (SMRA). Pathological LVH was evoked by transverse aortic constriction model (TAC) or induction of β1-adrenoceptors-activating-autoantibodies (ADRB1 model). These models were compared to physiological cardiac hypertrophy associated with voluntary wheel running (VWR).

NO-mediated relaxation was reduced in LCA of both hypertrophic models (Two-Way ANOVA: ADRB1: $P < 0.0001$, treated $n = 10$, control $n = 10$; TAC: $P < 0.0002$, Sham $n = 8$, TAC $n = 6$), as well as in aorta from TAC animals (Two-Way ANOVA: $P = 0.0208$, TAC $n = 7$, Sham $n = 8$). In LCA from ADRB1 treated mice, total endothelial relaxation decreased proportionally to the degree of LVH (Pearson; $P < 0.0001$, $R^2 = 0.98$, $n = 7$). Contractility of LCA was significantly increased in TAC animals in response to depolarization (*t*-test; 1.67 ± 0.10 versus 1.18 ± 0.11 mN/mm Sham treated, $P = 0.087$, $n = 6/5$) and to phenylephrine (*t*-test; 1.27 ± 0.10 versus 1.02 ± 0.05 mN/mm Sham, $P = 0.0404$, $n = 6/5$). Reduced EC50 to phenylephrine were observed in SMRA in the TAC model (1.04e-6 ± 0.08 versus 2.09e-6 ± 0.08 M Sham, $P = 0.0076$, $n = 9/9$) and LCA of ADRB1 (2.35e-8 ± 0.10 versus 5.81e-8 ± 0.12 M control, $P = 0.0018$, $n = 4/5$). These vascular alterations were not recapitulated in vessels from VWR animals.

Pathological LVH is associated with endothelial dysfunction and increased developed force to phenylephrine as a sensitization to this drug, suggesting that LVH does not only affect heart function and circulation but also induces modification in the entire vascular tree.

PO.017

Investigation of hemorheological parameters and oxidative stress in patients with cardiac syndrome X

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Introduction: Cardiac syndrome X (CSX) is an interesting clinical entity, yet the underlying pathophysiological mechanisms have not been fully elucidated. The most current pathophysiology suggests endothelial dysfunction and chronic low grade inflammation. The effects of hemorheologic parameters and oxidative stress on CSX remain unclear.The aim of the current study was to determine alterations in blood rheology (erythrocyte aggregation and deformability) and oxidative stress parameters [total oxidant/antioxidant status (TOS, TAS), oxidative stress index (OSI)] in patients with CSX.

Materials and methods: The study comprised 26 CSX patients (55.77 ± 12.33 years) and 37 age and sex matched (56.32 ± 11.98 years) healthy controls. Erythrocyte aggregation, and elongation index (EI), which is the indicator of erythrocyte deformability were measured by an ektacytometer. TOS and TAS were measured using a commercial kit and OSI was calculated.

Results: Erythrocyte deformability measured at 1.69, 3.00 and 30.00 Pa were lower in CSX patients than in the controls ($P = 0.001$, 0.017 and 0.006, respectively). Erythrocyte aggregation index (AI; 72.75 ± 7.65 versus 66.48 ± 6.63, $P = 0.002$); TOS (24.058 ± 7.833 versus 16.398 ± 7.963, $P = 0.001$); TAS (1.998 ± 0.327 versus 1.595 ± 0.549, $P = 0.0001$) were significantly higher in the CSX patients.

Discussion: In CSX, higher oxidative stress values appear to be related with lower erythrocyte deformability and higher erythrocyte aggregation. Abnormal hemorheological parameters and increased oxidative stress might be involved in the pathophysiology of CSX. Treatment modalities that decrease oxidative stress and modify rheological parameters might be beneficial for the management of CSX.

PO.018

Decreased erythrocyte deformability and increased erythrocyte aggregation in patients with pulmonary hypertension

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Introduction: Pulmonary hypertension (PH) has a multifactorial pathophysiology including obstructive remodeling in the pulmonary blood vessel system. The hemorheological properties of blood is one important factor behind the resistance to blood flow in the circulatory system. The aim of the current study was to determine alterations in blood rheology (erythrocyte deformability and aggregation) and oxidative stress parameters [total oxidant/antioxidant status (TOS/TAS) and oxidative stress index (OSI)] in patients with PH.
Materials and methods: The study comprised 20 PH patients (58.85 ± 14.83 years) and 35 age and sex-matched (57.11 ± 11.83 years) healthy controls. Patients were in class II (n = 7), class III (n = 12), and class IV (n = 1). Erythrocyte deformability and aggregation were measured by an ektacytometer. TOS and TAS were measured using a commercial kit and OSI was calculated.
Results: The average pulmonary vascular resistance (PVR) was 5.52 ± 3.4 Woods, mean right atrial pressure (mRAP) was 10.1 ± 5.97 mmHg, cardiac index (CI) was 4.31 ± 2.62 L/min/m^2, mixed venous O_2 saturation (SVO_2) was 65.26 ± 13.46%, and 6-min walk distance (6MWD) was 263.82 ± 138.71 m. Erythrocyte deformability measured at 3.00–16.87 Pa were significantly lower in PH patients (P = 0.035, 0.025, 0.021 and 0.030 respectively). Erythrocyte aggregation index (AI; 75.92 ± 5.93 versus 66.04 ± 13.93, P = 0.0001), and TAS (2.04 ± 0.45 versus 1.58 ± 0.56, P = 0.001) were significantly higher in patients with PH.
Discussion: The hemorheologic alterations observed (increased erythrocyte aggregation and decreased erythrocyte deformability) may theoretically increase the flow resistance through the pulmonary vascular system and may be of hemodynamic significance. These abnormalities therefore may represent logical pharmacologic targets in the future studies.

PO.019

High salt diet results in a hypertonic skin microenvironment not reflected in draining lymph

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Excessive sodium (Na^+) retention in the body is linked to hypertension. Although the kidney is the major regulatory system for body Na^+, it has also been shown that the skin is contributing in Na^+ homeostasis and can be a reservoir in situations of Na^+ retention. The skin microenvironment during such Na^+ retention has not been investigated in detail before. Therefore, we aimed to investigate the relationship between the osmolality of the skin and plasma upon salt loading. Rats received deoxycorticosterone acetate (DOCA) treatment (100 mg pellet) and 1% saline drinking water (DS) for 3 weeks or high salt diet chow containing 8% salt and 1% saline drinking water for 2 weeks (HSD). Control rats received low salt diet, <0.1% salt, and tap water (LSD) for 2 weeks. The osmolality of collected plasma, skin and skin lymph samples were measured using a vapor pressure osmometer. The average (± SD) skin osmolality (mosmol/kg) was significantly higher ($P < 0.05$, ANOVA) in the DS (319 ± 5, n = 6), and HSD (324 ± 4, n = 10) groups compared with LSD (308 ± 4, n = 10). The plasma and lymph osmolality did not differ significantly between the groups. In elution experiments we showed that there is a Na^+ gradient in skin itself, and that epidermis has higher osmolality than dermis regardless of the diet. These results show that excessive Na^+ accumulation in the skin generates a hypertonic microenvironment in the interstitium, which is not reflected in lymph draining the skin.

PO.020

Cardioprotective effects of Na^+/H^+ exchanger inhibition on reperfusion injury in rats

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Purpose: Increased H^+ and hyperactivation of Na^+–H^+ exchanger1 (NHE1) are the causes contracture and cell deaths induced by ischemia reperfusion (I/R) injury. The aim of this study is to investigate the effect of the NHE inhibitor (cariporide, CRP) on the

function and biochemistry of cardiac cells and to demonstrate the protective effect of CRP.
Methods: The study consisted of a control group with global I/R (ischemia time is 30 min and reperfusion time is 60 min) in isolated rat cardiac tissue (CONT, $n = 6$) and a second group with I/R with CRP treatment (10 μm/L; CRP, $n = 6$) during reperfusion. Left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP) were measured into the ventricule. Necrotic area was determined in the same cardiac tissues. Two new groups (CONT, CAR) were made for the evaluation of ECG and biochemical parameters ($n = 9$). ECG parameters of animals in each group were recorded and the hearts were homogenized, then malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and mitochondrial ATP levels were measured.
Results: CRP didn't prevent the increase in LVEDP and decrease the development of elongation in the QTc interval but prevented the increase of ST interval. MDA value was decreased ($P < 0.003$) and SOD and CAT activites were increased ($P < 0.006$). CRP caused more decrease in the mitochondrial ATP production compared to CONT group. Formation of necrotic area was decreased in CRP group ($P < 0.001$).
Conclusions: Cariporide decreased lipid peroxidation and increased the antioxidant defense mechanism however mitochondrial function was abolished due to intracelluler increased H^+ concentration.

PO.021

Angiotensin-(1–7) peptide and Mas receptor and angiotensin II- type 2 receptor are involved in aldosterone and cortisol production in human adrenocortical cell line (NCI-H295)

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Introduction: Aldosterone and cortisol secretion are tightly regulated by angiotensin II (Ang II) through angiotensin type 1 receptor (AT1R) in adrenal tissue. Although it is known that AT2R and Mas receptor, target of Ang-(1–7), can counteregulate AT1R effects in various tissues, their function in adrenal cortex remains to be elucidated.
Objectives: The aims of this study are to investigate: (i) the presence of AT2R and MasR in healthy human adrenocortical tissue and in Aldosterone-Producing Adenoma (APA); and (ii) if Ang-(1–7) could play a role in modulating aldosterone and cortisol production in a human adrenocortical cell line (NCI-H295) *in vitro*, through the quantification of *CYP11B1/CYP11B2* genes expression.
Materials and methods: We conducted several analysis using RT-PCR, immunoblotting, and immunohistochemistry to detect AT2R and MasR in different patient tissues. Moreover, in NCI-H295 cells, we stimulate MasR with Ang-(1-7), and AT1R and AT2R with Ang II. We also used irbesartan and A779 as blockers for AT1R and MasR respectively.
Results: AT2R and MasR are heterogeneously expressed in human adrenal cortex and in APA. Ang-(1–7) had no effect at low concentrations after 12 h, although higher concentrations significantly increased *CYP11B1* and *CYP11B2* transcriptional expression compared to control, and affected AngII effects. A779 did not significantly blunt the effects of Ang-(1–7) at high doses whereas irbesartan abolished its effects.
Discussion: AT2R and MasR are expressed in human adrenal cortex, albeit at much lower levels than the AT1R. This suggests a role of high local concentrations of Ang-(1–7) in the modulation of aldosterone and cortisol production.

PO.022

The crude leaves extract of adansonia digitata causes redox-sensitive endothelium-dependent relaxation involving no and EDHF in porcine coronary artery

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Introduction: *Adansonia digitata* leaves are used in the traditional treatment of the arterial high blood pressure in Senegal. The aim of this study was to determine the vasorelaxant effect of hydro-ethanol leaves extract of *Adansonia digitata* (ADF) in porcine coronary arteries and to investigate mechanism of this effect.
Material and methods: Porcine coronary rings were suspended in organ chambers for recording of changes in isometric forces. Rings provided by endothelium are incubated or not with various inhibitors. L-nitro-arginin (L-NA) an inhibitor of endothelial NO synthase; MnTMPyP, an inhibitor of intracellular production reactive oxygen species; Wortmanin, an inhibitor of redox-sensitive pathway PI3 kinase/Akt Src; apamin (APA) an inhibitor of small conductance potassium channels calcium-dependent (SKCa) and TRAM an inhibitor of intermediary conductance potassium channels calcium-dependent (IKCa); indomethacin (INDO), an inhibitor of cyclooxygenase. Thirty minutes after incubation with the inhibitors, vessels are contracted

with U46619 a mimetic analogue of the thromboxane A2 and relaxed with an increasing range of ADF. In some experiments, endothelium was removed before contraction with U46619 and concentration relaxation to ADF.

Results: ADF produced 100% relaxation at 10 μg/mL dose in endothelium intact arteries pre-contracted by U46619. ADF induces a redox-sensitive endothelium-dependent relaxation mediated by NO and endothelium-derived hyperpolarizing factors (EDHF) whereas prostacyclins has not appeared to play a role in the vascular effects.

Conclusion: *Adansonia digitata* induces vasodilation which may explain its antihypertensive effect and its use in traditional African medicine.

PO.023

Role of H_2S in frank-starling law realization in rat heart

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Introduction: H_2S is the third gaseous transmitter that play important role in vasorelaxation and myocardial function. Frank–Starling low is the most effective mechanism of heterometric regulation of heart function. Earlier we demonstrated the profound effect of H_2S donor NaHS at ability of myocardium to overcome increased left ventricular volume. However, the role of endogenos H2S is poorly studied.

Methods: We used L-cysteine (121 mg/kg) as the precursor of H_2S, propargylglycine (PAG, 11.3 mg/kg) as inhibitor of cystathionine gama-lyase, aspartate (17.1 mg/kg) as inhibitor of 3-mercaptopyruvate sulfurtransferase, buthionine-sulfoximine (BSO 22.2 mg/kg) as glutathione depletor. Isolated hearts of Wistar rats were perfused by Langendorff preparation. Functional reserves were evaluated studying P–V dependence. This was carried out with subsequent increase of latex balloon volume placed in the left ventricle with step by 34 μL.

Results: PAG increased LVDP by 20% at balloon volume 134 μL comparing to control. This effect was prolonged in PAG + L-cysteine group to 235 μL. Pretreatment with BSO abolished effect of PAG + L-cysteine, thus, the P–V curve repeated the control one. Inhibition of both way of H_2S synthesis (PAG + Aspartate) prevented increase of contractile activity of left ventricle right after first loading step (34 μL).

Discussion: Inhibition of both cytosolic and mitochondrial isoforms of H_2S production decreased heart functional reserves indicating involvement of H_2S in heterometric regulation of heart work. PAG + L-cysteine extended heart functional reserves and effectiveness of Frank–Starling low realization probably via directing L-cysteine to glutathione synthesis.

PO.024

Investigation of DNA damage with comet assay in patients with pulmonary hypertension

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Introduction: In pulmonary hypertension (PH), pathologic lesions involve increased oxidative stress and inflammatory processes. Comet assay is a sensitive, rapid and easy gel electrophoresis method currently used to demonstrate single and double strand DNA breaks. The purpose of our study was to investigate the potential contribution of DNA damage in PH.

Materials and methods: The study comprised 11 PH patients (63.82 ± 12.56 years) and 19 age- and sex-matched (59.68 ± 15.66 years) healthy controls. DNA damage was assessed using comet assay. Fifty cells per slide per sample were scored to evaluate DNA damage. Several parameters including head length, tail length, head intensity, tail intensity, tail moment, tail migration were evaluated for quantitative analysis of DNA damage.

Results: The average pulmonary vascular resistance was 5.86 ± 4.01 Woods, mean right atrial pressure was 9.09 ± 5.20 mmHg, cardiac index was 3.55 ± 1.27 L/min/m^2, mixed venous O_2 saturation was 62.2 ± 16.6%. There was no statistically significant difference between the PH and control groups in terms of DNA damage parameters such as head length (21.99 ± 4.86 versus 30.24 ± 10.64), tail length (30.93 ± 14.16 versus 29.47 ± 15.7), head intensity (73.29 ± 14.26 versus 77.57 ± 10.51), tail intensity (26.71 ± 14.26 versus 22.43 ± 10.51), tail moment (3.74 ± 2.97 versus 3.79 ± 2.79), tail migration (20.37 ± 14.18 versus 14.76 ± 11.6).

Discussion: Compared to the control group, we did not observe a significant increment in DNA damage in our PH patients. Due to advanced age of our sample in both groups, aging itself might have induced DNA damage in both groups. Larger studies with younger subjects are needed to have a better understanding of DNA damage in PH.

PO.025

Effects of melatonin on neuromuscular degeneration in early-phase sepsis

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Introduction: Melatonin has been demostrated to have anti-inflammatory, antioxidant, and also protective effects on mitochondrial function in both animal models and clinical studies. In the literature, few studies have reported the impacts of melatonin on energy metabolism in skeletal muscle in sepsis. In this study, we aimed to investigate the effects of melatonin on skeletal muscle structure, energy metabolism, and antioxidant levels in early-phase sepsis.
Material and methods: In our study, rats were grouped as control, lipopolysaccharide (LPS) (20 mg/kg, i.p), melatonin (10 mg/kg, i.p. three times), and melatonin + LPS. Melatonin was injected i.p. 30 min before and after LPS injection (two injections at 2 h intervals). Blood GSH level was measured using Ellman's method. Tissue sections were stained using modified Gomori trichrome (MGT), succinic dehydrogenase (SDH), and cytochrome oxidase (COX) for investigating muscle structure. Creatine, creatine phosphate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were evaluated using reverse-phase high performance liquid chromatography (HPLC) in muscle tissue.
Results: In the melatonin + LPS group, blood GSH levels were increased compared with the LPS group ($P < 0.01$). Melatonin reduced myopathic changes in the LPS group according to the histopathologic findings. In addition, ATP values were increased compared with the LPS group ($P < 0.05$).
Discussion: Our findings show that melatonin treatment may have beneficial effects on muscle damage in early-phase sepsis.

PO.026

The effect of deuterium-depleted water on NO/ROS balance in the heart

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Introduction: A concentration of deuterium in deuterium-depleted water (DDW) is 6–7 times lower than in naturally occurring water (20–25 ppm versus 150 ppm). When administered for a longer period, DDW can reduce the concentration of deuterium throughout the body, activating cellular mechanisms that depend on protons. The aim of study was to determine the effect of DDW on the balance between nitric oxide (NO) and reactive oxygen species (ROS) in the heart of rats treated with 15% fructose (FRU) for 3 weeks.
Material and methods: The experiment was carried on Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). They were divided into four groups: control, group treated with DDW or FRU and the group treated with DDW and FRU concomitantly. Blood pressure (BP) was measured by tail-cuff plethysmography. Total NO-synthase (NOS) activity was examined by measuring the rate of conversion from L-[^{3}H] arginine to L-[^{3}H] citrulline. Protein expressions of NOS isoforms were determined by Western blot analysis and concentration of conjugated dienes (CD) was measured spectophometrically.
Results: Neither DDW nor FRU had any significant effect on BP and relative heart weight in WKY or SHR. DDW increased cardiac NOS activity. However, no changes were observed in the protein expression of NOS isoforms. DDW decreased concentration of CD.
Discussion: DDW was able to increase cardiac NOS activity. Even, DDW decreased concentration of CD, a marker of oxidative damage. When DDW is administered in some pathological conditions for a longer period, it could leads to increased NO bioavailability.

PO.027

Terminalia avicennioides can reduce blood viscosity in drepanocytic subjects

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Objective: The aim of our study is to determine the effect of an hydroalcoholic extract of barks of

Terminalia avicennioides on the blood viscosity in drepanocytic subjects.

Material and methods: About 35 g of powder of the drug are wallowed in 175 mL of ethanol in 60%. After filtration, the hydroalcoholic extract so obtained is evaporated in the rotavapor to give a dry residue. This residue is resumed in some saline solution buffer to prepare a solution to 1 mg/mL of raw extract of barks of *Terminalia avicennioides*. Measures of the blood viscosity was performed on whole blood (AA, AS and SS) at baseline; after incubation with saline solution and buffer after incubation with the extract on *Terminalia avicennioides* hydroalcoholic solution (1 mg/mL). A viscosimeter cone-plan typifies Brookfied DVII allowed us to measure in 37°C the various speeds of cutting for the total blood viscosity.

Results: Our results show that the blood viscosity in drepanocytic carriers AS on baseline (5.82 ± 0.14 Pa/s) is superior to the average blood viscosity of the drepanocytics subjects SS (4.87 ± 0.15 Pa/s) and that of the normal subjects AA (4.06 ± 0.15 Pa/s). Our results show that also the hydroalcoholic extract of barks of *Terminalia avicennioides* is able to lead a significant reduction of blood viscosity in the drepanocytic carriers subjects and drepanocytic subjects.

Conclusion: These results show that the hydroalcoholic extract of barks of *Terminalia avicennioides* can decrease blood viscosity in sickle desease.

PO.028

Postconditioning – a clinically applicable form of cardioprotection and possibilities to improve its benefitial effect by molecular hydrogen

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The clinically applicable cardioprotective strategies which may improve prognosis of patients with infarction or subjected to larger cardiosirgical interventions are intensivelly studied. Hypoxic or ischemic postconditioning (HpostC, IpostC) belongs with this strategies becawse it is provided by short periods of reoxygenation or reperfusion after infarction which is always unannounced. Besides the prosurvival cell signalling pathways the postconditioning include an passive mechanisms based on gradual recovery of coronary flow or reoxygenation which prevents myocardial oxidative damage. Aim of our experiments was to evaluate if there is posibilities to improve this antioxidant effect of HpostC using molecular hydrogen (H_2). There were used isolated rat hearts perfused according to Langendorff with Krebs-Henseleit buffer (KHB) exposed to global 30-min ischemia (I) and 120-min reperfusion (R). HpostC was provided by four 1-min cycles of perfusion with oxygen-free (KHB). HpostC and H_2 coadministration was performed by perfusion with oxygen-free KHB saturated with H_2. HpostC decreased infarct size and improve functional parameters against non-HpostC controls. Morever H_2 even more improved the antiinfarct effect of HpostC. It seems that hydrogen treatment apears to be cardioprotective in a setting of HpostC. Molecular mechanism behind this effect remain to be elucidated.

PO.029

Morphological and functional alteration of human erythrocytes caused by some iranian vipers' venom: novel glance at the old problem

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Snake bites are an endemic public health problem in Iran, both in rural and urban area. Viper venom as a hemolytic biochemical 'cocktail' of toxins, primarily cause to the systemic alteration of blood cells. In the sixties and seventies, erythrocytes or red blood cells (RBCs) were extensively studied, but the mechanical and chemical stresses commonly exerted on RBCs continue to attract interest for the study of membrane structure and function. Here, we monitor the effect of *Vipera latifi, Macrovipera lebetina obtuse and Montivipera raddei* venom on human erythrocytes ghost membranes using phase contrast and fluorescent microscopy and changes in ATPase activity under snake venom influence in vitro. The ion pumps [Na^+, K^+]-ATPase and (Ca^{2+} + Mg^{2+})-ATPase plays a pivotal role in the active transport of certain solutes and maintenance of intracellular electrolyte homeostasis. We also describe the interaction of these venoms with giant unilamellar vesicles (GUVs) composed of the native phospholipid mixtures visualized by the membrane fluorescence probe, ANS, used to assess the state of membrane and specifically mark the phospholipid domains. To confirm molecular recognition of a collagen receptors on human erythrocytes by desintegrins from viper venoms, a surface acoustic wave-biosensor was applied. The data provide evidence for a direct confirmation of disintegrin binding to erythrocyte ghost membrane and thus, contribute to prove the presence of integtrins in the red cell membranes earlier neglected. Therefore, disintegrins are coming into light as attractive pharmacological tools for suggesting novel approaches to the generation of red blood cell's aggregation inhibitors.

PO.030

Effects of autonomic blockade on human cardiovascular regulation at rest and during exercise

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Introduction: The autonomic nervous system (ANS) is a key source of spontaneous heart rate variability (HRV). Pharmacological blockade of ANS helps in understanding this regulation. Assuming that ANS is the only modulator of the heartbeat, HRV during rest and exercise should be decreased with vagal or sympathetic blockades, and suppressed under double blockade. We investigated the effects of ANS blockade on HRV.
Methods: Seven healthy male subjects (age 24.3 ± 2.6) were tested during 5-min sessions at rest and 80-W exercise (EX), in four conditions: control (CTRL), atropine (ATP), metoprolol (bB) and atropine + metoprolol (double blockade, DB). We evaluated HRV by power spectral analysis.
Results: At rest, HR increased in ATP and DB. During EX, HR increased in ATP. Compared to rest, heart rate (HR) increased in CTRL, ATP and bB during EX. At rest, total power (Ptot) and low (LF) and high (HF) frequency components of HRV decreased with ATP and DB. LF/HF and normalised LF (LFnu) were higher in ATP than in DB. Normalised HF (HFnu) was unchanged. The same occurred in EX. Moreover, LF/HF and LFnu were lower and HFnu higher in DB. Compared to rest, Ptot, LF and HF decreased in ATP and DB during EX. LF/HF and HFnu increased in CTRL and bB. LFnu did not change.
Conclusion: Ptot, LF and HF showed positive values under DB at rest and EX: ANS is not the only responsible of heart beat modulation. LF under bB was the same as in CTRL, questioning the relation between LF and sympathetic modulation.

PO.031

Vitamin D – predictor of cardiovascular disease risk factor

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Background: Vitamin D is inversely associated with adiposity, glucose homeostasis, lipid profiles and blood pressure along with its role in calcium homeostasis and bone metabolism.
Objective: To evaluate the association between vitamin D and Cardiovascular disease risk factors in South Indian population.
Materials and methods: A total of 520 participants attending the Comprehensive health Check up clinic of **Amrita Institute of Medical Sciences; Kochi; Kerala; India** from January to March 2013 were enrolled in the Cross sectional study.Waist circumference,weight and height were measured and blood withdrawn for investigations including plasma 25 (OH) vitamin D.
Results: About 86.2% (448) of the people in the study had hypertension (HTN). Percentage of participants with HTN with severe, mild – moderate vitamin D deficiency, insufficiency and sufficiency were 77, 8.7, 6 and 8.3% respectively ($P < 0.001$). About 64% (333) of the people in the study had Dyslipidemia. Percentage of participants with Dyslipidemia with severe, mild – moderate vitamin D deficiency, insufficiency and sufficiency were 88.6, 8.4, 1.2 and 1.8% respectively ($P < 0.001$). About 45% (235) of the people in the study had Diabetes Mellitus (DM). Percentage of participants with DM with severe, mild – moderate vitamin D deficiency, insufficiency and sufficiency were 86.8, 6.4, 3 and 3.8% respectively ($P < 0.001$).
Conclusion: Vitamin D deficiency was highly prevalent in people with cardiovascular disease risk factors. Vitamin D deficiency is often clinically unrecognised, however laboratory measurements are easy to perform and treatment is inexpensive. Oral supplementation is the best tolerated and the most effective route of supplementation.

PO.033

NmMLCK a key player in intermittent hypoxia-induced inflammatory vascular remodeling?

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Introduction: Obstructive sleep apnea (OSA) is characterized by repetitive pharyngeal collapses during sleep leading to intermittent hypoxia (IH), the main contributor of OSA-related cardiovascular morbidity. OSA patients exhibit increased intima-media thickness (IMT) that correlates with circulating inflammatory markers and severity of nocturnal oxygen desaturation. In mice models, IH also induces hemodynamic alterations and cardiovascular inflammatory remodeling. The non-muscle myosin light chain kinase (nmMLCK) isoform contributes to endothelial cell-cell junction opening, monocyte migration and thus participates to inflammation. nmMLCK deficiency or inhibition has been reported to attenuate systemic, lung and atherosclerotic inflammation. The aim of the present study was to investigate the role of nmMLCK in the

IH-induced inflammatory vascular remodeling. We assess vascular remodeling/inflammation in different vascular beds (aorta, carotid and mesenteric arteries).
Methods and results: C57bl6 or nmMLCK knockout mice were exposed to 14-days IH or normoxia (N). IH was associated with an elevation of mean arterial pressure, which was prevented by the nmMLCK deletion. We demonstrated that IH induced an inflammatory response in the three vascular beds, characterized by an increased mRNA expression of CD45 and IFNγ, an increased expression of the macrophage marker F4/80 and an increased expression and activity of NFkB. Interestingly nmMLCK deletion prevented the IH-induced vascular inflammatory responses. On-going experiments are characterizing the effect of nmMLCK deletion on IH-induced structural vascular remodeling.
Conclusions: These results strongly suggest that nmMLCK participates to IH-induced vascular inflammatory remodeling and might represent an attractive target to fight against the occurrence of inflammation and the vascular outcomes of OSA.

PO.034

Mice with cardiomyocyte-specific, conditional loss of Akt1, in contrast to loss of Akt2, are shielded of cardiac hypertrophy induced by angiotensin II

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Introduction: Protein kinase Akt is involved in development of Angiotensin II (AngII) induced cardiac hypertrophy, but specific roles of its isoforms, Akt1 and Akt2, are not yet clearly defined.
Materials and methods: We generated tamoxifen-inducible, cardiomyocyte-specific knock out mice of Akt1 (iCM-Akt1KO) and Akt2 (iCM-Akt2KO). Pathological hypertrophy was induced by implantation of osmotic mini-pumps with AngII (1.5 mg/kg/day) or PBS for 14 days in transgenic and wildtypic (wt) male mice. Echocardiography was performed on days 0, 7 and 14. Cardiac fibrosis was studied by histological analyses.
Results: At day 0 there were no differences in cardiac function between wt and KO groups. AngII initiated cardiac hypertrophy in both wt and iCM-Akt2KO mice as seen by increased left ventricular mass and wall thickness at 7 and 14 days, but not in iCM-Akt1KO mice. The finding that only wt and iCM-Akt2KO mice develop cardiac hypertrophy was supported by an increased heart weight/body weight ratio after 14 days AngII in wt and iCM-Akt2KO, but not in iCM-Akt1KO mice. In contrast to iCM-Akt1KO mice, AngII treatment (7 days) diminished cardiac output and stroke volume in wt and iCM-Akt2KO mice. Sirius Red staining depicted that wt and iCM-Akt2KO mice developed fibrosis, whereas hearts of iCM-Akt1KO mice revealed development of fibrosis to a weaker extent.
Discussion: AngII treated wt and iCM-Akt2KO mice developed typical hypertrophy, while iCM-Akt1KO mice are protected from cardiac remodeling, clearly indicating that AngII induced cardiac hypertrophy is critically modulated by Akt1.

PO.035

Membrane capacitance changes in isolated rat cardiac myocytes

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Plasma membrane of cardiac myocytes is structurally active during growth and adaptation of myocardium. Nevertheless, its dynamics is not well known. Here we describe high resolution recordings of membrane capacitance changes in cardiomyocytes of rat heart. Similar records were instrumental in disclosing secretory and endocytotic mechanisms in simpler cells. We used a new method of membrane capacitance recording based on deconvolution of cell current responses to the square wave voltage stimulation (MAT-MECAS) that was applied the to whole-cell patch-clamped isolated cardiac myocytes. We observed spontaneous discrete capacitance events of 1–20 fF that could correspond to fusion and fission of single membrane bodies of 180–800 nm in diameter. In addition, large spontaneous increases of membrane capacitance of 80–250 fF were also observed. The increases were occasionally preceded by transient opening of a fusion pore that lasted for few hundreds of milliseconds. These might be related to the opening of the mouth of transversal tubules. Capacitance increases were also observed after stimulation by a train of calcium currents. In these experiments, the discrete capacitance increases of 50–160 fF occurred. This might reflect the calcium induced fusion of intracellular membrane bodies with the plasma membrane. When intracellular concentration of calcium ions was permanently increased the membrane capacitance showed a continuum of step-like capacitance events. These findings provide the evidence of membrane fusion/fission events related to physiologically relevant membrane activities in cardiac myocytes.

PO.036

Involvement of cyclooxygenase-2 – dependent mediators in the hyperemic response to spreading depolarization in the rat brain

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Introduction: Cyclooxygenase-2, a key enzyme of neurovascular coupling mediates, in part, the hyperemic response to somatosensory stimulation. We set out to investigate whether cyclooxygenase-2 also regulates the cerebral blood flow response to spreading depolarization (SD).

Materials and methods: Two cranial windows were prepared on the parietal bone of isoflurane-anesthetized rats. The rostral window was superfused with the cyclooxygenase-2 inhibitor NS-398 (100 μM in 1.5% DMSO in aCSF) ($n = 10$) or vehicle ($n = 9$). Ischemia was induced in half of the animals by ligation of both common carotid arteries. SDs were triggered by 1M KCl in the caudal cranial window. SD occurrence was confirmed in the rostral window by the acquisition of DC potential; the cerebral blood flow response was recorded with laser-Doppler flowmetry. Live, coronal brain slices of additional animals ($n = 5$) were prepared for the in vitro elicitation of SD by 1M KCl under NS-398 or vehicle treatment. SDs were evaluated on DC potential recordings.

Results: Cyclooxygenase-2 inhibition in the intact brain, or ischemia alone reduced the magnitude of the SD-related hyperemia to 60–63% of control value. Cyclooxygenase-2 inhibition under ischemia caused further reduction to 32%. At the same time, cyclooxygenase-2 inhibition decreased the amplitude of the SD-related negative shift of the DC potential in the intact cortex both *in vivo* (from -9.0 ± 2.4 to -6.8 ± 4.3 mV) and *in vitro* (from -11.5 ± 6.2 to -6.0 ± 3.3 mV), but had no such effect under ischemia.

Discussion: Cyclooxygenase-2 emerges as a significant mediator of the SD-associated cerebral blood flow response in the intact and ischemic rat brain.

PO.037

Identification of a new adenylyl cyclase 8 isoform – implication in vascular smooth muscle cells trans-differentiation and pathological vascular remodeling

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Atherosclerotic lesion expansion and post-angioplasty restenosis result mostly from a phenotypic transition in the medial vascular smooth muscle cells (Shankman, 2015, *Nature Med*, 21: 628). The cyclic AMP, whose production and degradation are operated by adenylyl cyclases and phosphodiesterases respectively, plays a central role in modulating VSMCs phenotype through integration of environmental stimuli and implementation of the related cellular responses (Yokoyama, 2010, *Circ Res*, 106: 1882).

We have demonstrated, *in vitro*, that the trans-differentiation of VSMCs into inflammatory/migratory cells depends on the *de novo* expression of AC8 (Clément, 2006, *J Cell Physiol*, 208: 495; Keuylian, 2012, *J Biol Chem*, 287: 24978). In human atherosclerotic lesions, pathological neointimal VSMCs display a high level of AC8 (Gueguen, 2010, *J Pathol*, 221: 331). Knocking AC8 in ApoE$^{-/-}$ mice decreased aorta inflammation and size of lesions; siRNA-mediated knock down of AC8 reduced neointima formation in a rat model of post-angioplasty restenosis (unpublished).

Recently, we discovered that 90% of total AC8 expressed in tdVSMCs consist of a newly identified splice variant lacking the first five trans-membrane domains: the AC8D. Immunocytochemistry and AC activity assays evidenced a profound change in membrane topology knocking the cyclase activity. Using FRET-based biosensors, we evidenced that AC8D provokes a dramatic increase in PDE4 activity that limits the accumulation of cAMP. In view of the inhibitory effect of cAMP on VSMCs proliferation/migration, the AC8D-induced PDE4 activity may participate in restenosis by maintaining pathological properties of tdVSMCs. Ongoing investigations aim at clarifying the mechanisms involved and evaluating the role of AC8D in pathological vessel remodeling.

PO.038

Epithelial Na^+ channel (ENAC) differentially contributes to shear stress-mediated regulation of the vascular tone in murine carotid and mesenteric arteries

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Introduction: A potential 'new player' in the control of vascular tone and response to flow and shear stress is the Epithelial Sodium Channel (ENaC). However, it is unknown if ENaC has different roles in conduit versus resistance arteries. We tested the hypothesis that intraluminal shear stress regulates endothelial ENaC activity in both types of arteries.
Materials and methods: Carotid and 3rd order mesenteric arteries were isolated from euthanized male C57Bl/6 mice, and maintained at a mean intraluminal pressure of 60 mmHg in a pressure myograph. Stepwise increases in intraluminal flow from baseline conditions (no flow) were induced and changes in the internal diameter were measured. Flow-protocols were also applied in the presence of amiloride (10 μM), L-Name (100 μM) and BQ-123 (1 μM) to determine if ENaC interferes with endothelial nitric oxide synthase (eNOS) and/or endothelin-1 ET_A receptor activity.
Results: Under no-flow conditions, amiloride dilated carotid ($13 \pm 2\%$, $P < 0.05$), but not mesenteric ($0.5 \pm 0.9\%$, $P > 0.05$) arteries. With intraluminal flow amiloride-sensitive effects were observed in both arteries, indicative of ENaC-mediated activity. In carotid arteries, amiloride augmented flow-mediated dilation ($9.2 \pm 5.3\%$) compared with control (no amiloride, $6.2 \pm 3.3\%$; $P < 0.05$). In mesenteric arteries amiloride induced a flow-mediated constriction ($-11.5 \pm 6.6\%$) compared with control ($-2.2 \pm 4.5\%$; $P < 0.05$). L-Name mimicked and prevented in both arteries the ENaC flow-mediated effects, and BQ-123 abolished the amiloride effect in mesenteric arteries.
Discussion: We conclude that ENaC is a crucial shear-sensor for flow-mediated responses in conduit as well as resistance arteries, and may influence different downstream signalling machineries in the different arteries.

PO.039

Magnetic nanoparticles for *in vitro* artificial blood development: glutaraldehyde linked hemoglobin molecules

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Introduction: Immobilization of biomolecules has been attracting attention due to advantages. In this study, we have investigated and characterized the redox properties of immobilized hemoglobin on glutaraldehyde-coated magnetite nanoparticles (GAMNP/Hb) for use as artificial blood substitutes.
Material and methods: In this study, hemoglobin (Hb) molecules were immobilized onto GAMNP. Constructs were analyzed by FT-IR, elemental analysis, SEM-EDX, XRD, TEM spectrometry. The structure and properties of immobilized Hb were investigated by cyclic voltammetry (CV) and the amount of immobilized Hb was analyzed by elemental analysis and Kjeldahl methods. Human umbilical vein endothelial cells (HUVEC) were used to study the material-cell interactions as well as the quantification of antioxidant enzymes produced in response to GAMNP/Hb.
Results: The FT-IR spectra of GAMNP/Hb showed that the characteristic peaks were observed at 3000–3700 cm^{-1} (O–H), 2908 cm^{-1} (C–H), 1633 cm^{-1} (C=O), 1524 cm^{-1} (C=N) and 559 cm^{-1} (Fe–O). The nanoparticle size was 26 ± 5 nm which was calculated from Debye–Scherrer equation according to XRD results. CV measurements revealed that -0.468 V cathodic potential and -0.06 V anodic potential were measured against Ag/AgCl standard electrode. GAMNP/Hb enhanced cell proliferation against control ($P < 0.005$), there was no significant change in antioxidant enzyme activity.
Discussion: New characteristic peaks at the FT-IR spectra clearly demonstrated the linkage of Hb to GAMNP. XRD, elemental analysis and TEM results supported these findings. Hb molecules conserved their oxidation and reduction states according to CV. It is considered that endothelium cells responded to oxygen delivered by GAMNP/Hb through increase in proliferation rate.

PO.040

Atrial fibrillation is associated with a marker of endothelial function and oxidative stress in patients with acute myocardial infarction

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Background: Atrial fibrillation (AF), whether silent or symptomatic, is a frequent and severe complication of acute myocardial infarction (AMI). Asymmetric dimethylarginine (ADMA), an endogenous eNOS inhibitor, is a risk factor for endothelial dysfunction. We addressed the relationship between ADMA plasma levels and AF occurrence in AMI.

Methods: A total of 273 patients hospitalized for AMI were included. Continuous electrocardiographic monitoring (CEM) ≥48 h was recorded and ADMA was measured by High Performance Liquid Chromatography on admission blood sample.

Results: The incidence of silent and symptomatic AF was 39 (14%) and 29 (11%), respectively. AF patients were markedly older than patients without AF (≈20 years). There was a trend towards higher ADMA levels in patients with symptomatic AF than in patients with silent AF or no AF (0.53 versus 0.49 and 0.49 μmol/L, respectively). After matching on age, we found that patients with symptomatic AF had a higher heart rate on admission and a higher rate of patients with LV dysfunction (28% versus 3%, $P = 0.025$). Patients who developed symptomatic AF had a higher ADMA level (0.53 versus 0.43 μmol/L; $P = 0.001$). Multivariate logistic regression analysis to estimate symptomatic AF occurrence showed that ADMA was independently associated with symptomatic AF [OR: 2.46 (1.21–5.00), $P = 0.013$] beyond history of AF, LVEF < 40% and elevated HR.

Conclusion: We show that high ADMA level is associated with the occurrence of AF. Although no causative role can be concluded from our observational study, our work further supports the hypothesis that endothelial dysfunction is involved in the pathogenesis of AF in AMI.

PO.041

Biomarker kinetic modelling to estimate the effect of conditioning therapies on myocardial ischemia-reperfusion injury

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Infarct size is a key predictor of subsequent cardiovascular events following ST-segment elevation myocardial infarction (STEMI). This relevant endpoint was used in every recent study exploring conditioning therapies. Generally assessed through serial blood sampling that gives approximate results, it can be accurately measured by imaging techniques such as cardiac magnetic resonance imaging with reduced availability in daily practice. We developed a mathematical biomarker kinetic model based on pharmacokinetic compartment models to easily and accurately estimate infarct size using the population of five clinical trials evaluating the impact of conditioning therapies in STEMI between 2005 and 2013. We used individual data from these studies focusing on the effect of ischemic postconditioning (3), pharmacological postconditioning with Cyclosporine A (1), and remote ischemic perconditioning (1) in STEMI patients presenting within 6 h of the onset of symptoms and treated by primary angioplasty. Serial blood sampling was available in all studies with data regarding creatine kinase (CK), CK specific of cardiomyocytes (CK-MB) and cardiac troponin I (cTnI). Our mathematical model allowed an accurate estimation of infarct size using all three biomarkers. It even showed a significant influence of conditioning therapy on cTnI release, that the original publications failed to report. This biomarker kinetic modeling approach identified CK-MB as the most sensible biomarker in determining infarct size and supports the development of limited sampling strategies, with total biomarker released amount being determined using a lower number of samples. It will certainly be a useful add-on to future studies in the field of STEMI and cardioprotection.

PO.042

Exercise capacity and cardiac hemodynamic response in ApoE/LDLR$^{-/-}$ mice: a paradox of preserved V'O_{2max} and exercise capacity despite coronary atherosclerosis

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Introduction: Atherosclerosis is usually associated with worse exercise capacity. Accordingly, exercise capacity of female ApoE$^{-/-}$ mice was impaired and their cardiac structure and function were reduced. However, no reports published so far provide evidence of impaired cardiac function of atherosclerotic ApoE/LDLR$^{-/-}$ mice and there are no data regarding their exercise capacity. Therefore, we investigated exercise capacity, function of coronary circulation and basal/dobutamine-stimulated cardiac performance of female ApoE/LDLR$^{-/-}$ versus age-matched C57BL6/J mice with respect to atherosclerosis development.

Material and methods: To estimate exercise capacity, we assessed maximal oxygen consumption (V'O_{2max}), maximal distance ($DIST_{max}$), maximal velocity (v_{max}) and running time (t) of young ApoE/LDLR$^{-/-}$ mice with first signs of atherosclerosis and their older counterparts versus age-matched C57BL6/J mice. Cardiac function at rest and under dobutamine stimulation was assessed *in vivo* by MRI imaging and NO- and PGI_2-dependent function of coronary circulation was assessed in isolated perfused hearts. Atherosclerosis progression was visualised in large and small coronary arteries by OMSB and ORO staining.

Results: We found that V'O_{2max} of ApoE/LDLR$^{-/-}$ mice was preserved and they displayed better running exercise capacity than healthy mice. It was associated with preserved NO-dependent function of coronary circulation and increased COX-2-dependent PGI_2 production in ApoE/LDLR$^{-/-}$ mice. Cardiac performance at rest as well as cardiac reserve were also preserved in ApoE/LDLR$^{-/-}$ mice.

Discussion: Robust compensatory mechanisms in coronary circulation, including increased vascular responsiveness to NO and increased generation of PGI_2, could, at least partly, account for excellent exercise capacity and preserved cardiac function of ApoE/LDLR$^{-/-}$ mice.

PO.043

Mechanical dispersion: a novel tool to diagnose pathological hypertrophic remodeling in athletes

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Background: Previous studies have demonstrated that global longitudinal strain (GLS) is altered in hypertrophic cardiomyopathy (HCM) patients in comparison to athletes. Nevertheless these results rely on studies comparing sedentary HCM to healthy athletes. The aims of the study were to confirm these findings in an appropriate group of athletes with HCM; and to study the additive value of novel parameters, that is mechanical dispersion and exercise GLS.

Methods: We included 36 athletes with HCM, matched on age with 36 sedentary HCM patients, 36 healthy athletes and 36 sedentary controls. Both athlete groups were matched on training duration and HCM groups on maximal wall thickness. All subjects underwent an echocardiography at rest and during submaximal exercise. GLS was assessed; standard deviation of time to maximum myocardial shortening of longitudinal strain was calculated as a parameter of mechanical dispersion.

Results: HCM sedentary group showed the lowest resting and exercise GLS. Resting GLS was not different between HCM athletes and the two control groups but exercise GLS enabled to differentiate HCM athletes from healthy athletes. Mechanical dispersion was higher in both HCM groups vs. both control groups at rest and during exercise. ROC analysis in the athlete groups demonstrated that resting mechanical dispersion (AUC = 0.949 ± 0.023) had better ability to identify HCM compared with GLS at rest (AUC = 0.644 ± 0.069; $P < 0.001$) or during exercise (AUC = 0.706 ± 0.066; $P < 0.005$).

Conclusions: Mechanical dispersion of longitudinal strain seems to be a promising tool for the diagnosis of HCM in athletes.

PO.044

Are *HFE* gene knock-out mice dying earlier than wild-type mice because of iron overload in heart tissue induce cardiac dysfunction and exercise limitation?

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Introduction: Mechanisms involved in cardiac dysfunction induced by iron overload are not fully elucidated. The goal of our study is to evaluate cardiac function in *HFE* gene knock-out mice and determine the potential impact of iron overload induce-cardiac dysfunction on exercise performance.
Materials and methods: Male wild type (WT, $n = 9$) and *HFE* gene knock-out (KO, $n = 6$) were used in this study. Cardiac function (echocardiography) was assessed at rest at 14, 18 and 20 months. Mice also underwent running exercises on treadmill and were sacrificed. Hearts were stored at −80°C.
Results: The shortening and ejection fractions were significantly reduced in KO mice compared to WT mice at 14, 18 and 20 months (in average from 14 to 20 months: −28.1 and −19.8% respectively, $P < 0.001$). The peak oxygen consumption (VO_{2peak}) was significantly lower in KO mice than in WT mice in average from 14 to 20 months (in mL/kg/h: 7.43 ± 0.33 versus 8.36 ± 0.17, $P < 0.01$). The two KO mice which died before 18 and 20 months where those which exhibited two of lowest shortening and ejection fractions and VO_{2peak} compared to the other KO mice.
Conclusion: Our study showed that KO mice exhibit cardiac dysfunction and exercise limitation. The heart iron overload could explain this cardiac dysfunction and both may be involved in the reduction of KO mice life span compared to WT mice.

PO.045

The effect of bioactive compound of aronia melanocarpa on cardiovascular system in experimental hypertension

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Introduction: The aim of the study was to analyze the effects of non-alcoholic concentrate from aronia melanocarpa (AM), a rich source of polyphenols, on blood pressure (BP), total NOS activity, cytokine level and concentration of conjugated dienes (CD) in the left ventricle (LV) of L-NAME-induced hypertensive rats.
Methods: The 12-week-old male WKY rats were assigned to control group, group treated with L-NAME (40 mg/kg/day), group treated with AM concentrate (1 mL/kg/day), and group treated with combination of L-NAME (40 mg/kg/day) and AM concentrate (1 mL/kg/day) in tap water. The experiment lasted 3 weeks. BP was measured by the tail-cuff-plethysmography. NOS activity was determined by conversion of ^{3}[H] Arginine to ^{3}[H] Citrulline in the LV. Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. Concentration of CD was measured spectrophotometrically.
Results: After 3 weeks of L-NAME treatment BP was increased by 28% than the control group. AM reduced BP by 21% in L-NAME + AM group in comparison to L-NAME group. Moreover, AM inhibited TNF-α and IL-6 production in the plasma in L-NAME + AM group in comparison to L-NAME group. NOS activity of LV in L-NAME group was decreased by 40%, on the other hand AM was able to increase NOS activity on 90% of control level. In addition, AM decreased concentration of CD by 40% in comparison to L-NAME group.
Discussion: Considering the results, active substances from AM may have positive effect on blood pressure, cytokine level, concentration of CD and NOS activity in L-NAME induced hypertension.

PO.046

Role of AC8 in atherosclerosis in the atherogenic ApoE knockout mouse model

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Atherosclerosis is a chronic inflammatory disease affecting the walls of large and medium-sized arteries. It is characterized by localized wall thickening (lesions), which may detach, leading to the formation of occlusive thrombi (clots). Change in the phenotype of the smooth muscle cells lining the arteries, resulting in a transition from a contractile/quiescent state to a proliferative/migratory/inflammatory state, contributes to atherogenesis. Our team demonstrated the key role of the Adenylyl Cyclase 8 (AC8) in this transition.

This project aims at studying the role of AC8 in atherosclerosis by following the formation of atherosclerotic plaques along aging in mice susceptible to the development of this disease ($ApoE^{-/-}$) in which the gene encoding AC8 had been invalidated.

Our results show that the average lesions size measured on aortic roots sections was not different

between ApoE$^{-/-}$ and ApoE$^{-/-}$ × AC8$^{-/-}$ mice whatever their age. However, invalidation of the AC8 gene decreased inflammation of the aorta and the area of the arterial wall displaying atherosclerotic lesions (*en face*) when compared to the age-related ApoE$^{-/-}$ mice. Indeed, the ratio of the leased surface/global abdominal aorta area was significantly smaller at 38 and 48 week-old; the expression of the transcripts coding for inflammatory markers (including IL-1β, CCL2, CCL3, ICAM-1, VCAM-1 and MSR1) tested is significantly decreased as soon as 20 week-old.

These results indicate that knocking out AC8 gene makes atherogenic mice less susceptible to developing atherosclerotic lesions in abdominal aorta likely due to the inflammation lowering effect.

PO.047

Beneficial effect of carotenoids to heart function associated with cell-to-cell communication via gap junctions after LPS administration

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The most widely civilization diseases of cardiovascular system are accompanied by inflammation. Connexin-40 is a protein of gap junctions which is expressed except the electrical conduction system of heart and atria in vascular endothelium, modulating cellular homeostasis and myoendothelial communication. Changes in Cx isoforms expression during pathophysiological conditions emphasize importance of Cx's as therapeutic targets in cardioprotection. Aims of our pilot study was to examine the effect of lipopolysaccharide (LPS) on expression of Cx40 in left ventricle (LV) and to investigate anti-oxidative effects of natural carotenoids of yeast biomass (Rhodotorula glutinis). Wistar rats were fed with carotenoids (10 mg/kg/day) for 10 days after LPS application, single dose of LPS (*E. coli*, 1 mg/kg, i.p.). LPS reduced Cx40 expression compared with controls whereas treatment with carotenoids normalized Cx40 expression. LPS elevated levels of measured inflammatory markers (NF-κB, MDA, NOS and NAGA activity) in plasma and LV, indicating the presence of moderate inflammation. LPS locally decreased glycogen phosphate (GIP) activity and caused rarefaction of capillaries of arterial bed as well. Carotenoids reduced levels of inflammatory markers and protected GlP activity and capillary bed against injury. LPS decreased pressure and increase tachycardia in LV. Our results indicate that LPS impairs the function of heart through blood vessels, which reflects changes in the cell-to-cell communication via gap junctions represented by Cx40. Treatment with carotenoids had beneficial effect to heart function.

PO.048

L-arginine supplementation alleviates post-prandial endothelial dysfunction when baseline fasting plasma arginine concentration is low: a randomized controlled trial in healthy subjects with risk factors for metabolic syndrome

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Introduction: Vascular endothelial dysfunction, which precedes atherosclerosis, is transiently induced by a high-fat meal. We aimed to study the effect of a low dose of a sustained release (SR)-arginine supplementation on fasting and postprandial endothelial function in healthy subjects with risk factors associated with the metabolic syndrome, according to their baseline arginine status.

Design: In a randomized, double-blind, two-period crossover, controlled by placebo trial (4 weeks of treatment, 4 weeks of washout), we compared the effects of 1.5 g t.i.d Placebo and SR-arginine on endothelial function [flow-mediated dilation (FMD) and reactive hyperemia index (fRHI)] in 33 healthy subjects with hypertriglyceridemic waist phenotype. Using subgroup analysis, we determined if the effect was related to the fasting plasma arginine concentration.

Results: The SR-arginine supplementation attenuated the postprandial decrease in FMD ($P < 0.0001$) but the fasting FMD was higher after placebo than SR-arginine. The effects of SR-arginine varied with plasma arginine concentration ($P < 0.05$), and in the subjects with the lower levels (<78.2 μmol/L), SR-arginine attenuated the postprandial endothelial dysfunction ($P < 0.05$) with a greater artery response at the end of the postprandial period, as assessed by both FMD and fRHI.

Conclusion: Low dose SR-arginine supplementation alleviates endothelial dysfunction in the postprandial state when fasting plasma arginine concentration is low. We suggest that a low baseline fasting arginine concentration reveals a configuration of arginine metabolism associated with a limitation of the bioavailability of endogenous arginine for NO synthesis during a postprandial metabolic challenge. This background metabolism would determine the benefit of arginine supplementation.

PO.049

Obesity in children and adolescents: a relation to endothelial function and arterial stiffness

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Introduction: Obesity is a growing global problem not only in adults but also in children and adolescents. The obesity is linked with an increased risk of atherosclerosis development. Our aim was to ascertain the presence of early atherosclerotic changes in obese adolescents and to compare the findings with their lean peers.

Methods: We used two non-invasive methods designated for the quantification of measures related to the early stages of the atherosclerosis development: RH-PAT method (Reactive hyperemia peripheral arterial tonometry – RHI index) – a method for non-invasive examination of endothelial dysfunction and a method for the arterial stiffness evaluation using CAVI index (Cardio-ankle vascular index). In 16 obese (7f, 9 m, age: 15.22 ± 2.2 years, BMI: 30.95 ± 2.5 kg/m^2) and 16 non-obese (7f, 9m, age: 16.22 ± 1.5 years, BMI: 20.74 ± 2.0 kg/m^2) adolescents were the indices RHI and CAVI assessed.

Results: We found a significant difference in RHI ($P = 0.018$) and CAVI ($P = 0.014$) between obese and lean participants. In contrast to our expectations, RHI was higher (↑RHI = less impaired endothelial function) and CAVI was lower (↓CAVI = lower arterial stiffness) in obese group compared to controls ($RHI_{ob} = 1.66 \pm 0.28$ versus $RHI_{cont} = 1.4 \pm 0.25$; $CAVI_{ob} = 4.57 \pm 0.92$ versus $CAVI_{cont} = 5.09 \pm 0.38$).

Discussion: In accordance with several recent studies, we found less expressed early atherosclerotic changes in obese adolescents – the mechanisms responsible for these findings require future study.

PO.050

In vitro exposure of human endothelial progenitors to sevoflurane improves their survival abilities

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Endothelial progenitor cells (EPCs) have important roles in vessel and tissue repair; however, their regenerative potential is impaired due to the poor survival in the ischemic microenvironment. Recent data suggest a promising potential of volatile anesthetics for improving stem cells biology. Thus, we hypothesized that sevoflurane could stimulate EPCs growth and viability.

Mononuclear cells were isolated from human umbilical cord blood by gradient centrifugation. After 5 days in culture, the cells were exposed for 1 or 2 h to sevoflurane 2 or 4% in air/5% CO_2, or only to air/5% CO_2 (control) in a modular chamber. 24 or 48 h post-exposure, viability, proliferation and apoptosis were assessed using lactate dehydrogenase (LDH) leakage assay, methyl tetrazolium salt assay and FITC-annexin V/propidium iodide staining.

LDH leakage was discretely lowered, whereas the levels of formazan were increased ($P < 0.05$ for 1 h incubation with 4% sevo at 24 h, and with 2% sevo at 48 h postexposure, $n = 4$) in treated vs control samples. A 2 h preconditioning protocol indicated a prompter expansion of cultures exposed to 2% than to 4% sevoflurane. Early ($P < 0.05$) and late apoptosis ($P < 0.05$ only for 2% sevoflurane, $n = 5$) were diminished in preconditioned samples.

In conclusion, sevoflurane has protective effects on viability and proliferation of human EPCs, suggesting a promising potential of anesthetic preconditioning for improving the regeneration of ischemic tissues.Preconditioning with 2% sevoflurane for 2 h seems to be an efficient preconditioning protocol.

PO.051

Nanoparticle-loaded aliskiren: beneficial effects on the cardiovascular system

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Introduction: Despite beneficial effects, clinical use of aliskiren is limited by short lifetime of this drug. We

aimed to determine the effects of nanoparticle-loaded aliskiren, with gradually realized drug, on blood pressure (BP), nitric oxide synthase (NOS) activity, and structural alterations developed due to hypertension.
Materials and methods: The 12-week-old male SHRs were divided to the untreated group, group treated with powdered aliskiren, or nanoparticle-loaded aliskiren (25 mg/kg per day), and nanoparticles only for 3 weeks by gavage. NOS activity including isoforms expressions, and collagen and elastin contents were determined in both heart and aorta. Wall thickness (WT), inner diameter (ID) and cross sectional area (CSA) were determined in the aorta.
Results: At the end of experiment, BP was lower in both powdered aliskiren and nanoparticle-loaded aliskiren groups with more pronounced effect in the second one. Moreover, nanoparticle-loaded aliskiren was able to decrease collagen content (by 11%) and CSA (by 25%) in comparison to the powdered aliskiren group, while it had no significant effect on the similar parameters in the heart. There were no significant changes in elastin content, WT and ID among aliskiren groups and control group. Polymeric nanoparticles, however, increased collagen and elastin contents and WT of the aorta. Only nanoparticle-loaded aliskiren increased the activity of NOS in the heart (7.42 ± 0.36 pkat/g) in comparison to untreated SHR (5.15 ± 0.27 pkat/g). In conclusion, nanoparticle-loaded aliskiren seems to be promising drug in large vessels protection. More appropriate and effective polymeric nanoparticles, however, are needed for better tissue protection.

PO.052

Novel approaches to mitigate ischemic injury: potential mechanisms of cardioprotection by 'remote' preconditioning

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Introduction: Application of ischemic preconditioning (IPC) in humans to ameliorate ischemia/reperfusion injury is limited by technical requirements (chest opening). However, non-invasive 'remote' PC (RPC) was already used in clinical conditions. Although its mechanisms are not completely clear, involvement of transcription factors PPAR has been proposed to play a role. We have previously shown that up-regulation of PPAR-α confers IPC-like cardioprotection linked with activation of pro-survival cascades, antioxidative and antiapoptotic effects.
Methods: The study aimed to assess the effect of RPC induced by three cycles of 5-min pressure cuff inflation (200 mmHg)/5-min deflation applied on hind limb of normotensive and hypertensive rats, with or without PPAR-α antagonist MK886 (3 mg/kg i.p., given prior to RPC), on the size of infarction (IS, TTC staining), recovery of LVDP (left ventricular developed pressure) and incidence of ventricular tachyarrhythmias in Langendorff-perfused hearts exposed to 30-min global I/120-min R. IS was expressed in percentage of area at risk size, while post-IR recovery of LVDP was evaluated in % of baseline values. In parallel groups, LV tissue was sampled for examination of PPAR-α (RT-PCR) and pro-survival protein kinase levels (WB).
Results: RPC significantly reduced IS, severity of arrhythmias and improved recovery of LVDP in both, normotensive and hypertensive hearts. Cardioprotective effects, as well as RPC-induced up-regulation of PPAR-α and PKCε were blunted by MK886.
Discussion: The results suggest the effectiveness of RPC in protection against ischemia/reperfusion injury in both, healthy and diseased myocardium, and the role of PPAR-α as one of potential cardioprotective mechanisms.

PO.053

Radiation induced heart disease. Molecular mechanisms of radiation injury and selected substances with potencial to ameliorate its toxic effect

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Therapeutic doses of chest irradiation in oncological patients represent a significant source of cardiovascular morbidity and mortality. Irradiation of normal tissues leads to intracellular signaling and altered cell function, resulting in organ dysfunction and ultimate failing of the heart. This process can be modulated by therapies directed at mitigating the cascade of events resulting from normal tissue injury.

The aim of the study was to explore the effects of a single dosage radiation (25 Gy) applied to the mediastinal area of normal adult Wistar rats on the heart functional parameters, selected molecular markers and the effects of selected drugs that might be involved in molecular mechanisms of tissue radiation injury.

Morphological examination revealed increased ultrastructural signs of both, endothelial cell degeneration/regeneration, microthrombi, activated fibroblasts, mast cells and monocytes. Gene expression of PPARα was significantly lower in left ventricular tissue of irradiated rats, while expression of microRNA-21 in these hearts was increased nearly 10-fold. Myocardial Cx43 was upregulated via reduced miRNA-1. miRNA-15b was downregulated almost by 42% and Bax protein decreased, indicating triggered adaptive mechanism. Activities of circulating 72 kDa MMP-2 was significantly increased. As compared to untreated control groups, irradiation caused a significant decrease in TNF-α. Enbrel and ASA decreased the level of TNF-α, however, in the Sildenafil group, there was an increase in the TNF-α levels.

These results suggest possible protective action of Enbrel and Tadalafil on the heart damaged by irradiation as demonstrated by changes in miRNAs and TNF-α levels.

PO.054

Electrocardiographic left ventricular hypertrophy: correlation with left ventricular mass by echocardiogram in obese patients

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Introduction: Left ventricular hypertrophy (LVH) and obesity are important cardiovascular risk factors.
Material: A cross sectional observational study of 101 healthy adults aged 24–60 years was performed. Anthropometric data, blood pressure, standard 12-lead ECGs and echocardiogram finding of LVH were collected and compared.
Results: The mean age was 37 years. Of the study population, 21% were presented as overweight and 52% as obese. Sensitivity of Cornell voltage and product was significantly higher in Obese and overweight patients comparing to Socolow–Lyon voltage and product. Body mass index (BMI) values showed significant correllations with LV mass. However M-mode echocarfiographic LV mass is superior to ECG criterias for clinical diagnosis of LVH in obese patients.
Conclusion: These abnormalities in LV structure may have important implication for explainning the increased cardiovascular morbidity and mortality caused by obesity.

PO.055

Influence of menstrual cycle on hemodynamics in young African women

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Introduction: Some differences observed in women compared to men are usually attributed to sexual hormones.The aim of this study was to determine in young African women, the influence of sexual hormones on hemodynamic profile at rest, peak effort and recovery.
Materials and method: Fourteen young black African women aged 24 ± 1.7 years, with a regular menstrual cycle and not taking contraception were selected. A supine rest and a stress test with 6 min recovery (3 active, 3 passive) were done on morning at each three phases of their menstrual cycle which were confirmed by estradiol and progesterone levels. Heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure were taken at rest, peak effort and recovery.
Results: At rest, estradiol level was significantly higher during follicular phase (356.6 ± 160.7 pg/mL) and progesterone level during luteal phase (16.38 ± 5.88 ng/mL). SBP were comparable during the three phases. At luteal phase, DBP was significantly lower (68 ± 6 mmHg, 71 ± 5, 70 ± 6, $P = 0.03$) while heart rate significantly higher (74 ± 13 mmHg, 69 ± 15, 68 ± 14, $P = 0.01$). At peak effort, HR, SBP and DBP were each comparable during the three phases. At the 3rd minute of passive recovery, SBP was significantly lower at follicular phase (106 ± 9 beats/min, 111 ± 10, 110 ± 9, $P = 0.04$).
Discussion: During luteal phase, progesterone and estradiol level could induce a vaso-dilatation with lower DBP and a more important sympathic activity could explain a higher HR. At follicular phase, an important parasympathic activity could explain a lower SBP.

PO.056

Influence of prolonged total light deficit during chamber rest on electrophysiological parameters of human heart

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Aim: Primary aim of the study is to evaluate effect of resting stay in absolute darkness on ECG parameters of young people. The results of treatment method, which is based on staying in absolute darkness to improve the health of people who are under constant stress, suggest that this method could affect electrophysiology of heart and decrease especially heart rate.
Methods: Evaluations were performed in 17 students aged between 19 and 26 years. They were placed for 72 h in a special room with an absolute darkness. The room met the optimal conditions for a comfortable individual stay and it was located in a quiet place. In the room there was only one person per stay. Participants received food and drink according to their needs. RR, PQ, QT, QTc intervals and heart rate were evaluated from II. bipolar limb record of ECG 1 day before entering the room and 1, 3 and 7 days after exiting every day at 07:30 am.
Results: RR, PQ and QT intervals nonsignificantly extended, with maximum during third day after exiting the stay. QTc interval nonsignificantly shortened and heart rate nonsignificantly decreased.
Conclusion: Resting stay in darkness for 72 h is too short period to have effect on electrophysiology of the heart.
Grant support: SGS10/FF/2016-2017 (Experimental study on cognitive and psychophysiological processes under chamber REST conditions.

PO.057

Effects of hyperthermia on the ECG of European sculpin (*Myoxocephalus scorpius*)

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Introduction: Ectothermic animals living at low temperatures are vulnerable to global warming since hyperthermia affects various organs including the heart. Hyperthermia was demonstrated to affect ionic currents and action potentials in fish myocardium. The present study aimed to elucidate effects of hyperthermia on heart function in non-narcotized free-swimming fish.
Materials and method: Two stainless steel electrodes were implanted into pericardial cavity of anesthetized (exposed to tricaine 0.17 g/L in seawater) animals. ECG recording was started after 48 h of recovery in 20 L tank with running seawater. During the experiments the temperature was elevated with 3°C/h rate from 12°C up to 26°C. In the experiments with autonomic blockade, atropine (2 mg/kg) and propranolol (2 mg/kg) were injected into pericardial cavity before ECG recording. All experiments were approved by local bioethical committee.
Results: Hyperthermia caused significant increasing of heart rate (HR) and decreasing of PR-, QRS- and QT-intervals [$P(T) < 0.05$, $n > 6$] till the breakpoint temperature (22°C); further warming caused opposite changes leading to the final cardiac arrest. Autonomic blockade increased maximum HR [$P(T) < 0.05$, $n > 6$) and decreased fish tolerance to high temperatures. At 26°C single P-waves and QRS-complexes were observed on ECG.
Discussion: Hyperthermia has profound effect on processes of impulse generation and conduction in sculpin heart. Presumably, it can cause arrhythmic events leading to cardiac arrest and death. Autonomic system plays important role in the adaptation to hyperthermia.

PO.058

Left atrial abnormalities on electrocardiogram and echocardiogram in obese healthy young adults

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Introduction: Obesity is associated with a wide variety of electrocardiographic (ECG) abnormalities. Some reflect alterations in cardiac morphology and others serve as markers for sudden death.
Methods: The study population consisted of 53 obese, 21 over weight and 27 normal weight subjects. P-wave duration and PR interval duration were calculated on the 12-lead ECG. As echocardiographic variables, left atrial diameter (LAD), left ventricular ejection fraction (LVEF), interventricular septum thickness (IVST), left ventricular posterior wall thickness (LVPWT), and left ventricular mass (LVM) of the obese and the control subjects were measured by means of transthoracic echocardiography.

Results: There were statistically significant differences between obese and non obese as regards to maximum P-wave duration ($P < 0.001$). Correlation analysis showed that P-wave duration in the obese patients was related to the clinical and echocardiographic parameters including BMI, LAD, IVST, LVPWT, and LVM.

Conclusion: Our data suggest that obesity affects P-wave duration and changes in PR interval duration may be closely related to the clinical and the echocardiographic parameters.

PO.059

Experimental cerebral ischemia in rats increases myocardial vulnerability to ischemia-reperfusion injury *ex vivo*

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For years, the relationship between cardiac and neurological ischemic events has been mainly attributed to overlapping pathophysiological mechanisms and common risk factors. However, acute stroke may induce dramatic alterations of cardiovascular function. The aim of this work was to evaluate how prior cerebrovascular lesions affect myocardial function *in vivo* and *ex vivo*, as well as myocardial vulnerability to ischemic injury.

Cerebral embolization was performed in adult Wistar male rats by the injection of microspheres into the left internal carotid artery. Left ventricular function, investigated *in vivo* using echocardiography (1 h, 24 h and 7 days after the embolization), was not significantly impaired; however, the heart rate was significantly increased in the stroke group (+7.2%). Epinephrine (E) and norepinephrine (NE) plasma levels increased in rats from the stroke group (E: 47.3 ± 2.1 versus 24.3 ± 8.7 and NE: 22.7 ± 4.2 versus 10.9 ± 3.7). One hour after stroke or sham embolization, hearts were isolated and perfused *ex vivo* in the Langendorff mode. In hearts from the stroke group, the baseline left ventricular developed pressure was diminished (−11%); moreover, a greater myocardial vulnerability to ischemic injury was observed, with impaired coronary flow recovery after 40 min of total global normothermic ischemia.

Our study provides original exciting data indicating that myocardial vulnerability to ischemia can be worsened by prior ischemic stroke, a situation that does not agree with the concept of remote preconditioning. The underlying molecular mechanisms of the stroke-induced myocardial alterations after cerebral embolization remain to be established, insofar as they may involve the sympathetic nervous system.

PO.060

Effects of artificial skin depigmentation on vascular function in Senegalese women

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Introduction: Artificial skin depigmentation is a widespread practice within sub Saharan Africa. Prolonged application of skin depigmenting substances exposes hypertension and diabetes. The aim of our study was to evaluate the vascular function in depigmented Senegalese women.

Methods and materials: Fifteen depigmented (experimental group) and 15 non depigmented black skinned women (control group), serving as a control group participated in the study. Their ages were 31 ± 6.9 and 29.73 ± 6.3 respectively.

Brachial artery flow-mediated dilation (FMD) was used to compare the vascular function between the two groups.Biochimical profile was also evaluated in our population.

Results: Our results showed that the depigmented subjects had a significantly weaker FMD at 60 s than the non depigmented black skinned subjects (2%; 13%' $P = 0.048$). It is significantly correlated to LDL cholesterol ($P = 0.01$; $r = 0.57$)

A significant elevation ($P = 0.025$) of fasting blood sugar was noted in our depigmented subjects in comparison to our no depigmented ones ($0.94 \pm 0.14 \neq 0.83 \pm 0.11$ g/L). However, an elevation ($P = 0.019$) of total cholesterol was reported in the non depigmented black skinned compared to the depigmented subjects ($2.26 \pm 0.33 \neq 1.99 \pm 0.45$ g/L).

Conclusion: Our results showed that vascular function of depigmented women would be impaired compared to subjects with black skin. These changes are accompanied by metabolic disorders such as hyperglycemia and hypocholesterolemia. Thus we recommend a good awareness of the harmful effects of depigmentation in women.

PO.061

Epicardial progenitors are source of adipocytes in human atria

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Epicardial adipose tissue (EAT) is associated with a high risk of atrial fibrillation, however its orgine is unknown.

Methods: Surgical specimen of human right atrial specimens were used for histological and biochemical studies ($n = 60$), as well as for harvesting epicardial progenitors derived cells (EPDCs; $n = 20$). EPDC were characterized using flow cytometry, proteomic and genic expression assays. Epicardial cell fate was studied using a *Wt-1-CreERT2-Rosa-tdT*$^{+/+}$ lineage tracing mouse model ($n = 7$).

Results: In the sub- and epicardial layer of atrial section, cells were positives for epicardic progenitor marker Wilm's tumor-1 (Wt1) and pre-adipocyte marker pre-adipocyte factor 1 (Pref-1) suggesting that EPDCs could engage in the adipogenic fate. *In vitro*, using human and mouse aEPDCs obtained from atrial samples; atrial epicardial cells underwent an epithelio-to-mesenchymal transition (EMT) and acquired mesenchymal phenotypes (aEPDCs), and could subsequently differentiate into osteocyte or chondrocyte. When cultured using an adipogenic medium, around 40% of aEPDCs cells showed lipid droplets stained with oil red and expressed mature adipogenic markers perilipin, PPAR and C/EBPα. These results were supported by the formation of lipid droplet^{+}-tomato^{+} adipocytes observed in murine aEPDC cultures induced by adipogenic medium. To follow the fate of Wt1^{+} epicardium in vivo, we used a lineage tracing *Wt-1-CreERT2-Rosa-tdT*$^{+/+}$ mouse model. We found that a number of adipocytes that compose the atrial EAT derived from aEPDC through an epicardial EMT process.

Conclusion: Atrial EAT derives, from an EMT process of progenitors present in the epicardium of adult human and mouse atria.

PO.062

Role of the sodium-hydrogen exchanger in the development of hereditary cardiomyopathy

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Cardiomyopathy is defined as a cardiac muscle disease that is characterized by necrosis, followed by hypertrophy associated with a progressive development of heart failure, which eventually leads to premature sudden death. Hereditary cardiomyopathy in the hamster, more specifically the UM-X7.1 colony, provides a unique possibility for studying the pathology and clinical course of primary congestive cardiomyopathies. Using this animal model, it was reported that heart failure is associated with an intracellular calcium overload. However, treatment with calcium blockers did not give the expected beneficial effects. In this study, we showed that an intracellular sodium overload takes place before the appearance of any visible pathological markers of cardiomyopathy. The early development of the sodium overload was associated with an increase in the density of the sodium-hydrogen exchanger. This took place in both the cardiac and vascular systems and led to hypertrophy, heart failure and eventually premature death. However, blockade of the NHE1 prevented sodium overload as well as all signs of heart failure and prevented early death. This work is supported by the Canadian Institutes of Health Research (CIHR).

PO.063

The effect of growth hormone and/or swimming exercise on PI3K, AKT, PTEN and miR21 expressions in rats

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Introduction: Human growth hormone (hGH) is a peptide hormone with anabolic and performance increasing effects. It has been known that recombinant hGH (r-hGH) increases in cardiac hypertropy. The role of r-hGH and exercise on cardiac PI3K/AKT/mTOR signalling pathway and miRNA21 were investigated in this study.

Materials and methods: Adult male Sprague Dawley rats were divided into sedantary control (SC, $n = 9$), swimming exercise (SE, $n = 8$), r-hGH (GH, $n = 10$) and swimming exercise-r-hGH (SE-GH, $n = 9$) groups. r-hGH were administered with 0.3 mg/kg/day during 8-weeks subcutaneously. Exercise groups completed 1-h swimming exercise 5 times a week during 8-weeks. Phosphatase and tensin homolog (PTEN), phosphoinositede-3-kinase catalytic alpha polypeptide (PIK3α), AKT and miR21 gene expressions were performed by Real-Time PCR in left ventricle muscle. Cardiac protein expression of PTEN, PIK3α and AKT determined with immunohistochemistry technique. Immunoreactivities were scored as mild, moderate, strong and very strong and results

were evaluated comparatively using ANOVA statistical test.
Results: PTEN was increased in SE, GH, SE-GH groups when compared with SC group (respectively, 4.3, 4.4 and 6.0; $P < 0.05$). AKT was increased 3.3 fold in SE-GH group when compared with SC group ($P < 0.05$). miR21 was up-regulated 2.10 fold in GH group when compared with SC group ($P < 0.05$).
Discussion: Our data indicated that GH administration is effective on PI3K/AKT/mTOR signalling pathway in rats exposed the swiming exercise. In additon GH administration increases miR21 gene expression in rat heart.

Respiratory Physiology

PO.064

New respiratory inductive plethysmography (RIP) method for evaluating ventilatory adaptation during 6-min walk test

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Introduction: The 6-min walk test (6MWT) is a valuable tool to assess exercise capacity in ecological conditions. However, it does not give any pertinent information about ventilatory limitation responsible for 6MWT distance (6MWD) decrease. In this context, we developed a new monitored 6MWT by using a thoracic respiratory inductance plethysmography (RIP) associated with nasal pressure signal to achieve respiratory pattern and minute ventilation during the test.
Materials and methods: Our method includes an algorithm to minimize gait-induced artifacts, and a calibration procedure. By comparison with standard pneumotachometer (PT), we validated our method with 30 healthy volunteers. In addition, we investigated the influence of body mass index (BMI) known to reduce 6MWD, on ventilatory adaptation with healthy subjects [high BMI (>30 kg/m^2; $n = 15$) and low BMI (<25 kg/m^2; $n = 10$)].
Results: Comparisons between RIP and PT revealed significant ($P < 0.001$) correlations for tidal volume (Vt; $r = 0.81$), inspiratory (Ti; $r = 0.92$) and expiratory (Te; $r = 0.94$) times. By comparison with low BMI subjects, high BMI subjects displayed a higher amount of artifacts eliminated by our algorithm with an increase in Vt ($P < 0.001$) and respiratory rate ($P = 0.003$) in association with a decrease in 6MWD ($P = 0.001$).
Discussion: These results indicate that ventilation of high BMI subjects was much solicited despite reduced locomotor activity by comparison with low BMI subjects. These outcomes obtained in healthy subjects suggest that ventilatory limiting factors could be diagnosed with this method in miscellaneous respiratory diseases.

PO.065

Increased firing rates of dorsal medulla inspiratory neurons during laryngeal expiration reflex in cats

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Introduction: The expiration reflex (ER) is an important defensive reflex of respiratory tract and its major role consists on preventing aspiration of foreign material into the lower airways and removing mucus from subglottal region. ER lacks inspiratory phase and is characterized by a brief expiratory effort and coordinated adduction and abduction of vocal folds. Even though there is no activity of diapraghm or phrenic nerve during ER, there is some information about inspiratory neuron participation during ER. The aim was to study whether inspiratory neurons of dorsal medullary respiratory network participate in the reflex.
Material and methods: Extracellular neuron activity of dorsal respiratory group neurons was recorded with a multi-channel electrode array in anesthetized, spontaneously breathing cats. Expiration reflex was elicited by mechanical stimulation of the medial margins of the vocal cords using thin polyethylene catheter. Electromyograms (EMG) were recorded from laryngeal, chest wall and abdominal muscles and intra-oesophageal pressure was recorded from mid-thoracic oesophagus.
Results: A total of 106 respiratory modulated neurons, including 64 inspiratory phasic (I) neurons, were recorded in five animals during breathing and ER. During ER, 22% of I neurons increased their firing frequency, 14% decreased their firing frequency, 62.5% (64%) were not active, and one I neuron was recruited.
Conclusion: The results confirm that inspiratory neurons participate in ER. The increased firing rate of I neurons during ER seem to be associated with

electrical activity in posterior cricoarytenoid muscle that opens the glottis during expulsive phase of ER.

PO.066

Effect of intravenous administration of glucocorticoid on inflammation in experimental model of acute lung injury

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Introduction: Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a critical illness characterized by diffuse alveolar damage, inflammation due to loss of alveolar-capillary membrane integrity, excessive transepithelial migration of inflammatory cells, and release of pro-inflammatory, cytotoxic mediators.

Treatment of ALI/ARDS is based on ventilatory strategy. The main goal of our study is to evaluate if intravenous administration of anti-inflammatory therapy influences inflammation in lung tissue in experimental animals with ALI/ARDS.

Materials and methods: In rabbits, ALI was induced by repetitive saline lung lavage (30 mL/kg, 9 ± 3 times). Animals were divided into three groups: ALI treated with dexamethasone *i.v.* (0.5 mg/kg, Dexamed; ALI + DEX), ALI without therapy (ALI) and healthy animals (Control). After 5 h of ventilation, total and differential counts of cells in bronchoalveolar lavage fluid (BAL) were measured. Lung edema was expressed as wet/dry weight ratio. Concentrations of interleukins IL-1ß, -8, esRAGE, S1PR3, TNF-α in the lung tissue were analysed. In right lung, apoptotic cells were evaluated by TUNEL assay and caspase-3 immunohistochemically.

Results: Dexamethasone therapy reduced leak of cells into the lung ($P < 0.05$), mainly neutrophils ($P < 0.001$), concentration of pro-inflammatory marker (IL-1β, $P < 0.05$) and marker of lung injury (esRAGE, $P < 0.05$), lung edema formation ($P < 0.05$), and apoptotic index in lung tissue ($P < 0.01$), but increased immunoreactivity of caspase-3 in lung tissue ($P < 0.001$).

Discussion: Intravenous dexamethasone therapy suppreses inflammation, lung injury and development of the lung edema.

PO.067

Is exercise-induced bronchoconstriction associated with respiratory symptoms among 213 Tunisian school children?

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Introduction: Many reviews that studied separately Respiratory Symptoms (RS) and Exercise-Induced Bronchoconstriction (EIB) showed that adolescents have a high risk of developing either symptoms.

Aim: The aim of this study is to evaluate the prevalence of RS and their relationships with EIB among Tunisian school children.

Methods: The study included 213 random school children (15–17 years) in Tunis which responded to the questionnaire about RS and medical history and underwent a resting spirometry testing that was repeated 5, 10 and 15 min after exercise.

Results: Statistical analyses were performed by 'khi-two' test. RS are present in 40 pupils (19%). EIB objectified by spirometry is present in 109 pupils (51.2%) and among those 37% presented RS. The relationships between EIB and RS were linear. Increasing RS is associated with a high risk of EIB ($P = 0.032$).

Discussion: We found high prevalence of association RS and EIB in school children. These data are concordant with many studies that have shown that any physical exercise can lead to symptoms in susceptible subjects. It has also been demonstrated that some exercises have a higher propensity to trigger bronchospasm.

Other studies highlighted the relationship between the perception of symptoms and the eosinophilic inflammation that causes EIB.

Conclusion: High RS is evidently associated with a high risk of EIB in school children. New policies and strategies for school medicine will be necessary for early diagnose and management.

PO.068

Paradoxical bronchoconstriction with salbutamol and terbutalin administered by metered-dose inhaler and nebulizer solution

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Introduction: Based on the effectiveness and relative safety of inhaled β_2 adrenergic agonists in the treatment of both acute and chronic asthma, their use is increasing.

Observation: We report a case of 50-year-old non-smoking male, followed since childhood for allergic asthma presented repetitive acute exacerbations episodes with limited responses to inhaled bronchodilators. He described severe respiratory attacks occurring after terbutalin and salbutamol inhalation and even leading to respiratory failure. In April 2015 he was admitted in the intensive care unit after terbutalin inhalation. In February 2016, basic spirometry performed revealed only distal airway obstruction. After Inhalation of 400 μg of salbutamol, the patient developed an explosive coughing, dyspnea, and a fall of FEV1 and FEV1/FVC respectively at amount of 25 and 21%. Administration of atrovent restores basic spirometry values. A week later, another spirometry was performed after an interrupted nebulisation of a standard dose of terbutalin, confirmed previous observations.

Discussion: Contrasting with extensive use of inhaled β_2 agonists, few clinical reports highlight the severity of respiratory symptoms induced by these drugs. In this case, we excluded bronchospasm induced by expiratory maneuvers because all parameters measured on basic spirometry were reproducible. It is not clear whether bronchospasm induced by this inhaled drugs is related to allergic reaction or rather nonspecific mechanismsin witch paradoxical bronchospasm may occur. Than, despite the fact that paradoxical bronchospasm resolve spontaneously we doesn't lose sight of the potential lethal reaction.

PO.069

Experimental and simulated functional connectivities of the motor cortex driving the respiratory muscles in chronic obstructive pulmonary disease (COPD) (I)

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Introduction: Patients with COPD have a decreased functional connectivity of the motor cortex with their contralateral counterparts and no connectivity with the brainstem, compared with controls. Transcranial magnetic stimulation (TMS) indicated that the motor cortex of the patients driving the respiratory muscles exhibits a low motor threshold and high excitability.

Methods: We sought support of these results (cerebral fMRI connectivity, TMS) by developing a mathematical model of neural network. We used a discrete version of the FitzHugh-Nagumo model. We studied mathematically: (i) the network connectivity having different motor threshold (parameter a) and excitability (parameter J), (ii) whether the synaptic parameters (strength K, transmission delay Δ) could influence the connectivity. We convolved the resulting spiking neurons with a hemodynamic response function using SPM-Matlab to replicate fMRI signal, and computed the Pearson's correlation coefficient of the two time series (neural clusters).

Results: We simulated the network in controls, using specific values for J and a. In patients, we used a higher value for J (excitability) and lower value for a (motor threshold) and found a resulting weaker connectivity. Reducing K and increasing Δ also diminished connectivity.

Conclusions: The mathematical model helps to understand which mechanisms, at the neuronal scale, induce the connectivity changes during COPD. These findings, evidenced at rest and inspiratory loading may explain why some patients are prone to acute respiratory failure.

PO.070

The retrotrapezoid nucleus is necessary to sustain respiration under anesthesia in mice

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Introduction: Congenital Central Hypoventilation Syndrome (CCHS) is a life-threatening disorder caused by mutations of the paired-like homeobox 2B gene (*PHOX2B*), generally alanine expansions. CCHS is characterized by hypoventilation during sleep. The anesthetic management of patients with CCHS is particularly challenging. Knock-in mice carrying the most frequent mutation found in patients ($Phox2b^{20/27Ala}$ mice) present main symptoms of CCHS and die soon after birth. Conditional knock-in mice that carry a $Phox2b^{27Ala}$ mutation targeted to the RTN survive without chemosensitivity. In both strains, the retrotrapezoid nucleus (RTN), a group of brainstem neurons pivotal to chemoreception did not form. Here, we tested whether the lack of RTN may cause

vulnerability to anesthesia by administering various anesthetics to conditional *Phox2b*$^{20/27Ala}$ mice.
Material and methods: We administered 150 mg/kg ketamine, 150 mg/kg propofol, or 1 mg/kg fentanyl intraperitoneally in isolation on 8-day and 14- to 15-day old *Phox2b*$^{27Alacki/+}$ pups and their wildtype littermates. We measured breathing pattern using whole-body flow plethysmography and assessed mortality.
Results: Mortality reached 78–100% in mutant pups, versus 0% in wildtype littermates whatever the anesthetics used. Blockade of opioid receptor by coadministration of naloxone did not prevent ketamine nor propofol anesthesia but abolished mortality, suggesting that the lethal effects of ketamine and propofol were at least partly mediated by their agonist effects on opioid receptors.
Discussion: Our data support that RTN is required to sustain breathing during anesthesia. The impairment of RTN development in CCHS patients may account for their vulnerability to anesthetic agents, especially opioids.

PO.071

Influence of neonatal hypoxia ischemia on ventilation in the newborn rat: implication of KCC2

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Introduction: Perinatal brain injuries result from several conditions, including neonatal encephalopathy (NE), encephalopathy of prematurity (EP) or perinatal systemic infections. The lack of oxygen due to hypoxia-ischemia (HI) around birth is a major cause of neurological disabilities in childhood. NE and EP are associated with a high morbidity and mortality and leads to motor dysfunction (breathing and locomotor). Motor dysfunction may results from excitation/inhibition imbalance in the respiratory network. A down-regulation of KCC2 expression increases intracellular chloride concentration and leads to a reduction of inhibition, which changes cell excitability and respiratory network processing.
Methods: EP is produced by stenosis using coils wrapped around intrauterine arteries in both uterine horns of pregnant rats at E17. We discriminate ischemic rats at birth on the basis of intrauterine growth restriction. NE is induced by thrombin injection in the left carotid artery followed hypoxia (8%) the day of birth.

Ventilation is evaluated by plethysmography. Western Blot and immunohistochemistry are used to evaluate KCC2 expression in the respiratory network.
Results: Ventilatory parameters are modified in pups exposed to neonatal to NE or EP. The occurrence of apnea is increased. The tidal volume, respiratory frequency and minute ventilation are decreased in NE or EP pups. KCC2 expression is decrease in both groups.
Conclusions: HI leads to ventilatory dysfunctions. Change in the expression of KCC2 in neurons of the respiratory network may be involved in the ventilatory dysfunction. Therefore, KCC2 becomes a key target to alleviate breathing alteration after neonatal HI.

PO.072

Phospholipids in synthetic surfactants are important for tidal volumes and alveolar stability in newborn rabbits with respiratory distress syndrome

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Introduction: In order to develop active synthetic surfactants, different phospholipid mixtures with analogues of either surfactant protein B or C have been tested. However, an optimal composition of phospholipids in synthetic surfactant is not known. The aim of the study was to investigate the effect of different phospholipid compositions in combination with SP-B and SP-C analogues on lung functions in an animal model of respiratory distress syndrome without using positive end-expiratory pressure.
Material and methods: Phospholipids were alone or together with SP-B and/or SP-C analogues instilled in ventilated premature newborn rabbits. Lung functions were evaluated.
Results: Treatment with egg yolk-phosphatidylcholine (PC) mixed with dipalmitoylphosphatidylcholine (DPPC) and palmitoyloleoylphosphatidylglycerol (POPG) gave small tidal volumes but after addition of SP-B and SP-C analogues VT was only somewhat lower and lung gas volumes (LGV) similar to that obtained with Curosurf®. Substitution of egg yolk-PC with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine, and combining them with DPPC, POPG and 2% each of the SP-B and SP-C analogue gave a completely synthetic surfactant with similar effects on VT and LGV as Curosurf®.
Discussion: The phospholipid composition is important for VT while the two analogues increase alveolar stability at end-expiration. Totally synthetic surfactant consisting of unsaturated and saturated phosphatidylcholines, POPG and the analogues of SP-B and SP-C gave a preparation with similar activity as Curosurf®

regarding VT and LGV in an animal model using preterm newborn rabbits ventilated without positive end-expiratory pressure.

PO.073

The aerosurf project: micron particles settlement in the lung during breath holding. What should be the expiratory concentration pattern?

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Introduction: The deposition of aerosol in the lung during a single breath manoeuvre could be used as a tool to know some characteristics of lung geometry and function such as the alveolar surface and the thickness of gas covering the walls of alveoli. A first step was to study with a model the decrease in particle concentration during the full expiration following a single breath manoeuvre and to compare the theory to experimental data.

Methods: Model. One alveolus was considered as a sphere with a given radius. Particles of 1 μm in the inspired air were supposed to settle by gravity on alveolar walls at a velocity of 33 μm/s.

Experiments. Expired flow was measured using a flow meter, after integration the volume signal was obtained. Particle concentration was measured with a Pegasor electrostatic device. A nebulizer (Atomisor© DTF, F) with a cyclone produced a one tenth million particles per mL at a 5 L/min rate.1 μm particles were made of an isotonic NaCl solution of water. Ten liters of aerosol were stored in a 15 L plastic balloon.

Results: According to the model the decrease in particle concentration should not be linear during the full expiration however its first part could be taken as linear, it is not exponential.

Experiments in four subjects confirmed the theory, the time courses of the expiratory concentrations were not exponential, some were nearly linear.

Conclusion: The present simple model will allow to calculate in a next step the effective surface of the lung.

PO.074

Osteopontin, a key mediator expressed by senescent pulmonary vascular cells and involved in pulmonary hypertension

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Background: Senescent pulmonary artery smooth muscle cells (PASMC) may contribute to the pathogenesis of pulmonary hypertension (PH) through the synthesis of secreted factors.

Objective: To explore the role of extracellular matrix proteins released by senescent PASMC on experimental and human PH.

Methods and results: Microarray analysis of human PASMC undergoing replicative senescence revealed upregulation of osteopontin (OPN), which mediated the stimulated PASMC growth and migration induced by senescent PASMC media and matrix. One year old mice compared to younger counterparts displayed elevated lung OPN levels, right ventricular systolic pressure, pulmonary vessel muscularization, and p16 and p21 stained PASMC number also stained for OPN. No such changes with age were observed in $OPN^{-/-}$ mice which developed attenuated PH during hypoxia. Compared to cultured young mice PASMC, PASMC from 1 year old WT mice grew faster, and similarly as PASMC from young mice stimulated by media or matrix from old PASMC WT mice, both effects suppressed by OPN antibodies. $OPN^{-/-}$ PASMC grew slower than WT PASMC, but were stimulated by WT PASMC media and matrix, and more from old vs young mice. In patients with COPD, lung OPN was elevated with age and pulmonary vascular remodeling, predominating in PASMC at sites of vascular hypertrophy. Cultured PASMC from COPD patients displaying early replicative senescence overexpressed OPN. Lung OPN levels were markedly elevated in idiopathic PH patients in proportion to age.

Conclusion: These results underscore the importance of OPN as key mediator of the interplay between senescent and non senescent PASMC during PH progression.

PO.075

GATA3-regulated polarization of t cells towards Th2 phenotype

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Introduction: The Th2 polarization pathway starts with the predominant Th2 citokine, IL-4, binding on its receptor followed by STAT6 phosphorylation and GATA3 activation. We aim to better define the role of GATA3 in polarization towards Th2 allergic response.
Material and methods: A group of 20 adult allergic patients to dust-mites (prick test positive to Der pter extract and specific IgE Der p1 > 0.70 UI/mL), without associated respiratory pathology, was selected.

$CD4^+$ cells were separated in Ficoll gradient from PBMC, by negative magnetic separation (Miltenyi Biotec). GATA3 expression was analysed by cDNA semiquantitative assay with RT of mRNA, with specific forward/reverse primers for GATA3 sequence (ACC-GGC-TTC-GGA-TGC-AA/TGC-TCT-CCT-GGC-TGC-AGA-C). Serum IL4, IL13, IFNγ were determined by ELISA.
Results: GATA3 expression was highly variable, from overexpression to lack of expression. Serum IL4 (0.2 ± 0.17 pg/mL) highly correlates with GATA3 ($r = 0.66$, $P = 0.002$), the maximum IL-4 titer (0.66 pg/mL) being associated with highest GATA3 expression. There was no correlation neither between GATA3 and IL13 (0.52 ± 0.72 pg/mL) or IFN-γ (12.6 ± 2 pg/mL), nor between the cytokines' titers.
Discussion: Although IL4 and IL13 are important cytokines defining Th2 phenotype and GATA3 is a transcription factor involved in Th2 differentiation, we demonstrated GATA3 significance only for IL4 production. Existing studies demonstrate the suppressor role of GATA-3 on IFN-γ expression, but the serum titer of IFN-γ was much higher than IL4 an IL13 titers, without significant association between the cytokines and transcription factor expression.

PO.076

Determinants of respiratory function in adult patients with primary ciliary dyskinesia

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Introduction: Primary ciliary dyskinesia (PCD) is a genetic disease characterized by abnormal ciliary function, responsible for chronic sinopulmonary disease, with or without *situs inversus* (SI). Adult respiratory function is poorly described. We aimed to study the determinants of FEV_1 in adults with PCD.
Methods: Retrospective study in two tertiary hospitals, focusing on adults with an asserted diagnosis of PCD. Clinical, functional, radiological, microbiological and ciliary features were recorded. Annual FEV_1 decline after 20 years of age was calculated for each patient using a linear equation. Study was approved by the IRB of the Société de Pneumologie de Langue Française.
Results: A total of 78 patients were included (median follow up 8.1 years). Respiratory function at end of study was significantly lower in women (median last available $FEV_1 = 60\%$ pred, IQR = 26 versus 77.5%, IQR = 33, $P < 0.05$) and in patients with chronic airway *P. aeruginosa* (PA, $n = 21$) infection (median $FEV_1 = 60.5\%$ versus 75.5%, $P < 0.05$). In univariate analysis, FEV_1 correlated independently with sex ($r = 0.34$, $P = 0.009$), chest CT score ($r = -0.40$, $P < 0.001$) but not with age at diagnosis, SI or BMI. In multivariate analysis (stepwise multiple regression), only sex ($r = 0.34$, $P = 0.009$) and chest CT score ($r = -0.40$, $P < 0.001$) correlated with FEV_1. FEV_1 decline was -11 mL/year (IQR = 62.3) and was greater in women (-28.3 mL/year, IQR = 43.4, versus -3.8 mL/year in men, IQR = 85.7, $P = 0.01$).
Conclusion: Alteration of respiratory function in PCD adults appears more severe in women and in patients with chronic PA infection. Although usually mild, FEV_1 decline is greater in women than in men.

PO.077

Influence of ovalbumine sensitisation on the down-regulation of cough during exercise in rabbits

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Introduction: The 'cough centre' exhibits plasticity at the sensor and the integration levels leading to modulation of the reflex, but little is known about interactions between cough and exercise. On a clinical point of view, cough is a major symptom of asthma frequently experienced during exercise. Our study was designed to determine whether exercise is able to modulate the cough reflex (CR) of ovalbumine (OVA) sensitized rabbits.

Materials and methods: Ten OVA rabbits and eight controls were studied. Exercise was mimicked by electrically induced muscular contractions. Tracheal stimulation (performed at rest and during exercise) was mechanically delivered via a catheter. Broncho-alveolar lavage (BAL) and OVA intradermal challenge were performed to evaluate inflammation.
Results: An overall of 494 tracheal stimulations were analysed (271 at rest; 223 at exercise). OVA rabbits showed higher total cell count ($P = 0.04$) and percentage of eosinophil count ($P = 0.008$) in BAL fluid compared to controls. Minute ventilation between rest and exercise increased from 36 and 35% in OVA and control rabbits respectively (P = NS). Control rabbits showed a decreased sensitivity of the CR during exercise compared to baseline ($P = 0.0313$) whereas the sensitivity of the CR was unchanged (P = NS) between both conditions (i.e. rest and exercise) in OVA rabbits.
Discussion: Our results support a down-regulation of cough during exercise in control rabbits that appeared to be abolished (i.e. absence of down-regulation) in OVA rabbits. The inflammation present in OVA rabbits may be involved (at a central and/or sensor levels) in this different (i.e. compared to controls) cough modulation during exercise.

PO.078

Respiratory and neurobehavioural characteristics of a murine model of congenital myotonic dystrophy type 1

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Introduction: Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease caused by CTG repeats in the myotonin-protein kinase. Transcribed mRNA accumulation leads to protein synthesis perturbation and multisystem symptoms. Phenotype severity is correlated to expansion size and reaches its peak in congenital form with hypotonia, respiratory failure, mental retardation and high mortality.

We aimed to characterize the respiratory and neurobehavioural phenotypes of a mouse model of DM1 with large CTG expansion (>1500 repeats, DMSXL).
Material and methods: Weight and temperature were recorded daily in wild-type, heterozygous and homozygous DMSXL pups from birth to postnatal day 30 (P30). Breathing variables and apnoea duration were measured using whole-body flow barometric plethysmography in air and in response to hypercapnia (8% CO_2) and hypoxia (10% O_2) on P1 and P8. Motor development was analysed using righting, cliff avoidance and negative geotaxis tests from P3 to P15 and gait quality assessment from P2 to P30. Behavioural development was assessed with object recognition tests on P40.
Results: Weight growth was significantly delayed in DMSXL mice, compared to WT mice. Morphological abnormalities were occasionally present. DMSXL showed significantly longer apnoea duration at P1 and P8. Otherwise breathing variables and response to hypercapnia and hypoxia were normal. Righting and cliff avoidance reflexes' acquisition were delayed but eventually achieved in DMSXL pups; gait quality was significantly lower compared to controls.
Discussion: MSXL mice display key features of DM1 and may serve as a model to analyse pathophysiological processes and test treatments.

PO.079

Adult scoliosis: spirometric data and comparative study of three methods for height correction

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Introduction: Interpretation of spirometry results of patients with scoliosis requires height correction. Objective: to analyze spirometric data of scoliotic adults in relation to anthropometric parameters and to compare three methods of height correction.
Methods: We made a retrospective study within 10 years. Spirometric measurements were forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), FEV1/FVC and peak expiratory flow. The height (H) was corrected using a relation to arm span (AS). Method 1 did not take account of sex (H = AS/1.6) contrary to the method 2 (H = AS/1.01 for women; H = AS/1.03 for men). The third method was already applied to a same aged Afro-Caribbean population: H = 66.9 + 0.57 AS and H = 54.9 + 0.66 AS for men and women respectively. We compared height corrected values and also adjusted body mass index (BMI) and assessed relationship between spirometric and anthropometric data.
Results: The height by method 2 was significantly higher. More half of subjects presented a ponderal

deficit. Lung restriction was found in 67% of patients while bronchial obstruction in 6%. The adjusted BMI by method 2 but especially by method 3 was significantly correlated with the spirometric parameters.
Conclusion: The integration of gender or better, the use of the regression equations would give more precision to the formulas of height adjustment in scoliotic patients.

PO.080

Dose–response to bronchodilator and dose-finding in asthmatic young children

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Introduction: Inhaled short-acting beta2 agonists such as salbutamol are used for clinical purposes or to evaluate Lung Function (LF) reversibility in asthmatic subjects. In opposite to asthmatic adults, preschool wheezers (2 years 6 months to 6 years 11 months) is a heterogeneous population characterised by an inconsistent effect of asthma medication. We looked for a dose-effect of salbutamol in preschool wheezers tested for routine LF and determined the relevant dose of salbutamol to administer with respect to effectiveness and side-effects.
Materiel and methods: Prospective multicentre study (NCT01470755) conducted in 4 hospitals using the interrupter technique to measure the resistance (Rint) of the respiratory system in preschool wheezers. Each child received two successive doses of salbutamol in four designs allowing Rint measurement after 100, 200, 400, 600 and 800 μg. Using MONOLIX 4.3.3 to model the dose-effect, 90 children were required to estimate pharmacokinetic constants Imax (mean maximal effect), D50 (dose achieving 50% of Imax) with an accuracy of 8.9 and 25.7%, respectively.
Results: Results from 102 children showed an adequate adjustment with the prediction of Imax sigmoid model. Imax was 0.31 (SRE 5%), D50 84 μg (SRE 24%). The dose achieving 90% of Imax (D90) was 258 μg which corresponded to a 27.9% decrease in Rint.

Simulation in 5000 subjects showed that the proportion of children with Rint reversibility (-35% decrease) increased rapidly up to 400 μg (18.2%) then levelled off (21.8% for 800 μg).

Conclusion: Wheezy preschoolers exhibit a dose-effect of salbutamol, the optimal dose for testing LF reversibility is 400 μg.

PO.081

Intrapulmonary lymphoid neogenesis induced by prolonged bacterial airway infection in mice

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Introduction: Lymphoid follicles (LF) are absent in normal lungs, but are described in lungs of subjects with cystic fibrosis (CF) or non-CF bronchiectasis, suggesting a role for bacterial infection in lymphoid neogenesis. We aimed to study the dynamic of pulmonary lymphoid neogenesis (LN) during bacterial infection.
Methods: C57BL/6 mice were instilled intratracheally with *P. aeruginosa* (PA)O1- or *S. aureus*-coated (1.106 CFU/mouse) agarose beads (which produced prolonged airway infection) and compared to controls (sterile beads or no instillation) (Martin et al., ERJ 2011). Mice were sacrificed on day (d)1, d4, d7, and d14 after instillation for immunohistochemistry (histomorphometry), qPCR, ELISA.
Results: Chronic pulmonary infection with PAO1 or *S. aureus* induced organised LF in 14 days after a single challenge with PAO1- or *S. aureus*-coated beads. Bacteria-induced LF were exclusively localized in the subepithelium of infected airways. CXCL13 mRNA and protein were augmented in the lungs of infected mice from day 1. Staining for phospho-EGFR, CXCL12 and CXCL13 was weak in airway epithelium of controls, but was positive in airway epithelium (phospho-EGFR, CXCL13) from day 1, and in LF (both) of infected mice at 14 days. Treatment with a phospho-EGFR tyrosine kinase inhibitor (gefitinib), anti CXCL12 or anti CXCL13 Antibody did not reduce LN induced by PAO1 infection.
Conclusion: Chronic bacterial infection and respiratory epithelium could contribute to LN in chronic airway diseases. Our unique model allows studying mechanisms for the formation and maintenance of lung LF.

PO.082

Identification of breathing-related cortical activity in hyperventilation syndrome: preliminary data on healthy subjects

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Ventilation is the only vital function that is equally dependent on both voluntary and involuntary control, and can be colored by emotions. Hyperventilation syndrome (HVS) is a debilitating and poorly understood disease that can affect this function. Among the proposed pathophysiological mechanisms underlying HVS there is an insufficient neural sensory gating of afferent respiratory signals.

To further explore this hypothesis, this study was designed to assess the respiratory electroencephalographic (EEG) activity in healthy subjects undergoing voluntary hyperventilation (HV).

We hereby report the validation of a novel specific experimental set-up designed for this study, in which, to avoid the potential influence of the presence of a mouth piece on afferent EEG signals, ventilatory data was recorded using a nasal cannula.

EEG pre-inspiratory potential (PIP) and nasal end-tidal carbon dioxide (PetCO2) level were obtained on ten healthy subjects during tidal breathing, hyperventilation and sniff maneuvers.

Validation of the setup was confirmed by the absence of PIP during tidal breathing (negative control) and its presence during sniff (positive control). Six subjects showed PIP during the recovery period. Three subjects showed a particular pattern of breathing with apneas during recovery from hyperventilation.

The presence of PIP during recovery from HV in some subjects is a novel and intriguing finding that suggests a role of the cortical control of breathing even in hypocapnia. Further studies are needed to confirm this preliminary finding and to explore its mechanisms. Moreover, the application of this experimental set-up to patients with HVS could provide additional insight on its pathophysiology.

PO.083

Prediction of maximal oxygen uptake at high altitude

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The maximal oxygen uptake (VO_2max) at high altitude is a key quantity to understand in alpine medicine but has not been yet explained in terms of the physiological parameters – ventilation, cardiac frequency, PvO_2 and inhaled gas O_2 pressure – that govern respiration.

A novel theoretical approach (Kang, M.-Y. et al. *Respir. Physiol. Neurobiol.* 2015; 205: 109–119) that has allowed the prediction of VO_{2rest} and VO_{2max} at sea level, is used to predict the altitude dependence of VO_{2max} from the above parameters values. By solving the equations for O_2 convection-diffusion dynamics in airways and O_2 saturation dynamics in the capillaries, the method yields the corresponding value for VO_{2max}.

The ventilation and cardiac output parameters are obtained by a quadratic fit of ventilation and perfusion data from the literature. PvO_2 is assumed to be 20 mmHg. Using these values, VO_2max at different altitudes is computed for different altitude yielding a theoretical prediction. In the figure, it is shown as a percentage of sea level value. The predicted VO_2max display the usual decrease with increasing altitude, but exhibit a very good agreement with experimental data. A 80% decrease in VO_2max at Mt. Everest altitude relative to sea level is predicted in agreement with experiment.

Further investigation shows that the influence of ventilation on VO_2 becomes more significant at high altitude. This explains why hyperventilation is the most important feature of acclimatization to altitude.

PO.084

Prediction of post-pneumonectomy respiration

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Introduction: Recent results on oxygen capture during the respiratory cycle have shown that this capture exhibit a complex dependence on ventilation VE and cardiac output Q (Kang, M.-Y. et al. *Respir. Physiol. Neurobiol.*2015; 205: 109–119). In particular, it was found that *the ratio* VE/Q *does not govern* oxygen capture. This result is obtained by solving, for the first time interactively, the equations for O_2 convection-diffusion dynamics in airways and O_2 saturation dynamics in the capillaries. This yields an ensemble of abacus of given VO_2 values as a function of VE and Q (not shown here).

Materials and methods: The method is based on the idea that the resection of a fraction of the lungs volume does not modify strongly the cardiac output so that the blood velocity in the remaining volume is increased accordingly.

Results: Consider, for example, the case where half the lungs volume has been resected. Consequently, the

local blood velocity in the remaining part is doubled but the local ventilation keeps approximately the same if the diaphragm motion is not modified. So, from the abacus, one predicts the VO_2 of the remaining part after surgery and what adaptation is needed. In this case it is shown, without any new calculation, that normal ventilation is insufficient to get normal VO_2 from the non-resected part only.

Conclusion: At constant cardiac motion, ventilation increase or artificial support is needed to recover normal VO_2. The method can be extended to any resection fraction and to COPD patients that exhibit impaired ventilation before pneumonectomy.

PO.085

Respiratory responses to voluntary hyperventilation test (VHT) for diagnosing hyperventilation syndrome (HVS)

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There is a need to clarify the use of the VHT in the evaluation of HVS. Sixty-seven patients (91% female, 42.4 years, BMI 23.6) were evaluated for symptoms suggestive of HVS. They were all referred by a pulmonologist after a standard evaluation including a normal pulmonary function test, EKG and heart exam. They were compared to 46 normal voluntary subjects from whom reference values of the VHT were obtained. They all fulfilled the Nijmegen questionnaire and the VHT in 1 min. All the procedure was monitored with a breath by breath analyzer. A positive result for any of the parameters was defined using the *Z*-score (*Z*).

The Nijmegen score was 29.7 ± 10, it was >22/64 in 79, and 91% using $Z > 2$. The recurrence of the main complaint by the 1′HV was present in 75%. The $PetCO_2$ at rest was <30 mmHg in 28%, and did not return to baseline 5′ after the 1′HV in 96%. Using the $Z < 2$, only 16% of the patients were positive for the PetCO2 at rest. This was even worse for the $PetCO_2$ at 5′ after HV (9%) and for the $PetCO_2$ at the end of the test it was positive in 43%.

Reference values of the VHT are provided to help the comparison with patients. The use of $PetCO_2$ does not seem to add any relevant information to the Nijmegen questionnaire. There is still a need to confirm which functional parameter from the test could be additive in the diagnostic approach of HVS.

PO.086

Inefficiency of influenza vaccination in copd patients is associated with defect of B cell differentiation and decrease of IFNγ $CD4^+$ T cells production

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Introduction: Infection with influenza virus is an important comorbidity in patients with chronic obstructive pulmonary disease (COPD). Unfortunately, influenza vaccination effectiveness is impaired in these patients and the mechanisms remain unknown.

Materials and methods: We analyzed specific humoral and T cell responses to influenza vaccination and performed characterization of B and T cells subsets in COPD patients.

Results: Increase of antibody titers 30 days post-vaccination was lower in COPD patients compared to control for two of the three influenza vaccine strains. T cell responses to Flu vaccine were also disturbed: IFNγ-but not IL-2 or TNF- production by $CD4^+$ T cells were reduced in COPD patients compared to controls. Beside specific responses to Flu, we found a more general perturbation of B cell homeostasis with an increase of the naïve/memory ratio that was correlated with DL_{CO} and emphysema score. Switched B cells ($CD27^+IgD^-$) were lower in COPD patients and correlated with DL_{CO} and emphysema score. Concerning T cells, no difference was observed between the two groups regarding naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), memory effector (CD45RA-$CCR7^-$) $CD4^+$ and $CD8^+$ T cells.

Discussion: In COPD patients, a defect of B cell differentiation and an inability of $CD4^+$ T cells to produce IFNγ after influenza stimulation may explain the inefficiency of vaccine response in these patients.

Exercise Physiology

PO.087

Performance & metabolism in mice

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Introduction: The notion of maximum oxygen consumption (VO_{2max}), as the real absolute maximum capacity of cardiovascular system to carry oxygen,

was applied to man in the nineteen-twenties by the Nobel Prize Archibald Hill. These measurements are intrinsically interesting in order to initiate comparative physiologic's analyses and they are frequently of critical importance for investigations of evolution of physiological traits. The *Performance & Metabolism in Mice (PMM)* facility provides comprehensive, standardized or advanced, customized characterization of performance and metabolism in mouse models.

Materials and methods: Ten Swiss and 15C57 female mice (3–4 months old) were tested for oxygen consumption (VO_2) on a treadmill. Our equipments allow simultaneous analysis of VO_2 and carbon dioxide production (VCO_2) (measured every 1 s). VO_{2peak} was determined as the highest value of VO_2 achieved over 15 s and expressed relatively to body and lean mass (measured by RMN) in mL/kg/h. All mice performed an incremental test (0.01 m/s increment each 15 s).

Results: Swiss VO_{2peak} relatively to lean mass (11.38 ± 0.61 mL/kg/h) were significantly different from C57 (9.57 ± 0.52 mL/kg/h; $P < 0.01$).

Discussion: The metabolic exploration facility of *PMM* performs specific, on-demand experiments as well as comprehensive metabolic analyses to evaluate *in vivo* the energy balance in mouse. This exploration is performed under either basal condition or energy challenges using standardized techniques for the detection of phenotype in energy metabolism. It provides technical and scientific support to design experimental protocols as well as to develop new techniques to investigate metabolism.

PO.088

Time dependent hemorheological and oxidative stress responses to repeated sprint test and wingate anaerobic power and capacity test

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Introduction: Numerous field tests have been developed to assess the physical capacities of athletes. Although time course of hemorheological alterations of different recovery tests to assess the anaerobic capabilities of athletes have been well-defined, no information is available about the effects of wingate anaerobic power and capacity test (WAnT) and repeated sprint test (RST) which are currently used to assess anaerobic performance in football players. Current study aim was to determine alterations in hemorheological and oxidative stress parameters in response to WAnT and RST in football players.

Materials and methods: Eleven football players (mean age 21.72 ± 3.60 years; mean body weight 72.09 ± 5.54 kg) participated to the study. Blood samples were collected before the tests and immediately after, 2 and 24 h after the tests. WAnT and RST were applied with 1 week break. Erythrocyte deformability and aggregation were measured by an ektacytometer. Total oxidant/antioxidant status were measured using a commercial kit and oxidative stress index was calculated.

Results: There was no statistically significant difference between the tests in terms of erythrocyte deformability and oxidative stress. On the other hand, erythrocyte aggregation index was significantly increased immediately after WAnT and decreased again at 2 h.

Discussion: Our results demonstrate that WAnT and RST may be used interchangeably in terms of hemorheological parameters and oxidative stress. On the other hand, according to blood lactate and performance responces of the tests they may need different performance abilities.

PO.089

Metabolic and inflamatuar effects of voluntary physical activity in male rats fed with fructose rich diet

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Introduction: The aim of this study was to investigate the effect of voluntary physical activity on metabolic and inflammatory alterations result from the fructose-rich diet.

Materials and methods: Adult male Wistar rats, were separated as control group ($n = 7$), fructose group which was fed 10% fructose to drinking water ($n = 7$) and fructose-active group ($n = 7$) which was fed 10% fructose in drinking water and housed with a running wheel during 10 weeks. Serum glucose, triglycerides, total cholesterol, HDL, LDL were assessed using enzymatic method. Insulin, TNFa and IL6 levels were determined by ELISA method after feeding period. Daily fluid intake and body weight of rats were measured weekly. Kruskal Wallis and Mann-Whitney U test were used for statistical comparisons.

Results: Serum glucose, insulin, triglycerides, total cholesterol, HDL, LDL, TNFa and IL6 levels were not differ between groups after 10 weeks of feding period. Weight gain between the 1 and the 10 weeks were

significantly lower for fructose active group (95.1 ± 14.3 g), in comparison to fructose group (109.0 ± 6.6 g) and control group (113.4 ± 10.9 g; $P = 0.04$ and $P = 0.03$). Total weight of the heart, lung, right ventricle and left ventricle, and heart weight/tibia length ratio were not found significantly different between groups.

Discussion: Our study indicated that this experimental model that was added 10% fructose to drinking water during 10 weeks in rats may not result in significant metabolic and inflammatory change in rats. However, the findings of this study shows that voluntary physical activity effective for decreasing weight gain in rats.

PO.090

Effects of a single bout of strenuous exercise on platelet activation in ApoE/LDLR$^{-/-}$ mice

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Introduction: Strenuous physical exercise leads to platelet activation that is normally counterbalanced by the production of endothelium-derived anti-platelet mediators including prostacyclin (PGI_2) and nitric oxide (NO).

Material and methods: In the present work, we evaluated platelet activation at rest and following a single bout of strenuous treadmill exercise in young (3-month-old) and old (7-month-old) ApoE/LDLR$^{-/-}$ mice with atherosclerosis compared to age-matched WT mice. In sedentary and post-exercise groups of animals, we analyzed TXB_2 generation and expression of platelet activation markers in whole blood *ex vivo* assay. We also measured 6-keto-$PGF_{1\alpha}$ and nitrite/nitrate plasma concentration in plasma.

Results: Sedentary 3- and 7-month-old ApoE/LDLR$^{-/-}$ mice displayed significantly higher activation of platelets compared to age-matched wild-type (WT) mice. Interestingly, in ApoE/LDLR$^{-/-}$ mice but not WT strenuous exercise partially inhibited TXB_2 production, expression of activated GPIIb/IIIa receptors and fibrinogen binding, with no effect on P-selectin expression and vWf binding. In ApoE/LDLR$^{-/-}$ mice but not in WT, an age-dependent increase in the basal plasma concentration of 6-keto-$PGF_{1\alpha}$ was observed. At the same time, an age-dependent decrease in the basal plasma concentration of nitrite (NO_2^-) was found in both ApoE/LDLR$^{-/-}$ and WT mice, whereas the basal plasma concentration of nitrate (NO_3^-) was lower in ApoE/LDLR$^{-/-}$ compared to age-matched WT mice.

Discussion: The overactivation of platelets in ApoE/LDLR$^{-/-}$ as compared to WT mice is not further increased by exercise but seems to be attenuated in ApoE/LDLR$^{-/-}$ but not WT mice. This phenomenon could be linked to compensatory up-regulation of PGI_2-dependent anti-platelet mechanisms in ApoE/LDLR$^{-/-}$ mice.

PO.091

Effect of high versus moderate intensity of intermittent work exercise program (IWEP) on endurance parameters and blood pressure values among seniors

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Introduction: Physical activity offers primary and secondary prevention of several chronic diseases among seniors. Few studies have compared high- versus moderate-intensity exercise programs for seniors. The aim of this study is to compare the effect of high- versus moderate-intensity of IWEP on endurance parameters and blood pressure among seniors.

Materials and methods: Sixty patients over 60 years were evaluated before and after 9-week of cycling program for 30 min, twice a week. Participants were divided into high-intensity training group (HITG: $n = 30$) and moderate-intensity training group (MITG: $n = 30$). The HITG performed six 5-min stages consisting of 4-min at first ventilatory threshold (VT1) and 1-min at 90% of maximal tolerated power (MTP) and the MITG performed six 5-min stages consisting of 4-min at VT1 and 1-min at 50% of VT1. Outcome measurements included VT1, heart rate (HR) at pre-training VT1, total workload, systolic (SBP) and diastolic blood pressure (DBP).

Results: Both training programs have decreased the SBP (HITG: −3.3% and MITG: −2.9%, all $P < 0.05$) without significant change in DBP. HITG was more effective than MITG only on total workload (HITG: 28.4% versus MITG: 23.6%, $P < 0.05$), whereas no significant change was observed between groups on VT1 (HITG: 25.7% and MITG: 23.3%, all $P < 0.05$), and in HR at pre-training VT1 (HITG: −5.5% and MITG: −4.9%, all $P < 0.05$).

Discussion: Moderate- and high-intensity cycling program, may lead to similar improvements in endurance parameters and SBP among seniors. The underlying

mechanisms of these results highlighted that seniors can benefit from a moderate-intensity of IWEP.

PO.092

Effects of eccentric exercise on different slopes

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Introduction: Eccentric muscle contraction occurs when the muscle lengthens under the tension. Muscle damage is seen after eccentric exercise experienced by the sedentary individuals. The purpose of this study is to investigate the muscle damage responses to different slopes for soleus muscle that predominantly works as eccentrically while running downhill.
Materials and methods: Thirty-two male Wistar rats were divided into four groups according to slope; control, −16°, −8° eccentric exercise (downhill running), 0° (horizontal running) groups. For practising; exercise groups trained for 10 days, 15 min with the motorized treadmill, following 3 days rest, rats exercised 90 min with the speed of 20–25 m/s for 5 days. After 48 h the last exercise, rats were sacrified and plasma creatine kinase (CK), heat shock protein 70 (HSP70) levels were examined. Plasma and soleus muscle total oxidant- antioxidant status (TOS-TAS) and histological changes of soleus muscle were assesed.
Results: CK increased for downhill running group −16° and TOS increased for −16° and −8°. There was a correlation with slope and muscle damage. Mononuclear cell infiltration and capillaries increased in soleus after eccentric exercise and there was a correlation with slope. Muscle splits were seen for −16° group. There was no significant change for plasma TAS/TOS, HSP70 and soleus TAS responses to eccentric exercise.
Discussion: Muscle response to eccentric exercise for different slopes provides information for daily life and training regimen for athletes. For HSP70 as a muscle response, further investigations will be needed.

PO.093

Spirometric profile of young adults: comparison between sedentary and practitioners of a regular physical activity

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Works on improving cardiopulmonary function in athletes are legion. Conversely, they are rarely studies on the respiratory function of African athletes who do not participate in sports competitions but whose practice is regular. This prospective and cross-sectional study was designed to compare in a homogeneous African population, the spirometric profile of sedentary subjects and the ones of those practicing a regular physical activity (RPA).

A total of 273 young adults, aged from 18 to 28 years, all belonging to the same class of medical students participated to this study. They filled out a standardised questionnaire, administered by trained interviewers, on their usual physical activity and their personal and family history respiratory symptoms during the preceding 12 months. After this, Each was subject to: -a pulmonary function testing (spirometry), -a bronchodilator test with salbutamol, -an exercise challenge testing. The atmospheric parameters were measured during the tests. Unlike the two Bronchial hyperresponsiveness tests which showed no statistical significant difference between sedentary subjects and those practicing a RPA, the spirometry revealed for the latter a significant increase, in the forced expiratory volume in 1 s, in the peak expiratory flow rate and in the maximal voluntary ventilation. The improvement of these lung flows in subjects practicing a RPA was an important argument to encourage campaigns against the increase of physical inactivity observed in our cities, becoming a major public health problem.

PO.094

Effects of exercise training on anxiety in diabetic rats

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Introduction: Diabetes mellitus coincides with anxiety disorders. Exercise has many beneficial effects such as maintenance of health, and management of disease and disability. Our goal was to investigate healing effects of exercise on anxiety in diabetic animals.

Materials and methods: Thirty-two female Wistar Albino rats were divided into four groups including control, diabetes, exercise, diabetes + exercise. Diabetic rats were applied streptozotocin (50 mg/kg) intraperitoneally. Exercise protocol was maintained on treadmill for 6 weeks, and subsequently open field test (OFT) and elevated plus maze (EPM) were conducted. Behavioural tests were recorded as video. Two different researchers analyzed duration in central/periferal zone, total traveled distance and rearing numbers in OFT, time spent in open/closed arms and total arm entries in EPM.
Results: Duration in central zone and rearing numbers increased in exercise group vs all groups ($P < 0.01$, $P < 0.05$). Total traveled distance increased in exercise group compared to diabetes and diabetes+exercise groups ($P < 0.01$). Duration in periferal zone reduced in exercise group vs all groups ($P < 0.01$). As for EPM; time spent in open arms increased and time spent in closed arms decreased in exercise and diabetes + exercise group vs control and diabetes groups ($P < 0.01$). Total arm entries increased in exercise group versus diabetes + exercise group ($P < 0.05$).
Discussion: Results suggest that exercise has anxiolytic effect both in exercise and diabetes+exercise groups. Exercise improved the locomotor activity in exercise group but not in exercise+diabetes group. In order to reveal the exercise effects on anxiety, different exercise protocols and sex genders should be included to the experimental design.

PO.095

BDNF signaling and endothelial no production in rats subjected to physical training

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Introduction: Brain-derived neurotrophic factor (BDNF) signaling is largely involved in neuroplasticity and cognition. Surprisingly, while physical training improves both endothelial function and cognition, the control of BDNF signaling by cerebral endothelium-derived nitric oxide (NO) has been poorly investigated.
Materials and methods: BDNF and TrkB receptors (unphosphorylated from and phosphorylated form at tyrosine 816) as well as eNOS and eNOS phosphorylated at serine 1177 (as markers of endothelial NO production) were measured in the cortex and hippocampus in three groups of rats ($n = 6$ each): sedentary rats, rats subjected to physically training, rats subjected to physical training after previous definitive occlusion of common carotid arteries. Physical training consisted in a daily (30 min) walking (18 m/min) activity on a horizontal treadmill for 7 consecutive days. BDNF levels were also measured in brain slices exposed for 24 h to the NO donor glycerol trinitrate (GTN).
Results: When induced in rats with patent carotid circulation, physical training increased NO production, levels of BDNF and of both form of TrkB receptors irrespective of the regions considered. These effects were completely abolished when training was induced in rats with carotid arteries occlusion. Moreover, GTN (5 and 10 μM) significantly increased BDNF production by brain slices.
Conclusion: Activation of brain BDNF signaling elicited by physical training is dependent on cerebrovascular NO production. This data provide new insight for the comprehension of the mechanisms involved in the procognitive effect of physical training.

PO.096

High intensity interval training improves glucose homeostasis in diabetic db/db mice

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The aim of this work was to compare the effects of High Intensity Interval Training (HIIT) with a traditional Moderate Intensity Continuous Training (MICT) on glucose metabolism and mitochondrial function in diabetic mice.

30 db/db mice aged 6 weeks were subdivided into MICT group, HIIT group, or control group (CON). Animals in the training groups ran on a treadmill 5 days/week during 10 weeks. *In vivo* starch and insulin tolerance tests were performed at the end of the protocol. *In vitro* measurements included high resolution respirometry, assessment of mitochondrial density markers and insulin signaling by enzymatic assays and western blot in muscle and liver.

HIIT lowered fasting glycemia (−40%) and % HbA1c (−20%), and improved response to starch and insulin tolerance tests compared to CON group. No changes were noted in MICT group regarding the glucose homeostasis. Assessment of muscle and liver mitochondrial density markers and *ex vivo* gastrocnemius mitochondrial respiration showed no differences between the three groups. Western blot analysis revealed a significant twofold increase of muscle Glut4 content only in HIIT group. Similarly, only HIIT increased liver Foxo1 phosphorylation ratio and decreased JNK phosphorylation ratio.

This study showed that HIIT may improve glucose metabolism more efficiently than traditional MICT in diabetic mice by *mechanisms independent of* mitochondrial adaptations. This effect could be partly explained by an increased muscle GLUT4 content, and a decreased activity of JNK/FoxO1 pathway in the liver, both involved in the pathophysiology of insulin resistance.

PO.097

A non-steroidal antagonist of the mineralocorticoid receptor protects from coronary reserve loss and diastolic dysfunction in mice with cardiomyocyte-specific overexpression of the mineralocorticoid receptor

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Mice with cardiomyocyte-specific mineralocorticoid receptor overexpression (MR-Cardio) were previously characterized by arrhythmias (ventricular extrasystoles and tachycardia) and impaired NO-mediated relaxation of coronary arteries. We tested whether impaired heart functional parameters aggravated by treadmill exercise in MR-Cardio mice are improved by 4 weeks oral treatment with the non-steroidal MR antagonist BR-4628 (1 mg/kg/day).

We performed (i) ECG telemetry at rest and during treadmill running, (ii) MRI measurements of left ventricular (LV) coronary reserve, and (iii) invasive LV hemodynamic.

In MR-Cardio mice compared to controls (Ctrl), ECG telemetry at baseline indicated increased ST-elevation, increased number of sinus arrests and ventricular extrasystoles, and increased prevalence of ventricular tachycardia after epinephrine injection (2 mg/kg). While running and during the subsequent resting phase, ECG telemetry showed increased QTc duration (from speed 21 cm/s) and exacerbation of ST-elevation.

Coronary reserve was decreased in MR-Cardio mice, but improved by treatment with BR-4628 (Ctrl 5.2 ± 0.7; MR-Cardio 2.4 ± 0.7, $P < 0.05$ versus *Ctrl*; MR-Cardio + BR 3.9 ± 0.8 mL/min/g, *NS* versus *Ctrl*). Moreover, hemodynamic studies showed impairments of LV-End-Diastolic-Pressure (LVEDP, i.e. filling) and LVEDP-Volume-Relation (LVEDPVR, i.e. compliance) in MR-Cardio mice, which were improved by BR-4628 [(LVEDP: Ctrl 5.10 ± 0.57; MR-Cardio 8.94 ± 0.88, $P < 0.01$ versus *Ctrl*; MR-Cardio + BR 6.06 ± 0.56; *NS* versus *Ctrl*) and (LVEDPVR: Ctrl 2.33 ± 0.33; MR-Cardio 4.94 ± 0.51, $P < 0.001$ versus *Ctrl*; MR-Cardio + BR 2.74 ± 0.52, *NS* versus *Ctrl*)].

In mice, increased cardiac MR expression (reported in several human pathologies) causes coronary reserve impairment, diastolic dysfunction and arrhythmias, resulting in inability to exercise. Treatment of MR-Cardio mice with the MR antagonist BR-4628 improves coronary reserve and diastolic function.

PO.098

Effect of exercise intensity on microarn expression in rodent

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Introduction: Exercise is known to induce several physiological changes in skeletal muscle that are responsible for muscle plasticity. However very little is known about the involvement of microRNA in this process. Our hypothesis is that microARNs are involved in the early stage of muscle gene expression reprogramming inducing muscle plasticity in response to exercise, this involvement being specific depending on exercise intensity.

***Experimental approach*:** In this study, we analyzed miRNA profiles of gastrocnemius muscle after an exhaustive exercise bout. We investigated three different intensity of exercise: maximal speed, critical speed and 150% of critical speed, and two different time point: just after the exercise or 2 h after.

Results and discussion: We found that a large number of microRNA were up or down regulated at the two time point post exercise, compared with control, in a specific exercise intensity manner. These microRNA profiles are supposed to be regulated by the upstream metabolic pathways involving hormones response to exercise (insulin, GH), and might regulate the downstream PI3K/AKT and AMPK signaling pathways. Most interestingly, this analysis allows us to define a signature for each exercises intensity, which can be very useful to better understand the specificity of microRNA in the tight regulation of muscle physiology.

Conclusion: The muscle microRNA expression after an acute bout of exercise is intensity-dependent. This

study suggest that microRNA play an important role in muscle physiology, allowing post-transcriptional regulation quickly after changes in muscle environment and so participate to a preadaptation before muscle remodeling by exercise training.

PO.099

Erythrocytes and plasma lactate change during exercise in humans

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Lactic acid is a nutrient involved mainly in muscular and hepatic metabolism. Our aim is to investigate the participation of erythrocytes to blood transport of lactate during physical effort.

Lactic acid erythrocyte transport is studied *in vivo* in nine subjects during 5 min of physical exercise on a ergocyclometer at 120–130 W, 40–60% VO_2 followed by a 130 min recovery. Lactic acid is assessed by amperometric method, it is first oxidized by LDH in the presence of hexacyanoferrate III and cytochrome b_2. Erythrocyte lactic acid (LG) is determined from plasma (LP) and whole blood lactic acid.

The overall lactate evolution during recovery could be accurately described by a sum of two exponential terms. At the end of exercise, LP (5.19 ± 2.42 mmol/L) is two times higher than LG (2.64 ± 0.94 mmol/L). LP and LG reach a peak after the end of exercise at 3 and 7 mn, respectively. Recovery with return of LP and LG to initial values lasted 105 mn. LG dropped between 0–2 mn of exercise in all subjects. Erythrocyte transport represents 45% of that of plasma ($r = 0.90$). Plasma lactic acid determine a LP-LG gradient to favor an erythrocyte lactic acid afflux [LG = 0.84*(LP–LG); $r = 0.90$].

Erythrocyte transport of lactic acid may be considered as a regulatory factor in acido-basic balance during exercise and acts as a well of lactate which increase muscular efflux of lactic acid.

PO.100

The effect of fructose rich diet and voluntary physical activity on cardiac hemodynamic responses during ischemia and reperfusion of isolated rat heart

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Introduction: Cardiac hemodynamic responses were determined following cardiac ischemia and reperfusion in rat hearts that were subjected to fructose rich diet and voluntary physical activity.
Materials and methods: Male Wistar rats were divided into control (C; $n = 7$), fructose (F; $n = 7$) and fructose-active (FA; $n = 7$) groups. Fructose groups were fed 10% fructose in drinking water for 10 weeks. FA group housed with access to running wheels. Hemodynamic measurements were made after hearts were perfused with Krebbs Henseleit solution for 15 min. Left ventricular developed pressure, maximum and minimum rate of change and heart rate were recorded after global ischemia (35 min) and 40-min-reperfusion. Left and right ventricle thicknesses were measured by macroscopic and microscopic assessment. Microscopic features of cardiac hypertrophy were evaluated with hematoxylin eosin stained slides.
Results: The maximum rate of change of baseline measurement was higher in FA group (2351.6 ± 442.2) than F group (1320 ± 542.2) and C group (1756 ± 468.7; respectively, $P = 0.01$ and $P = 0.05$). However, no difference was observed for left ventricular developed pressure, maximum and minimum rate of change and heart rate values among the groups before ischemia and 1, 10, 20, 30 and 40 min of reperfusion. Wall thicknesses were similar in all groups and hypertrophic changes were not detected in any animals.
Discussion: Fructose rich diet and voluntary physical activity did not alter cardiac hemodynamic responses after ischemia and reperfusion. But, voluntary physical activity may be an effective factor for increasing cardiac contractility in rats that were feeding with fructose rich diet.

PO.101

Extracellular beta endorphin concentration in the anterior cingulate cortex during rest, and following acute exercise in sedentary and exercise-trained rats

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Introduction: Exercise is known to stimulate beta endorphin (BE) release in central, or peripheral tissues. The purpose of this study is to investigate the alterations of extracellular beta endorphin (BE) concentration in the anterior cingulate cortex (ACC) following acute exercise in sedentary, and exercise-trained rats.
Methods: Male Wistar rats were assigned to sedentary control (C), or exercise-trained (T) groups. T group was subjected to 8 weeks of treadmill exercise, while C group received familiarization exercise. All animals were run on a motor-driven treadmill with a gradually increasing protocol until exhaustion. Rats were implemented in a stereotaxic frame with a microdialysis probe positioned in the ACC. Artificial cerebrospinal fluid was pumped at a rate of 2.0 mL/min in awake, freely moving animals and the dialysate was collected at 30-min intervals for 2 h. Extracellular BE concentration in the ACC of the animals were measured in microdialysate samples at rest, and following acute exercise.
Results: There were no statistically differences in BE levels between C and T groups in resting, or post-exercise state. Although BE levels in T group were found to be increased following exhaustive exercise, it did not reach statistically significance. However, a significant increase in BE levels in C group were observed in samples at 60, and 90 min following exercise compared with the resting levels.
Discussion: In conclusion, our results suggest that acute exercise-induced BE release in the ACC is more prominent in sedentary rats than exercise-trained animals.

PO.102

Exercise training potentiates the beta endorphin relase in the anterior cingulate cortex of rats under functional pinealectomy

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Introduction: In this study we explore the alterations of beta endorphin (BE) concentration in plasma, cerebrospinal fluid (CSF) and extracellular fluid (ECF) from the anterior cingulate cortex (ACC) in exercise trained and functional pinealectomized rats.
Methods: Male Wistar rats were assigned to one of four groups: sedentary control (C), sedentary pinealectomized (Px), exercise-trained (T), and exercise-trained under pinealectomy (T-Px) groups. Exercise trained groups were subjected to 8 weeks of motor-driven treadmill exercise. C and T groups exposed to normal light/dark cycle (12:12-h light/dark cycle) and rats in Px and T-Px groups exposed to continuous light for 8 weeks. BE concentration in plasma, CSF and ECF from the ACC of all rats were measured. Artificial cerebrospinal fluid was pumped at a rate of 2.0 mL/min in awake, freely moving animals and the dialysate was collected at 30-min intervals for 2 h.
Results: Although there were no differences among groups of BE concentration in the CSF, Px group showed an increase in plasma BE concentration compared with the other groups. BE levels in the ACC of Px and TPx groups were found to be increased in all samples of microdialysates compared with the C and T groups. Furthermore, BE levels in the ACC were found to be elevated in TPx group compared with the Px group.
Discussion: In conclusion, our results showed that functional pinealectomy induces beta endorphin release in the ACC, and exercise training potentiates this elevation.

PO.103

Hypoxic incremental cardiopulmonary exercise testing (hiCPET) and the risk of developing acute mountain sickness (AMS): results from the IRMS des pays de Loire

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A total of 74 consecutive subjects (M/F: 41/33, 56.5 years, BMI: 23.9) fulfilled an incremental CPET in normoxia, followed by a hiCPET (FiO_2: 0.12) 1 h after in the IRMS before a trip at high altitude. Hackett's score was obtained after returning at sea level. Peak VO^2 was 31.7 ± 9.7 (120 ± 23.5%). Compared to the constant level hCPET from Richalet, the Heart response was abnormal in 51% and the Ventilatory response in 41% of the subjects due to a more pronounced desaturation with the hiCPET. Peak VE/VO^2 from CPET in normoxia provides the best correlation with the Hackett's score ($r^2 = 0.092$, $P = 0.01$). The model that best predicts Hackett's score included the weight, VE/VCO^2 from CPET, VE and HF at rest between normoxia and hypoxia ($r^2 = 0.245$, $P = 0.001$).

Physiological parameters at rest and CPET in normoxia are valuable in the evaluation of subjects at risk of experiencing AMS.

PO.104

The evaluation of the exercise physiological effects on man

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Vibration exercise is a new neuro-muscular training method that is applied in athletes as well as in prevention and therapy of osteoporosis. The present study prospected the physiological mechanisms of fatigue by vibration exercise in 37 young healthy subjects. Exercise and cardiovascular data were compared to progressive bicycle ergometry until exhaustion. Vibration exercise was performed in two sessions, with a 26 Hz vibration on a ground plate, in combination with squatting plus additional load (40% of body weight). After vibration exercise, subjectively perceived exertion on Borg's scale was 18, and thus as high as after bicycle ergometry. Heart rate after vibration exercise increased to 128/min, blood pressure to 132/52 mmHg and lactate to 3.5 mM. Oxygen uptake in VE was 48.8% of VO_{2max} in bicycle ergometry. After vibration exercise, voluntary force in knee extension was reduced by 9.2%, jump height by 9.1%, and the decrease of EMG median frequency during maximal voluntary contraction was attenuated. The reproducibility in the two Vibration exercise sessions was quite good: for heart rate, oxygen uptake and reduction in jump height, correlation coefficients of values from session 1 and from session 2 were between 0.67 and 0.7. Thus, VE can be well controlled in terms of these parameters. Surprisingly, an itching erythema was found in about half of the individuals, and an increase in cutaneous blood flow. It follows that exhaustive whole-body vibration exercise elicits a mild cardiovascular exertion, and that neural as well as muscular mechanisms of fatigue may play a role.

PO.105

The effects of aerobic exercise in some physiological parameters in young and middle aged women

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The purpose of this study was to determine the effects of 12 week aerobic (jog-walk) exercise program on some physical and physiological parameters in young and middle aged women.

The groups were composed of the women who had not done exercise regularly, who had not followed a special diet and who did not have any health problems preventing them doing any kind of exercise. During 12 weeks, both groups participated in a jog-walk exercise program with the duration of 30 min. and 3 times per week. The intensity of the exercise was %70 that was determined by Karvonen method. Before and after the training measurements of body weight, body mass index, rest pulse, blood pressure, vertical jump, flexibility, aerobic power and anaerobic power were done. Mean age of groups were determined as 45 years for middle aged group ($n = 6$) and 22 years for young women ($n = 6$).

The results indicated that there was a significant decrease in body weight, body mass index and rest pulse values of both groups ($P < 0.05$), and there was a significant increase in vertical jump, anaerobic power, aerobic power and flexibility values.

Consequently, it is understood that a 12-week aerobic exercise develops aerobic and anaerobic powers and decreases rest pulse in young and middle aged women; however, it does not cause any significant change in their blood pressures.

PO.106

Effects of parental moderate treadmill running on spatial learning and synaptic plasticity in the hippocampus of offspring

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The aim of this study was to analyze the effects of parental exercise during pre-gestational (father) and gestational (mother) periods in the neurodevelopment, spatial learning and memory and synaptic plasticity in the DG hippocampus of Wistar rat offspring. Male wistar rats were submitted to walking on a treadmill at 14.43 m/min for 20 min/day (60% VO_{2max}), 5 consecutive days/week in a total period of 22 days prior to conception. Pregnant rats were submitted to walking on a treadmill at 22.2 m/min for 20 min/day (60%VO2max), 5 consecutive days/week during the whole pregnancy time. The pups were assessed for neonatal developmental milestones from postnatal day 1 to 21 and weighted at day of their birth (P0), and at P7, P14 e P53. The expression of synaptophysin in the hilus of dentate gyrus of the hippocampus was analyzed in the software Image Pro Plus 6.0 by from immunohistochemistry. According to the statistical analysis, there was no significant difference between

groups regarding absolute or relative weight of adrenal glands from the mothers, body weight of the offspring and day of presentation of each developmental milestone, neither for the performance in the water maze and synaptophysin content in the hilus of dentate gyrus of hippocampus of the offspring. These results suggest that moderate parental exercise protocol used in this study does not harm the development of the offspring and hippocampal synaptic plasticity of puppies, evaluated by synaptophysin immunohistochemistry. The spatial learning data are yet preliminary due to the need to increase the sample.

PO.107

The relationship between physical activity level and psychological distress in university students

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We aimed at assessing the association between physical activity level and psychological distress in Moroccan university students.

A cross-sectional study with 150 Moroccan healthy students was conducted. The mean age of the sample was 21.3 ± 1.8. Percentage of women was 61%.

We used The International Physical Activity Questionnaire to assess physical activity (IPAQ); and The Kessler Psychological Distress Scale (K10) (varying between 10 and 50) to assess a psychological distress. The study was performed far from examination periods to avoid any unusual stress.

The IPAQ levels were low (<600 MET-min/Week), moderate (a minimum of at least 600 MET-min/week) and high (a minimum of at least 3000 MET-min/week) in respectively 15.9; 76.8; 7.3% of students.

The mean K10 scale was 19.8 ± 7.3. The students who likely to be well (score under 20) represents 56.2% of the sample; And who likely to have a severe mental disorder (score 30 and over) represents 13.1%.

Low physical activity was significantly associated with a risk of having a severe mental disorder ($P = 0.001$).

This study suggests that a low physical activity level in university students seems to be associated with a higher risk of depression and anxiety. Improving physical activity should be encouraged to avoid eventual psychological disease among the Moroccan student population.

PO.108

Physical performance assessment by an accelerometer Myotest®

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Objective: To assess physical performance among university students using an accelerometer type Myotest® and to analyze factors linked to a high physical performance in this population.

Materials and methods: A total of 147 students following enrolled in their first year of pharmacy school (mean age 21.4 ± 1.2 years, female 61.9%) participated in the study.The skinfold thickness was measured using the clamp Harpenden. Physical fitness was assessed by the Ruffier–Dickson test and physical activity was assessed by the validated arabic version of the short form of self-questionnaire of IPAQ (International Physical Activity Questionnaire). Myotest examination included

Results: The mean of BMI was 21.7 kg/m^2 ± 3.2 (16.5–36.3). The median thickness of fat assessed by skinfold method was 22.8 (15.3–27.1). A bad capacity to adapt to a physical effort was found in 38.8% of students. The median total score of the IPAQ was 1417.5 (693–2653.1) METs-min/week.

The summary of the results of Myotest®exam: Jump height (cm) 23.7 ± 7.8 (11–46.4); Power (W) 43.1 ± 14.4 (24.8–97.6); Speed (cm/s) 210.8 ± 47.2, (145–407); Force (Nw) 28.3 ± 3.8 (18.2–41.7); Reactivity (kN/m) 2.1 (1.8; 2.6); Stiffness 20.6 (14.2; 29.5). Higher physical performance assessed by Myotest was related to male sex, regular practice and duration of physical activity. Positive correlation was found between physical performance parameters, physical fitness and IPAQ scores.

Myotest seems to be a simple tool to calculate in a quick manner different parameters of physical performance. Factors related to a higjher physical performance are especially a high physical activity and male sex.

PO.109

Longterm cycling training preserves VO_{2max} over 50 years old by increasing both central and peripheral factors: a non-invasive study

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Aging is characterized by a progressive decline in maximal oxygen uptake (O_{2max}). Improvements of O_{2max} have been well documented following endurance training in young and old subjects but the adaptation of O_{2max} on long-termcycling training and the respective contribution of central and peripheral components to this adaptation has been poorly investigated, especially in subjects over 50 years old. The purpose of this retrospective study in sedentary subjects and road cycling subjects from 18 to 80 year old (yo) was to examine the respective contribution of maximal cardiac output (Qc_{max}) and arterial-venous oxygen difference [(a-v)DO_2] to changes in DO_{2max}. Healthy volunteers ($n = 114$) were divided in sedentary or trained 18–30, 31–50 or 51–80 year old groups in function of their aerobic exercise frequency and duration. O_{2max}, peak power output (PPO), Qc_{max} and expiratory flow (E_{max}) were measured during incremental exercise test until exhaustion on a cycle ergometer, using fully noninvasive measurements with portative materials and (a-v)DO_2 was calculated. Training improved PPO but also O_{2max} (+56.6% in trained *vs* sedentary 51–80 year-old subjects, $P < 0.01$), which was higher than in 18–30 sedentary subjects (+19.7%; $P < 0.05$), by increasing both Q_{Cmax} and (a-v)DO_2. The increase of Qc_{max} can be attributed mainly to an increase in maximal stoke volume. E_{max}, maximal breath frequency and expiratory volume were also improved by training. In conclusion, cycling training improves aerobic capacities and force development in subjects over 50 years by increasing central and peripheral components of O_{2max} and consequently can contribute to improve lifestyle.

Endocrinology

PO.110

Maternal viral mimetic administration during fetal hypothalamic development does not affect leptin and corticosterone levels in female rat offspring

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Introduction: Viral infections are widespread and it is known that viral mimetics harm the brain of the offspring when given maternally. Therefore, we hypothesized that viral mimetics might perturb hypothalamic development and influence stress and adiposity markers of the offspring.

Materials and methods: To test this hypothesis, a viral mimetic polyinosinic: polycytidilic acid (poly i:c) was injected (10 mg/kg) to the pregnant rats during the beginning (days 12–13 of pregnancy, B) or at the end of this time window (days 14–15 of pregnancy, E) and female pups born to these mothers were taken into the experiment ($n = 10$ for each group). Simultaneously, for control groups, other pregnant rats were injected with sterile saline solutions on days 12–13 or 14–15 and female pups born to these mothers were taken into experiment ($n = 10$ and $n = 9$ respectively for each group). Following weaning, the pups were followed until when they showed two sequential estrous cycles. Plasma samples collected at the luteal phase were used for leptin and corticosterone analyses.

Results: Maternal poly i:c injection on day 12–13 and 14–15 of pregnancy rat pups did not significantly affect leptin and corticosterone levels ($P = 0.05$).

Discussion: Data suggest that maternal viral infections during the beginning or at the end of fetal hypothalamic do not affect stress and adiposity axis of the female rat offspring.

PO.111

Development and validation of a corticosterone enzyme immunoassay for rat plasma

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Introduction: Corticosterone is the main hormone of the hypothalamo-pituitary-adrenal axis of the rodents. Its secretion is associated with stress and, thus, with almost all physiological variables. The aim of the current study was to establish an enzyme immunoassay that was suitable for corticosterone measurement in rat plasma.

Materials and methods: Corticosterone: BSA conjugate produced in our laboratory was used to produce antibodies in New Zealand strain rabbits ($n = 4$). The conjugate was used as solid phase and the steroid in the liquid phase competed for the antibodies. Secondary antibodies conjugated to biotin were used as detection of the primary antibodies. Streptavidin peroxidase and substrate was used for color formation and the reaction was stopped by sulfuric acid and read spectrophotometrically. Standard curve, sensitivity, repeatability, parallelism, accuracy and precision were studied.
Results: With the series of incubation times and reagents, a successful test was established. The test lasted 3 h and sensitivity, sensitivity, repeatability, parallelism, accuracy and precision values were all in the acceptable range. Standard curve's dynamic range was between 10 and 2000 ng/mL. Sensitivity of the test was 10 ng/mL. Intra- and inter-assay coefficients of variations were <15%. High samples were diluted with zero standard and was shown to be parallel to the standard curve.
Discussion: A corticosterone enzyme immunoassay was successfully produced and it can be used for research purposes.

PO.112

Loss of Slc26a9 anion transporter results in reduced pancreatic fluid secretion in young female mice

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Slc26a9 is a member of the Slc26 multifunctional anion transporter family. Expression studies suggest that it may function as a chloride conductance interacting with CFTR. Polymorphisms in Slc26a9 are associated with an increased incidence of diabetes in cystic fibrosis patients.

We investigated the expression of Slc26a9 in the pancreas and liver and elucidated its role in pancreatic and biliary ductal electrolyte and fluid secretion and in pancreatic endocrine function.

The mRNA expression of Slc26a9 was low in pancreatic parenchyma but 20 fold higher in microdissected pancreatic ducts. No Slc26a9 mRNA expression was detected in the liver, while bile ducts displayed low Slc26a9 expression. Pancreatic and biliary fluid and bicarbonate secretion were assessed in anesthetized Slc29 knockout mice and age- and sex matched wild type (WT) littermates. Blood glucose levels were measured after an i.v. glucose bolus over a period of 2 h. Significantly reduced basal as well as secretin-stimulated pancreatic fluid secretory rates were observed in young adult (6–8 weeks) female Slc26a9 KO mice. In young male mice, as well as male and female old mice (>1 year), no significant difference in pancreatic ion secretion was observed. Blood glucosa levels declined slightly slower after 2 g/kg i.v. glucose bolus in Slc29a9 female mice. Biliary fluid and bicarbonate secretion were not affected by loss of Slc26a9 expression.

The results demonstrate that deletion of Slc26a9 is associated with a reduction in pancreatic fluid secretion in young female mice. They underline the importance of Slc26a9 in these epithelia particularly at young age.

PO.113

Stimulation of insulin secretion by a novel peptide that is active only at high glucose concentrations

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Introduction: The aim of this study was to examine the effect of a newly developed, chemically synthesized peptide (S1D2P) designed to block ATP-sensitive potassium channels preferentially in more depolarized beta cells. This avoids insulin release of beta cells at low glucose values with the final intention to prevent hypoglycemic shocks in patients that may occur in treatments with sulfonylurea drugs.
Material and methods: Whole cell path clamp experiments, intracellular calcium measurements, insulin release experiments as well as apoptosis assays were done with rat insulinoma cells (RINm5f).
Results: The peptide depolarized the membrane potential and decreased K_{ATP}-channel current. There was a higher pharmacological potency of the peptide, when the cells were initially more depolarized. Both intracellular calcium concentration and insulin release increased under S1D2P influence, however, in contrast to the sulfonylurea glibenclamide, only in presence of elevated potassium or glucose concentrations. Apoptosis assays showed less apoptotic cells than after treatment with glibenclamide.
Discussion: The peptide exhibits an antidiabetic (insulin secretion increasing) effect on insulinoma cells with the desirable property that is inactive at low glucose concentrations.

PO.114

A fast and reliable protocol for isolating functional rodent islets

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Introduction: Pancreatic islets have been a hallmark focus for unraveling new pathophysiological pathways and potential treatment targets in diabetes research. Therefore, developing practicable islet isolation protocols is of great interest for conducting reliable investigations. In this study, we describe, in details, an expeditious protocol for obtaining functional rodent islets.
Methods: Pancreases from male adult mice were cut and digested using a low specific activity collagenase then purified on a percoll solution. Islets were maintained into culture for 5 days, then viability and function assessed by fluo-4 calcium imaging, insulin secretion assays and glucose uptake measurements using 2-NBDG, a fluorescent glucose analog.
Results: This protocol was reproducible when many rodent species were used, and neither sex nor age affected the cell yield; 990 ± 112 islets were obtained from C57BL/6 male mice, 930 ± 96 from BALB/C male mice and 2610 ± 192 from Wistar male rats. Glucose-stimulated calcium elicited a biphasic cytosolic response with a rapid spike followed by slower oscillations; besides, insulin secretion displayed a similar response with two phases. Throughout the duration of culture, islets retained a healthy status as demonstrated by the stable percentage of living cells and the expression of pro- and anti-apoptotic as well as islet-specific genes. 2-NBDG uptake was inhibited by d-glucose indicating that it enters cells via glucose transporters and that the latter remained intact after pancreas digestion and islet culture.
Discussion: In conclusion, we propose an easy method for isolating functional rodent islets that could be useful for diabetes research purposes.

PO.115

The uterine and vascular actions of estetrol, a fetal estrogen, delineate a distinctive profile of estrogen receptor a modulation, uncoupling nuclear andmembrane activation

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Estetrol (E4) is a natural estrogen with a long half-life produced only by the human fetal liver during pregnancy, and has less action of the liver than the other classic estrogens when administrated by oral route. The crystal structures of the estrogen receptor a (ERa) ligand-binding domain bound to 17b-estradiol (E2) and E4 are very similar, as well as their capacity to activate the two activation functions AF-1 and AF-2 and to recruit the coactivator SRC3.

In vivo administration of high doses of E4 stimulated uterine gene expression, epithelial proliferation, and prevented atheroma, three recognized nuclear ERa actions. However, E4 do not promote endothelial NO synthase activation and acceleration of endothelial healing, two processes clearly dependent on membrane-initiated steroid signaling (MISS). Interestingly, E4 antagonized E2 MISS-dependent effects in endothelium but also in MCF-7 breast cancer cell line.

This profile of ERa activation by E4, uncoupling nuclear and membrane activation, characterizes E4 as a selective ER modulator. E4 thus represents a new pharmacological tool and As E4 could have a potential new medication in oral contraception and in hormonal treatment of menopause.

PO.116

Melatonin and L-carnitine ameliorates endothelial dysfunction in type 2 diabetic rats

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Diabetes mellitus (DM) is associated with significant morbidity due to diabetes related microvascular and macrovascular complications such as ischemic heart disease, stroke and peripheral vascular diseases. Endothelial dysfunction is thought to play a major role in the development of diabetic cardiovascular complications. Nitric oxide (NO) is an important mediator for regulation of vascular tone. Melatonin improved the NO pathway in diabetic animal models. L-carnitine has been shown to induce endothelial dependent relaxation by increasing the production of NO.

Diabetes induced with high fat diet (HFD, for 8 weeks) and multiple low doses intraperitoneal injection of STZ (twice, 30 mg/kg/d i.p). Thirty-five male Wistar rats were divided into five groups: Control group (C), STZ-induced diabetic group (HFD + STZ), HFD + STZ diabetic group received melatonin (HFD + STZ + MLT; 10 mg/kg/d i.p), HFD + STZ diabetic group received L-carnitine (HFD + STZ + LC) (0.6 g/kg/d i.p), and HFD + STZ diabetic group received glibenclamide (HFD + STZ + GB) (5 mg/kg/d, oral). The serum fasting blood glucose and insulin

levels were tested. NO levels in rat liver were determined. Acetylcholine induced endothelium-dependent relaxation and sodium nitroprusside induced endothelium-independent relaxation were measured in aortas for estimating endothelial function. The results suggest that melatonin and L-carnitine treatment decreased severity of hyperglycemia and especially melatonin significantly improved the diabetes-induced decrease in acetylcholine relaxations. According to this study; L-carnitine and melatonin treatment restore the vascular responses and endothelial dysfunction in diabetes.

PO.117

Effects of selected fungicides on steroid synthesis by bovine luteal cells

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Fungicides can cause adverse effects on fertility and luteal steroidogenesis. The present study was designed to evaluate effects of mancozeb, metalaxyl and tebucanazole on bovine luteal cell steroidogenesis.

Cell isolation was performed in aerated (O_2) culture media (DMEM/F12) containing collagenase, DNase, bovine serum albumin and antibiotic/antimycotic solution in an erlenmeyer flask, fixed on a shaking water bath at 37°C. The cells were incubated without treatment for 18 h and then with serum-free media containing mancozeb (0.01, 0.1 and 1 μM), metalaxyl (100, 500 and 2500 μM) or tebucanazole (1, 10 and 100 μM) for additional 4 days. The medium was replaced on day 1 and 3; and the retrieved medium was stored at −20°C until progesterone assay. Steroid accumulation in the used culture media was determined using a commercial Radioimmunoassay kit.

Incubation of the cells with 1 μM mancozeb, 10 μM tebucanazole and 100 μM metalaxyl resulted in significant reduction ($P < 0.05$) on progesterone production both on days 3 and 5. Suppressive effects of fungicide on steroid synthesis escalated parallel to increasing doses. Incubation of cells with fungicides resulted in significant reduction in steroid production ranging between 12 and 70%.

Present results suggest that stated concentrations of all three fungicides studied have adverse effects on luteal cell steroidogenesis. Suppressive effects of these three fungicides on luteal steroidogenesis are as metalaxyl < tebucanazole < mancozeb.

PO.118

Cortisol awakening response is lower during menstrual stage in cyclic women with premenstrual syndrome

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Introduction: Incidence of premenstrual syndrome, typically characterized by depression, anxiety and nervousness, is as high as 90% in cyclic women. These symptoms might be associated with cortisol as its receptors are widely distributed in the brain areas associated with behavior. The current study aimed at assessing cortisol awakening response (CAR) throughout the menstrual cycle.

Materials and methods: CAR was assessed by measuring salivary cortisol at 0, 15, 30 and 60 min following awakening in the same individuals ($n = 60$; age = 18–30) at different stages of the menstrual cycle (menstrual, periovulatory, luteal and premenstrual periods). Progesterone and estradiol concentrations were also determined in saliva samples to assess cyclic changes. DRSP (Daily Records of Severity of Problems) was used to assess presence of premenstrual syndrome and all the volunteers met the premenstrual syndrome criteria.

Results: Estradiol was significantly elevated during periovulatory period and progesterone was increased during luteal period ($P < 0.05$). CAR was lowest during menstruation ($P < 0.05$). Positive and significant correlations were found between cortisol and estradiol ($R^2 = 0.300$; $P < 0.001$); cortisol and progesterone ($R^2 = 0.160$; $P < 0.001$); estradiol and progesterone ($R^2 = 0.600$; $P < 0.001$). Premenstrual symptom scores at premenstrual and menstrual phases were higher than periovulatory and luteal periods ($P < 0.001$).

Discussion: Assessing CAR in the same individual throughout different phases of menstrual cycle reveals that (i) premenstrual symptoms are more severe when ovarian steroid levels drop, and that (ii) CAR is lower during menstrual phase suggesting that cortisol has a phase-specific role in the regulation of menstrual cycles.

PO.119

Changes of c-AMP level during oestrus cycle in normotensive and sponataneous hypertensive rats

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The mammalian pineal gland is under adrenergic control; however, the physiological oscillations of gonadal steroids could strongly affect the melatonin synthesis

and secretion by acting on the pre- and postsynaptic levels and by modulation of the target cells replay. The aim of this study was to determine the basal levels of cAMP in the pineal gland during the various phases of oestrus cycle in normothensive (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the histological finding of the pineal gland tissues. Two hundred female mature rats (100NTR and 100SHR) were investigated. They were divided in four groups according to the phases of the oestrus cycle (diestrus, proestrus, estrus and metaestrus). The phase of oestrus cycle has been determined by microscopic analysis of the vaginal smears. The level of cAMP (RIA) in the pineal gland was the parameter of its intracellular activity. The pineal gland tissues were stained on HaEo. In SHR there is a slight shortening of the oestrus cycle. In NTR there was an increase of the cAMP level from proestrus to metaestrus, contrary to the dramatic decrease in SHR. Histological findings of pineal glands showed the presence of many changed pinealocytes with picnotic nucleuses, while the neuroepithelial cells, in the upper parts of the glands, were separated in gland-like islets. There was a normal pineal histology in NTR. This study indicated significant neurohormonal differences between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.

Integrative Biology

PO.120

Clove extract ameliorate glucose and lipid levels by improving kidney antioxidant enzyme activities, in streptozotocin induced diabetic rats

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The effect of exposure to diabetes on the kidney appears to be modulated by elevated level of oxidative stress and lipid peroxide production, increased susceptibility to diabetic nephropathy. We also used experimental models of diabetes to further explore the effects of oral clove treatment on glycemia, lipid profiles and evaluated influences on kidney oxidative status. Diabetes was induced intraperitonially by a single injection of streptozotocin (STZ; 55 mg/kg BW). Diabetic rats ($n = 12$), weighing 280 ± 5 g, were randomly divided into two groups fed a casein diet supplemented or not with *Syzygium aromaticum* (D-*Sa*) (2 g/kg BW), for 4 weeks. Control group ($n = 6$) received 0.23–0.25 mL of citrate buffer and was fed a standard diet during the experiment. Treatment with *Syzygium aromaticum* once daily for 30 days resulted in a signicant decrease in blood glucose, glycosylated haemoglobin with a rise in plasma insulin level ($P < 0.05$). The activity of alanine and aspartate transaminase and acid and basic phosphatase was decreased respectively by −52, −77, −43 and −58%, in treated diabetic rats. The antihyperlipidemic activity as evidenced by significant decrease in plasma TC (−58%), TG (−43%), LDL-C (−62%), VLDL-C (−78%) concentrations coupled inversely with increase of HDL-C (+41%). In kidney, treatment *Sa* extract improved enzymatic activities of superoxide dismutase (SOD; +62%), glutathione peroxidase (GSH-Px; +54%), glutathione reductase (GSSH-Red; +67%) and catalase (CAT; +58%). *Syzygium aromaticum* extract possess antidiabetic, antihyperlipidemic and antioxidant activities, which act by improving insulin secretion and the alterations in the carbohydrate and lipid metabolism. Furthermore, the clove extract also has renal protective properties.

PO.121

Genetic risk factors of chronic mountain sickness

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Introduction: Chronic mountain sickness (CMS) is a pathological condition resulting from the loss of adaptation to high altitude. The syndrome is characterized by an excessive number of red blood cells (Excessive Erythropoiesis) associated with a high concentration of hemoglobin, hypoxemia and sometimes pulmonary hypertension. In the Peruvian Andes, the estimated prevalence of this condition is higher than 10% in the adult population living above 2500 m. The pathophysiology is not yet clearly established. Alteration of the control of ventilation, especially during sleep could induce a severe hypoxemia that would trigger excessive secretion of erythropoietin (EPO), leading to increased red cell production.
Material and methods: We collected clinical and physiological data and blood samples in a cohort of

143 CMS patients and 101 control subjects living at high altitude in Cerro de Pasco (4300 m). Leukocytes DNA was extracted by salting out procedure, DNA was quantified and qualified with NanoDrop™ 2000. We performed a genome wide association study using an Illumina HumanOmni5 array (4 301 332 SNPs).

Results: CMS was associated with low arterial O_2 saturation and high body mass index. We evidenced several genes that could be related to a susceptibility to develop CMS or pulmonary hypertension (ATM, NPAT, PPARGC1A, ATP9A, WWOX).

Discussion: These pathways revealed as submitted to recent positive selection. Moreover, the admixture level of European low lander ancestry in the genome of this population seemed to influence the occurrence of CMS and the polymorphism of selected genes. A replication study is planned to confirm our preliminary results.

PO.122

Role of the rapidly exchangeable calcium pool in bone in calcium homeostasis

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Bone plays a special role in calcium homeostasis by storing large amounts of calcium. The existence of a fast exchangeable pool of calcium in bone has been proposed and may be essential for the rapid regulation of plasma calcium concentration. However, calcium fluxes between bone and plasma are still poorly understood.

To characterize both pools, we first quantified the total amount of calcium in the skeleton of C57Bl/6J mice. We then determined the size of the rapidly exchangeable pool by injecting ^{45}Ca intravenously and measuring the quantity of radiolabeled ^{45}Ca in plasma, bone and urine at 5, 15 and 30 min in mice previously treated with pamidronate (an inhibitor of resorption) or vehicle (total $n = 16$ and 17 mice respectively).

We found that the mean total calcium content of the skeleton of 10 mice was 6.02 ± 0.57 mmol Ca per animal of 20 g and 55 days old, or 0.28 g of Ca/gBW. The plasma concentration of radiolabeled ^{45}Ca reached equilibrium 15 min after intra-venous injection, whereas ^{45}Ca was still being incorporated in the bone at 30 min. In pamidronate-treated mice, plasma ^{45}Ca reached steady-state with the same dynamic as vehicle-treated mice; however, bone did not incorporate further ^{45}Ca later than 5 min following injection.

Our data show that calcium is very rapidly incorporated in bone after injection (<5 min) and that pamidronate blocks further incorporation after the initial bone calcium deposit. Further work characterizing the size of the rapidly exchangeable pool and efflux rate is warranted.

PO.123

Obesity-associated alterations of glucose metabolism are ameliorated after chronic stimulation of abdominal vagus nerve

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Obesity alters glucose metabolism with specific reference to insulin resistance. We previously demonstrated that chronic stimulation of abdominal vagus nerve (VNS) was able to restore insulin sensitivity in obese animals (Malbert et al, Obesity facts, 2015). The aim of this study is to evaluate the capability of vagal stimulation to recover the altered glucose metabolism induced by obesity.

Fifteen adults minipigs were divided into three groups: lean (33 ± 1.6 kg), obese (49 ± 1.1 kg) and obese with VNS (47 ± 1.3 kg). Obesity was induced by 3 months of obesogenic diet. One obese, the obese and obese-stimulated groups were fitted with electrodes around the abdominal vagi using laparoscopy. VNS was applied in obese-stimulated group. Glucose metabolism were evaluated using indirect calorimetry during euglycemic clamp. Brain, hepatic and skeletal muscle glucose metabolism were quantified as insulin-mediated glucose uptake using FDG PET imaging. Regional glucose uptake was obtained by kinetic modeling.

Glucose oxidative metabolism was unchanged in lean, obese and obese-stimulated animals. Non oxidative glucose metabolism was significantly reduced by obesity and partially restored by VNS (4.5 ± 0.65, 2.9 ± 0.26 and 4.1 ± 0.19 mg/kg/min for lean, obese and obese stimulated, $P < 0.01$). Whole brain glucose metabolism was also reduced by obesity, a feature restored by VNS to lean condition. Liver and skeletal muscle glucose metabolism followed the same trend.

Chronic vagal stimulation was able to restore whole body glucose metabolism in obese. This restoration was equally effective on the main organs responsible for glucose metabolism demonstrating that restoration of insulin sensitivity by VNS was organ independent.

PO.124

A theoretical approach to physiological dysfunction in sodium regulation

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As sodium is essential for life the physiology of its regulation must involve systems that ensure optimum management. However, high salt intake is linked to cardiovascular disease, glucose intolerance, and even cancer which argues against this. Sodium regulation involves intake, distribution throughout body, and (mainly) kidney excretion under the influence of the hormones angiotensin II and aldosterone. Physiologically angiotensin is released in response to extracellular dehydration or hypovolaemia and stimulates thirst, i.e. the intake of water. Along with aldosterone it stimulates also sodium intake, at least in rodents. Furthermore, increased levels of both hormones are observed in cardiovascular disease, as well as in overweight, and obese, young adults and both health problems and hormones are associated with chronic kidney disease. Published data will be presented indicating that (i) urine sodium may not be a good representation of ingested sodium, (ii) thirst and sodium appetite are normal behaviours to physiologically restore decreased blood volume, (iii) human intake of sodium may be in response to the increased levels of angiotensin and aldosterone, and (iv) that increased ingestion of water would decrease blood levels of these hormones and increase elimination of any excess sodium in the urine. This data supports the hypothesis of chronic, mild, hypovolaemia (extracellular dehydration) in the general population.

PO.125

Cerebrovascular modifications induced by hypergravity: effects on blood brain barrier

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Aim: During spaceflights, one of the most remarkable effects of microgravity on astronauts is blood volume redistribution inducing 'puffy face', due to the increase of blood volume toward cephalic part. Previous studies suggested that the increase of intracranial pressure induced a reduction in the integrity of the blood-brain barrier (BBB) in microgravity. In pathological conditions, the BBB disruption could induce cognitive impairments. Protocols of hypergravity (2G), achieved by centrifugation or through vibrating plate, were shown to couterbalance the deficits induced by microgravity even if the effects on vascular functions are not deeply investigated. The aim of the present study is to determine whether the hypergravity obtained by centrifugation or vibration may affect the BBB integrity.
Methods: C57BL/6 mice were exposed either to centrifugation (2G) for 50 days or to vibrating platform for 63 days (different protocols increasing G force from 0.5 to 2 G). Immunoglobulins G (IgGs) immunolabelling was performed on hippocampal slices and IgGs accumulation in the parenchyma was quantified.
Results: In centrifuged mice, hypergravity increased the percentage of IgGs labelled surface compare to the control group. In accordance to our first finding, animals submitted to vibrating platform showed an IgGs accumulation that increases proportionally with the G force.
Conclusions: Since BBB breakdown leads to IgGs accumulation in parenchyma, our results suggest that both hypergravity protocols used in this study are effective in altering BBB integrity. Future studies will be aimed to shed light on the role of gravity changes in hippocampal dysfunctions affecting learning and memory performance.

PO.126

Cerebrovascular modifications induced by hypergravity: effects on calcium signalling in pial arteries

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Aim: Cerebral and cognitive functions closely depend on oxygen availability; and oxygen is brought to neurons by cerebral blood flow, which is regulated by vascular reactivity that is regulated by contractility of vascular smooth muscle cells (VSMC). This mechanism depends on calcium signaling. Microgravity exposure as hindlimb suspension can alter VSMC calcium signaling. We proposed that increasing the hypergravity could differentially modify the contractility of VSMC by changing the levels of expression of RyRs, IP3Rs and associated proteins.
Methods: Adult C57Bl/6J male mice were bred under different levels of gravity (1G, 2G, 3G) for 3 weeks. Cellular responses were then studied in pial arteries VSMC by determining the level of expression (RT-qPCR) of proteins implicated in calcium signaling and the evoked calcium signals, recorded with confocal microscopy coupled with fluo8-AM.
Results: Increasing the level of gravity induced in adult mice bred under high gravity conditions (3G) the decrease of RyR2, IP3R2 and CD38 expressions, suggesting lower calcium signals and potentially a decrease of myo-vascular reactivity. These signals were correlated with calcium signals.

Conclusions: Altogether, our results highlight the differential effects of gravity environment alteration on cerebro-vascular reactivity in mice.

Renal Physiology

PO.127

Primary hyperparathyroidism and renal stone disease patients: follow up after parathyroidectomy

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Introduction: Parathyroidectomy (PTx) is currently performed in hypercalciuric stone formers with primary hyperparathyroidism (PH). However, data related to persistent risk factors and calcium phosphate homeostasis following surgery remains mostly unknown.

Material and methods: Prospective observational follow up (31 patients) with a diagnosis of PH selected among 1448 hypercalciuric stone patients referred in our department. Patients with no PTx, or without any biological follow up were not included. Clinical and biological data (including morning urine sample, 24 h urines collection and a calcium load test) were collected during an outday clinic before and following surgery [3–15 months].

Results: Before surgery high ionized calcium were detected following calcium load in 100% of patients, with only 67% before calcium load (compared respectively to 3 and 0% after surgery). Interestingly, PTH after calcium load was increased in 35% and normalized in all patients after surgery. Comparison before and after surgery also showed a decreased stone risk factor (assessed by supersaturation indexes) mostly explained by a decreased calcium excretion (though a remaining hypercalciuria was detected in 35% of cases). Fasting urine calcium under calcium restricted diet was also decreased altogether with BALP and Cross laps suggesting a lower bone turn-over after PTx with no change in serum calcitriol or FGF23.

Discussion: PTx appears safe and effective for normalization of calcium, PTH, bone turn-over and decrease of stone recurrence assessed by urine supersaturation indexes. However, 35% of patients remain prone to stone recurrence assessed by a 'idiopathic' hypercalciuria which would deserve a long term follow up.

PO.128

A (very) incomplete Fanconi syndrome

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Introduction: Light chain proximal tubulopathy (LCPT) is a rare disease characterized by cytoplasmic inclusions of light chain immunoglobulins, typically associated with a Fanconi syndrome. Proximal tubule dysfunction can be incomplete, or even absent in exceptional cases. We report a case of LCPT where the only sign of tubular dysfunction is a loss of tubular secretion of creatinine.

Observations: A 39-year-old woman with monoclonal IgG κ (14 g/L) and free monoclonal κ light chains was diagnosed with LPCT (kidney biopsy). Serum creatinine was 120 μM with no tubular dysfunction except for tubular proteinuria. Three years later, she was referred in our renal physiology unit because creatinine had increased to 160 μM (MDRD-derived eGFR 31 mL/min/1.73 m²). Measured GFR was 57 mL/min/1.73 m^2, as assessed with urinary clearance of ^{51}CrEDTA during six consecutive 30-min periods, thus markedly higher that eGFR. Besides tubular proteinuria, she displayed no proximal tubular dysfunction: uricemia 236 μM (fractional excretion 11%), phosphatemia 1.3 mM (TmPi/GFR 1.1 mM), no aminoaciduria, tubular acidosis, or glycosuria. Creatinine clearance was determined simultaneously as mGFR, and tubular secretion of creatinine was calculated as creatinine clearance-mGFR. Unexpectedly, secretion of creatinine, a function of the proximal tubule, was completely abolished, when it was 15.3 ± 4.9 mL/min/1.73 m^2 ($P < 0.01$) in 25 control subjects matched for mGFR, age, sex and ethnicity.

Discussion: The prevalence of the inhibition of tubular secretion of creatinine in patients with LCPT remains to be determined. This particular and so far unreported proximal tubule damage leads to a clinically relevant underestimation of GFR by the creatinine-derived equations.

PO.129

Role of connexin 43 in acute kidney injury (AKI)

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Background: We have previously reported that connexin (Cx) 43 plays a crucial role in chronic kidney disease. In this study we investigated its role in renal ischemia/reperfusion (I/R), which is of major interest in AKI and renal transplant graft dysfunction.

Methods: I/R was induced in wild type (WT) and Cx43 heterozygous (Cx43+/−) mice (25 min ischemia after contralateral nephrectomy and reperfusion for 3–72 h). Kidneys were assessed for histological evaluation, immunohistochemistry and RT-PCR.

Results: Plasma creatinine (Cr) was elevated in WT I/R mice at 24 h (104.8 ± 18.8 μM versus C 6.7 ± 4.6 μM, $P < 0.01$). Severe tubular necrosis occured in the outer stripe (OS) at 24 h (histological scores 3.63 ± 0.12 versus C 0.06 ± 0.02, $P < 0.01$), with accompanying massive neutrophil infiltration (GR1 staining $2.89 \pm 0.53\%$ versus C $0.008 \pm 0.003\%$, $P < 0.01$). However, vigorous tissue repair followed, and the main site of lesions shifted to the inner stripe (IS) at 72 h (4.13 ± 0.07 versus C 0.08 ± 0.05, $P < 0.01$), while macrophage infiltration progressively increased (F4-80 staining $1.77 \pm 0.91\%$, versus C $0.02 \pm 0.01\%$, $P < 0.05$). Cx43 was de novo expressed in the proximal tubules after 24 h. Cx43+/− I/R mice showed improvement of OS lesions (2.10 ± 0.44 versus WT 3.65 ± 0.41, $P < 0.05$) without changes in IS lesions, decreased Cr (54.9 ± 17.4 versus WT 135.8 ± 24.5, $P < 0.01$), and a significant decrease in NGAL, HIF-1α, and Gal-3 mRNAs.

Conclusions: Cx43 is upregulated in renal I/R, and its partial deletion improved both renal structure and function, indicating a key role in AKI.

PO.130

Estrogen protects against acute kidney injury after prolonged hemorrhagic shock in mice by modulating cell death

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Women appear protected from both chronic kidney disease progression and Acute Kidney Injury (AKI). This phenomenon seems related to sexual hormones, as protection is lost after menopause or following ovariectomy. In this study, we have used a model of pressure-controlled hemorrhagic shock to assess the renal impact of estradiol during AKI.

Female C57BL/6 mice underwent 2 h hemorrhagic shock (35 mmHg mean arterial blood pressure) or were sham-operated. Experimental groups included ovariectomized mice, ovariectomized mice with chronic estradiol restoration and non-ovariectomized mice receiving a single dose of estradiol during resuscitation. Renal impact of AKI was investigated 1 day, 21 days and 4 months later.

Estrogen depleted mice present increased cell death and Kidney Injury Molecule 1 (KIM-1) expression at day 1 and develop renal fibrosis at day 21 without GFR alteration. These outcomes were not prevented by chronic estradiol restoration. By contrast a single dose of estradiol prevented the increase in KIM-1 expression at day 1 and renal fibrosis at day 21 and 4 months. Moreover, a single estradiol injection prevented the activation of caspase 3, 8, 1 and 11 accounting for the downregulation of pyroptosis and apoptosis. Estradiol injection was also associated with an increase in M2 macrophages markers (CD206, Ym1).

Our data indicate that estrogen depletion increases short-term and long-term impact of AKI. Although chronic administration seems inefficient, a single bolus of estradiol during resuscitation appears strongly renoprotective. This effect is due, at least in part, to the inhibition of both apoptosis and pyroptosis and M2 polarization of macrophages.

PO.131

Signification of distal urinary acidification defects in hypocitraturic patients

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Background and objectives: Hypocitraturia has been associated with metabolic acidosis and mineral disorders. The aim of this study was to investigate the occurrence of urinary acidification defects underlying hypocitraturia.

Design and setting: This retrospective observational study included 67 patients, with hypocitraturia (<1.67 mmol/24-h), nephrolithiasis, nephrocalcinosis, and/or bone demineralization, referred to our center from 2000 to 2015. We aimed to assess renal distal acidification capacity, prevalence/mechanisms of urinary acidification defects. Patients with low plasma HCO_3^- were studied by bicarbonate loading or furosemide/fludrocortisone tests. Patients with normal plasma HCO_3^- had an ammonium-chloride challenge test. A normal response was a decrease in urinary pH (UpH) <5.3 and an increase in urinary NH_4^+ (UNH_4^+) ≥33 μEq/min (idiopathic hypocitraturia).
Results: Eleven patients had low HCO_3^- and overt distal acidification defect. Three had a mutation in the gene encoding AE1,4 had Gougerot-Sjögren syndrome. Fifty-six patients had normal HCO_3^-; of those 33 had idiopathic hypocitraturia. Among the 23 remaining patients, 12 were unable to increase UNH_4^+-excretion (8 were able to decrease UpH, 4 were not) whereas 11 were able to increase UNH_4^+-excretion but unable to decrease UpH. These 11 patients had higher fasting urinary calcium, reflecting bone resorption.
Conclusions: Patients with hypocitraturia and normal plasma HCO_3^- frequently show a latent acidification defect that can be further dissected into several subtypes based on UpH and UNH_4^+-response to acid load. Those patients with impaired urine acidification capacity but preserved NH_4^+-excretion exhibit high calciuria and should be identified for treatment.

PO.132

Relative quantitative proteomic analysis of CNI-induced toxicity in a porcine proximal tubule cell line using iTRAQ technique

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Cyclosporine A and Tacrolimus, members of the Calcineurin Inhibitors (CNI) class of immunosuppressive drugs, are widely used to prevent allograft rejection in solid organ transplantation, by inhibiting the T lymphocyte activation pathway. However, their high efficiency is counterbalanced by nephrotoxic side effects leading to kidney loss of function. Even if the mode of immunosuppression is well described, out of scheme partners and impacted pathways remain largely unknown.

Here, we aimed at a tool designed to monitor global proteome evolution upon CNI exposure within model LLC PK-1 proximal tubular cells. To do so, we developed an untargeted approach based on drug exposure-related relative protein quantification using iTRAQ labelling allied to MS/MS analysis.

Automatized processing of relevant data from five independent experiments gave us a first glimpse at CNI exposure aftermath on a whole proteome scale. Identified proteins belonged to actin cytoskeleton dynamics, ribosome-linked protein synthesis, protein maturation, energetic metabolism and cell cycle processes. Relative quantification unveiled significant patterns of proteome evolution more or less unique to cyclosporine A and tacrolimus exposure.

While sample preparation and MS steps have been rapidly optimized, the lack of satisfying tools for iTRAQ data sorting and integration was a hindrance. It led us to develop our own algorithm to unlock the bioinformatics stages of our protocol, the keystone for further all-new hypothesis or illustration of results from targeted approaches. And now, we can provide a full, custom-made and personnalized process to monitor and compare proteome status and evolutions.

Neurophysiology

PO.133

Two-photon Ca^{2+} imaging reveals different effect of tonic inhibition on different types of LTP

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Long-term potentiation (LTP) is considered as one of the cellular mechanisms for learning and memory. Spike time-dependent (STD) plasticity is a rule for associative LTP in cortical networks. High frequency repetitive presynaptic bursts mimicked by theta-burst stimulation (TBS) trigger same pathway LTP. Both types of LTP require voltage-dependent Ca^{2+} influx (e.g. through NMDA receptors). Here we investigated the effect of tonic $GABA_A$ conductance on these two forms of LTP. We addressed this question using whole-cell recording, two-photon Ca^{2+} imaging with glutamate uncaging in CA1 pyramidal neurons in mouse hippocampal slices and mathematical modelling. Tonic $GABA_A$ conductance induced by 10 μM GABA had very little effect on regenerative events such as action potentials (APs), but reduced non-regenerative EPSPs by shunting. Thus, tonic conductance did not significantly affect APs triggered by

somatic current injections during STD protocol. Therefore, STD induced LTP was not significantly affected by tonic conductance. In contrast, during TBS protocol tonic $GABA_A$ conductance shunted the EPSPs thus reducing the probability of APs generation. This resulted in reduced NMDA receptor mediated Ca^{2+} entry in stimulated dendritic spines and suppression of TBS induced LTP. Our findings suggest that tonic $GABA_A$ conductance differentially regulates same pathway and associative LTP. Since ambient GABA concentrations depend on the level of network activity, this phenomenon may play an important role in contrasting the acquisition of different forms of memory in activity dependent manner.

PO.134

Why autistic people resist uncertainty? Electrophysiological evidence

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Resistance to change is often reported in autism and may arise from an inability to predict events in uncertain contexts.

Using EEG recorded in 12 adults with autism and age-matched controls, we characterized the influence of a certain context or an uncertain context on behavior and electrophysiological markers of predictive processing in a visual target detection task.

During an uncertain context, adults with autism had faster reaction time, enhanced CNV to all random stimuli, and earlier N2 to targets, indexing enhanced preparation and faster information processing compared to controls.

During a certain context, both controls and adults with autism presented an increased P3 amplitude to predictive stimuli, an enhanced CNV amplitude preceding predictable targets, and a reduced target-P3 latency, suggesting efficient extraction of predictive information to generate predictions. However, adults with autism displayed a failure to decrease mu power during motor preparation accompanied by a reduced benefit in reaction times to predictable targets.

The data reveal that patients with autism overanticipate stimuli occurring in an uncertain context, in accord with their sense of being overwhelmed by incoming information. These results suggest that adults with autism cannot flexibly modulate cortical activity according to changing levels of uncertainty.

PO.135

Candesartan promotes AT2 stimulation by angiotensin II, which prevents sensory small-fiber neuropathy induced by resiniferatoxin in mice

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Introduction: We have recently developed an experimental model of purely reversible functional sensory small-fiber neuropathy induced by resiniferatoxin (RTX) in mice. This model can in particular mimic what is observed in some patients who experiment transient neuropathic pain in the course of chemotherapy. Recent preclinical studies indicate that angiotensin II [deriving from the conversion of angiotensin I by angiotensin-converting enzyme (ACE)] via its receptor (AT1 and AT2), which are well known to control arterial pressure, are also involved in nerve protection and peripheral regulation of nociception. In this report, we aimed to determine whether angiotensin II activity modulation could prevent sensory small-fiber neuropathy induced by RTX.

Material and methods: Control and RTX-treated mice received no treatment, candesartan (AT1 blocker, 0.5 mg/kg/day) or ramipril (ACE inhibitor, 0.5 mg/kg/day) 1 day before vehicle or RTX administration respectively, and each day following.

Results: At day 7, in RTX mice, candesartan prevented thermal hypoalgesia; moreover, candesartan reduced significantly neuropeptide depletion in small fibers, whereas ramipril had no beneficial effect. So, AT1 receptor blocking protects against neuropathy induced by RTX. To go further, we tested the effects of an AT2 blocker (EMA 200, 3/kg/day) in RTX mice. AT2 blocker did not prevent thermal hypoalgesia and candesartan had not beneficial effect when it is administrated simultaneously with EMA 200, suggesting that AT2 receptor stimulation could protect sensory nerve deficit induced by RTX.

Discussion: Our finding that candesartan prevents nociception deficit and neuropeptide depletion encourages evaluation of its therapeutic potential in patients receiving chemotherapy.

PO.136

Obstructive sleep apnea – affecting serotonin signaling without affecting serotonin?

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Obstructive sleep apnea (OSA) is a respiratory disorder characterized by repetitive occlusion of the upper airways during sleep. It results from insufficient muscle strength against negative air pressure, which induces repetitive episodes of hypoxia/hypercapnia causing physiological and cognitive damages. Clinical and experimental data suggest involvement of the serotoninergic systems at the level of both airway muscles and respiratory neuronal network but the underlying mechanisms are poorly understood. Interestingly, OSA is more commonly found in men than in women. Now, among these, it is known that sex hormones modulate serotoninergic systems.

In both male and female mice, chronic intermittent hypoxia (CIH) was used to simulate apneic events. The immunohistochemical detection of the long-term activity marker FOSB was made in order to identify the affected brainstem structures. The co-staining of 5-HT was performed to characterize the FOSB-positive neurons.

Preliminary results 1/ underlined that CIH induced an increase in FOSB-positive cells in classically involved cardiorespiratory areas such as the nucleus of the solitary tract, 2/ suggest cell activation in the dorsal, magnus and median but not in the pallidus and obscurus raphe nuclei and 3/ surprisingly, did not identify the FOSB-positive cells of the dorsal, magnus and median raphe nuclei as serotoninergic neurons.

Against all expectations, CIH did not seem to directly modulate serotoninergic neurons. This finding sets an important milestone in understanding the pathological mechanism in OSA. Precise comparison between male and female and further experiments will be done in order to determine the origin of FOSB immunoreactivity.

PO.137

Thymelaea lythroides extract inhibits microglia activation in the adult hippocampus and behavioral alterations induced by early postnatal immune stimulation in male rats

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Introduction: *Thymelaea lythroides* is used in traditional Moroccan medicine as a general tonic to treat any inflammatory disease. Several studies have demonstrated that polyphenol and flavonoids compounds constitute a therapeutic approach for the treatment neuro-inflammatory disarrangement.

In the present study, we have assessed the efficacy of a methanolic extract of the *Thymelaea lythroides,* to counteract hippocampal microglia activation and depressive-like behaviors in adulthood in male rats that were injected with LPS (lipopolysaccharide) at 14 days of age.

Material and methods: At PND14, the pups' males were divided into four groups, group control received PBS (phosphate buffer saline). Others groups were injected with LPS (250 μg/kg), two of them were treated after 6 h from the LPS injection with minocycline or *Thymelaea lythroides* during 3 days. The behavior tests were assessed at the adult age (PND 90). At the end of the behavioral test, the animals were sacrificed. The postnatal effect of *Thymelaea lythroides* extract was compared to the effect of minocycline, a molecule known to inhibit microglia activation.

Our findings indicate that *Tl* treatment after LPS injection, showed in adulthood a low level of TNF α, Iba1 and GFAP immunoreactivity in the hippocampus and significantly decrease of depressive-like behavior in forced swimming test.

The results showed that *Tl* has beneficial effects on inflammation and depression like behavior induced by LPS, by modulating the astro-microglia cells activation. *Thymelaea lythroides* extract and minocycline had similar actions in counteracting the effects of perinatal LPS.

PO.138

Pregabalin have dose responsible anxiolytic effects on animal anxiety tests

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Introduction: Pregabalin is an antiepileptic drug which binds to $\alpha 2\delta$ unit at presynaptic voltage dependent calcium channels. Thus results in the inhibition of excitatory neurotransmission and so inhibits the triggerring of corticostriatal, talamocortical and amigdala in anxiety. Our goals are to investigate pregabalin effects on anxiolytic behaviour pattern by different animal anxiety tests.

Materials and methods: 56 male Wistar Albino Rats were divided into seven groups as control, vehicle and five different dose groups (5, 10, 30, 60, 100 mg/kg intraperitoneal pregabalin administration for 2 weeks). Subjects were conducted open field test (OFT), elevated plus maze (EPM), light dark box (LDB). Behaviour tests were recorded via a camera and evaluated by two different resarchers. We analyzed central zone entries, duration in periferal zone and total traveled distance in OFT, time spent in open arms and number of open arms entries in EPM, time spent in light/dark zones in LDB.

Results: Central zone entries increased in 60 and 100 mg/kg groups compared with control and vehicle ($P < 0.001$). Duration in periferal zone decreased in 100 mg/kg compared with control, vehicle and 5 mg/kg ($P < 0.05$). Total traveled distance increased in 60 mg/kg versus control, vehicle and 5 mg/kg ($P < 0.05$). Time spent in open arms increased in 60 ($P < 0.05$) and 100 mg/kg ($P < 0.001$) and open arm entries increased in 100 mg/kg ($P < 0.05$). Time spent in light zone increased and time spent in dark zone decreased among all Pregabalin groups versus control and vehicle ($P < 0.001$–0.05).

Discussion: Pregabalin have dose dependent anxiolytic effects in different tests.

PO.139

Neuronal correlates of uncertainty

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Uncertainty is a cognitive process that influences our decisions in everyday life. In obsessive-compulsive disorder (OCD), the high level of uncertainty alters the decision-making process. This work aimed to a better understanding of pathophysiological aspects of uncertainty using subthalamic (STN) and caudate nucleii local field potentials (LFPs) recordings in operated pharmacoresistant OCD patients during the realization of a delayed matching-to-sample task.

Six OCD patients were recorded. The task was delivered using the E-prime2 ®, Psychology Software Tools (Pittsburg, USA). The STN and caudate LFPs were recorded using a EEG recorder (Deltamed) with a sampling rate of 512 Hz. LFP activity (evoked potential and time frequency) was analyzed off-line, according to the different paradigm events and using the Matlab® tool EEGlab 12.2.2.4b (Delorme & Makeig, 2004 – MatLab 9.0).

The severity of OCD was attested by the Y-BOCS score before surgery (32.4 ± 4.3). LFP recordings were performed in the STN (2 patients) and in the caudate nucleus (4 patients). Our data reveals that uncertainty was associated with an increased amplitude of evoked responses in the subthalamic nucleus but not in the caudate nucleus. For one patient, gamma activity was increased during uncertainty in the STN only. One the other hand, amplitude of evoked potentials was increased in the ventral striatum during performance evaluation.

Our preliminary findings confirm the role of the subthalamic nucleus in OCD pathophysiology and in the mechanisms underlying the occurrence of pathological uncertainty. The ventral striatum seems to be more involved in performance evaluation.

PO.140

Check or go? Anxiety-related checking behavior in rhesus monkey

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Checking compulsions is among the most common behavioral features of obsessivecompulsive disorder (OCD). It is thought to be a behavioral attempt to relieve anxiety caused by high levels of doubt and uncertainty. Numerous studies suggest that alteration of prefrontal cortices would lead to compulsive checking behavior. However the physiology of checking behavior remains poorly understood. The present behavioral study tends to characterize physiological doubt and checking behavior in nonhuman primates (NHP) and to study electrophysiological correlates of decision making, doubt and checking.

We trained two Macaca mulatta on the CheckorGo task while recording their frontal EEG activity. Saliva samples were collected to correlate cortisol

concentration along sessions to anxiety with checking behavior. Our behavioral paradigm allows the animal to multiple check and potentially change the availability of the reward before taking the final decision leading to that reward.

By manipulating the ambiguity of the visual cue embedding the reward status, we successfully modulated animal uncertainty and created doubt. Behavioral results showed that the animal uncertainty level influenced not only performances and reaction times but also checking behavior rate. Fronto central EEG potentials were also modulated by visual cues' ambiguity level and checking decision. Daily cortisol quantification revealed a positive correlation between monkeys' checking behavior and anxiety level.

Taken together, our study demonstrated that the CheckorGo task is a valid behavioral tool to study the impact of ambiguity on doubt and the subsequent adaptive checking behavior and will thereby help us provide new insights into OCD mechanisms.

PO.141

The ultimatum game: proposer and responder disparities in ERP components

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The Ultimatum Game is a typical paradigm to investigate economic decision-making. Although human behavior in this task is well established, the underlying cognitive processes remain poorly understood.

Neuronal bases of the proposer and responder conditions were examined using event-related potentials (ERPs), independent component analysis (ICA), and source reconstruction.

Three major ERP components [P2, feedback-related negativity (FRN), and late positive component (LPC)] were observed in both conditions.

A negative deflection around 180 ms (N2) was identified in the proposer only. Additionally, an independent component was observed and source reconstruction showed higher activity in the anterior cingulate cortex (ACC) for the proposer condition in this N2 time-range.

The responder condition revealed a significantly decreased amplitude and delayed latency for P2 (3.50 versus 5.78 μV and 244.5 versus 226.5 ms, respectively; both $P < 0.01$), an increased amplitude and shortened latency for FRN (-1.46 versus -0.77 μV, $P < 0.05$; 342.9 versus 330.4 ms, $P < 0.01$), and a higher mean activity of LPC (1.23 versus 0.35 μV; $P < 0.01$).

Our findings indicate that the distinction between your own and the perception of another individual's choice is based on the engagement of multiple neuronal systems. The timeline of involvement of the affective-based ACC activity is the functionally relevant dimension and differs depending on the condition of the participant (with an earlier activation in the proposer), although the goal is the same for both tasks. Finally, the intensity of the activation of neuronal bases for cognitive-based judgements reveals a higher involvement of workload for the responder condition.

PO.142

Rectal tone is increased in patients suffering from spina bifida

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Introduction: Spina Bifida (SB) is a congenital spinal cord agenesy associated with bowel dysfunction that may involve impaired rectal adaptation to distension. The aim was to prospectively assess anorectal responses to rectal isobaric distension in adults with SB.

Methods: Anal and rectal adaptations to rectal isobaric distension of 22 consecutive patients between June 2015 and February 2016 (M/F: 15/7; mean age 40 $\pm$ 11.3 years) with SB were assessed and compared to 12 healthy volunteers (HVs) (M/F: 7/5; mean age 43 $\pm$ 1.3 years). The two groups did not differ with respect to age and sex ratio. Regarding patients with SB, 72.7% (16/22) patients had SB apperta and lumbar neurological level was predominant [68.2% (15/22)]. Rectal compliance was defined by the difference between the volume at resting state and the initial volume measured when the preselected pressure was just reached. Rectal tone was defined as maximal volume at the end of distension – initial volume.

Results: Rectal compliance was comparable between the two groups (SB: 69.0 mL/mmHg [45.1–117.8] versus HV: 112.7 mL/mmHg [62.9–135.7]; $P = 0.0961$). Rectal tone was significantly decreased in patients with SB (SB: 27.2 mL [13.8–36.0] versus HV: 54.1 mL [45.3–70.6]; $P = 0.0003$). In patients with

SB, two patients had normal rectal tone (defined as ≥IQR 25 of normal value i.e. 45.3). They both had SB occulta and neurological level S3.
Discussion: Patients with SB experiment rectal tone disorders without significant change of rectal compliance. These suggest impairment of viscoelasticity of the rectal wall.

PO.143

Diurnal cortisol pattern rather than cortisol awakening response is higher during menstruation in cyclic women

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Introduction: Cortisol secretion follows a diurnal rhythm, increasing following awakening in the morning (cortisol awakening response, CAR) and decreasing towards midnight. It is known that phasic behavioral changes occur throughout menstrual cycle. However, it is not well established whether diurnal cortisol secretion differs throughout the menstrual cycle. Therefore, the aim of the current study was to assess diurnal cortisol levels throughout menstrual cycles in cyclic women.
Materials and methods: Salivary cortisol was measured following awakening (0, 15, 30 and 60 min, cortisol awakening response, CAR) and at 12:00 pm, 17:00 pm, 22:00 pm (as diurnal rhythm) from healthy and normally cycling women (18–31 year-old, $n = 15$). The assessments were repeated in the same individuals three times over one menstrual cycle, namely at menstruation, luteal and premenstrual phases, respectively. Area under the curve (AUC) was calculated for CAR and diurnal cortisol.
Results: There were no differences between the phases in terms of cortisol concentration or AUC for CAR ($P > 0.05$). But CAR was significantly higher than the diurnal cortisol ($P < 0.05$). Additionally, menstrual diurnal cortisol AUC was higher than luteal and premenstrual phase diurnal AUC ($P < 0.02$). A significant positive correlation was detected between the CAR AUC and diurnal AUC in all phases ($R^2 = 0.210$; $P = 0.002$).
Discussion: The present data points out that instead of cortisol awakening response, cortisol secretion pattern towards night is higher in the menstrual phase of the cycle.

PO.144

The effect of maternal L-thyroxine treatment during lactation affects long-term potentiation in adult rat progeny

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Purpose: Numerous animal studies have shown that thyroid hormone imbalance affects cognitive functions such as learning and memory. The goal of present study was to investigate hippocampal long-term potentiation (LTP) response in maternally-induced and adult-onset hyperthyroid rats.
Methods: Animals were kept for 1 week before mating (M/F = 2:1). The pregnant rats were randomly assigned into three groups ($n = 8$ per group): control, maternal hyperthyroid (MH), and adult-treatment (AT) group. MH consisted of male rat offspring from these mothers treated with L-thyroxine (0.2 mg/kg body mass, 1 mL). When pups were grown to age of 39 day, male rat offspring from AT group were treated with L-Throxine for 21 days and they were consisted of AT group. Control group consisted of male rat offspring from control mothers. Field potentials were recorded from the dentate gyrus in response to stimulation of the medial perforant pathway *in vivo*. Measurements were all done in rats aged 60–66 days.
Results: An ANOVA on the PS-LTP and the EPSP-LTP revealed a significant group effect ($P = 0.001$ and 0.014, respectively). The *post hoc* analysis showed that the magnitude of PS-LTP at the last 5 min of recording was lower in the AOH group ($P = 0.01$) and MH group ($P = 0.001$) than that control group. There was also significant difference between AT and MH group ($P = 0.001$). When considering EPSP-LTP data, only difference was found between control and MH group ($P = 0.014$).
Conclusion: These results suggest that perinatal hyperthyroidism has longstanding effects on hippocampal function and may account for memory problems experienced by adolescents with congenital hyperthyroidism.

PO.145

Autonomic response to acute cognitive and emotional stress in healthy students

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Introduction: Autonomic nervous system continually exerts regulatory effects on peripheral organs. Alteration of this central-peripheral pathway increases the risk of stress related disorders. Understanding of the pathophysiology requires detailed information about physiological stress reaction. Therefore, we studied the autonomic response to different cognitive and emotional stressors in healthy students.

Materials and methods: Continuous recording of ECG and electrodermal activity (EDA) was performed in 40 participants (20 women; 22.9 ± 0.1 years, BMI: 21.7 ± 0.4) during baseline, Stroop test (S1), rest, arithmetic test (S2), rest, negative emotion (S3), and rest. Evaluated parameters: EDA amplitude [μS], heart rate variability: RR interval, spectral power in high frequency band (HF; cardiovagal control); symbolic dynamics – 0V% (cardio-sympathetic index), 2LV% (parasympathetic regulation).

Results: Significantly shortened RR-interval was found during cognitive (S1, S2: $P < 0.001$) and emotional (S3: $P < 0.01$) tasks. All stressors evoked significant decrease in logHF and 2LV% (both S2: $P < 0.001$; S1, S3: $P < 0.01$). Significant increase of the 0V% and EDA was found during cognitive tests (both S1, S2: $P < 0.001$). EDA was significantly higher during all recovery phases compared with baseline ($P < 0.001$), and decreased after negative emotion compared with stress period ($P < 0.001$).

Discussion: We found distinct autonomic responses to acute cognitive and emotional stress. Cognitive tests were characterized by decreased parasympathetic and increased sympathetic regulation. Emotional stress was associated only with vagal withdrawal. Elevated EDA could indicate sympathetic arousal during complete protocol.

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PO.146

Changes of sympathetic regulation after long-lasting psychogenic load in healthy students

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Introduction: Long-term mental stress is associated with increased risk of psychosomatic disorders. The pathomechanism could result from complex dysregulation of neuro-endocrine-immune interaction. One of the key components mediating the effect of allostatic load on health is sympathetic nervous system. The aim of this study was to assess the changes of sympathetic marker electrodermal activity (EDA) after long-term mental stress associated with university exams.

Materials and methods: The stress profile was assessed using EDA (μS) in 20 male university students (age 22.5 ± 0.3 years, BMI 23.5 ± 0.6 kg/m^2) at the beginning of semester (P1) and a day before the last exam (P2). Examination protocol: baseline (T1), Stroop test (T2), rest (T3), mental arithmetic (T4), rest (T5), negative emotion (T6), rest (T7); each period: 5 min.

Results: The EDA was significantly higher during all tasks and recovery periods (T2-T7) compared with baseline (T1) in both P1 and P2 (all: $P < 0.001$ except T7 in P2: $P = 0.011$). The amplitude of EDA significantly decreased in P2 compared with P1 (T2: $P < 0.001$; T1, T3-T7: $P < 0.01$).

Discussion: The increased EDA in response to acute stressors and during recovery (in both semester and examination period) could reflect prolonged sympathetic stress reaction. Unexpected finding of lower sympathetic activity after long-term mental load could be affected by activated coping mechanisms or subtle alteration of physiological stress response. Detailed study could help to understand distinct sympathetic-mediated effects of stress.

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PO.147

The dynamics of epileptiform discharges in neocortex and hippocampus during sleep waking cycle

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The purpose of our work was investigation of the changes of hippocampal and neocortical epileptiform discharges at different stages of sleep-waking cycle (SWC)

In vivo experiments were carried out on Wistar rats. Under ketalar anesthesia Stimulating and recording electrodes, were implanted in the neocortex and dorsal hippocampus (DH). Bipolar electrodes were also implanted in cervical muscles and orbital cavity for the recording of muscular tone and eye movements. The epileptiform discharges (EDs) which were induced by high-frequency electrical stimulation (30 Hz) of dorsal hippocampus were recorded with 8 channels eeg (Medicor 8 S; Hungary).

Result: It was found that EDs of hippocampal origin were more durable at the stage of slow sleep than in wakefulness. Consequently during slow sleep the hippocampal epileptogenic threshold was lowered. And consistently epileptiform discharges increases. According to the data obtained one of important factors increasing susceptibility of hippocampus to EDs, at the stage of slow sleep, should be the weaking of tonic inhibitory influence of the cortex upon hippocampus, result of which is decrease of epileptogenic threshold in the latter. Epileptogenic thresholds of neocortex were lower during wakefulness than in stages of slow sleep and epileptiform discharges increases during wakefulness, than in stages of slow sleep.

Conclusion: Icreases of neocortical epileptiform discharges in stages of wakefulness should be due to facilitatory influence of neocortical α-adrenoceptors upon the principal neurons in neocortex, while increase of hippocampal epiletogenic threshold may be conditioned either by direct action of inhibitory β-adrenoceptors on principal hippocampal neurons.

PO.148

An inverse agonist effect of tetraiodothyroacetic acid on the long-term potentiation *in vivo*

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Purpose: Tetraiodothyroacetic acid (tetrac) is a T4 analog that inhibits binding of iodothyronines to the integrin $\alpha v\beta 3$ receptor. We showed that intra-hippocampal T4 infusion attenuates the long-term potentiation (LTP) in the synapses between the perforant pathway and the dentate gyrus. However, the effect of tetrac on the attenuated LTP induced by T4 remains unexplored.

Methods: Field potentials were recorded from the dentate gyrus in response to stimulation of the medial perforant pathway by high- frequency stimulation (HFS). Infusions of saline, T4 and tetrac, either alone or together, were made during the stimulation protocol. The averages of the excitatory postsynaptic potential (EPSP) slopes and population spike (PS) amplitudes after HFS was used as a measure of the LTP magnitude.

Results: The magnitude of EPSP-LTP revealed a significant effect of T4 ($F_{1,28} = 13.75$; $P = 0.001$), a significant effect of tetrac ($F_{1,28} = 10.70$; $P = 0.003$), and a non significant interaction between these drugs ($P > 0.05$). The magnitude of LTP within the last 5 min of recording was lower in T4 infused experiments ($115 \pm 3\%$ of baseline) than non infused experiments ($133 \pm 5\%$) and significantly greater in tetrac infused experiments ($132 \pm 4\%$) than non-infused experiments ($116 \pm 4\%$).

Conclusion: Although tetrac is normally considered to be inactive in reference to intracellular thyroid hormone functions, this study shows that binding of tetrac to the $\alpha v\beta 3$ integrin receptor does not only block the effects of binding T4 but also activate the basal activity of the integrin $\alpha v\beta 3$ receptor. However, the molecular mechanisms mediating these effects need to be resolved.

PO.149

Hypothyroidism induced endoplasmic reticulum stress in rat amygdala

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Neurotransmitter systems are affected by deficiency or excess of thyroid hormones in adult rat brain. Various diseases have been shown to be associated with impairment of endoplasmic reticulum (ER) function. It is important to know more about the relationship of hypothyroidism with amygdala since thyroid state promotes psychiatric symptoms and disturbances like psychotic, depressive, and rapid cycling bipolar disorders. Therefore in this study, we aimed to determine the ER stress in amygdala of hypothyroid rats.

Male Wistar Albino rats (12 weeks) were randomly divided into two groups as control ($n = 6$) and thyroidectomized ($n = 12$). Hypothyroidism was induced by surgical thyroidectomy. Four weeks after

thyroidectomy, amygdala and blood samples were taken from rats. Serum concentrations of thyroid stimulation hormone (TSH) and free tri-iodothyronine (fT3) were assessed by autoanalyzer. The protein expressions of ER stress markers GRP78/Bip, ATF6 and PERK were determined by western blot analysis.

Hypothyroidism was confirmed in the thyroidectomized group by elevated TSH and decreased fT3 levels in serum. GRP78/Bip and ATF6 protein expressions were found to be upregulated, while PERK protein expression did not changed significantly in the thyroidectomized group. These results showed that hypothyroidism leads to activation of ATF6 signaling.

Hypothyroidism may cause an increase in unfolded protein response and ER stress. However, future investigations need at transcriptional level for clarify the underlying molecular mechanisms.

PO.150

The effects of usnic acid on the penicillin induced epileptiform activity on rats

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Aim: Aim of this study was to investigate effects of usnic acid using on experimental penicillin-induced epilepsy model in rats electrocorticographically.

Materials and methods: In this study 70 adult male Wistar rats were used. Rats were divided into 10 groups, control (penicillin), only usnic acid, diazepam (positive control), before or after penicillin administration doses of 50, 100 and 200 mg/kg of usnic acid. All of the substances were administered intraperitoneally except penicillin. After rats were anesthetized with administration of the 1.25 g/kg dose urethane, the left part of the cortex was opened and the electrodes were placed on somatomotor area. Electrocorticogram recordings were performed by Powerlab System. epileptiform activity was induced by penicillin which was applied intracortically. The time to onset of first spike wave latency, spike-wave frequency and spike-wave amplitude of epileptiform activity were analyzed statistically.

Results: Any epileptiform activity was not observed before penicillin administration in all groups. In the doses of before and after penicillin administration usnic acid had not reduced significantly both frequency and amplitude of epileptiform activity according to control and diazepam groups (except some time periods) ($P > 0.05$). According to latency there was no statistically significant difference between the groups ($P > 0.05$). However, diazepam decreased the frequency and amplitude of epileptiform activity versus after penicillin injection groups (except some time periods) ($P < 0.05$).

Conclusion: Usnic acid has not effect on spike-wave frequency, amplitude and latency in penicillin induced epilepsy model in rats. Further studies may reveal effects of usnic acid on epilepsy.

PO.151

Pregabalin alters climbing behaviour in forced swimming test

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Introduction: Pregabalin is a calcium channel ligand in central nervous system. Binding of Pregabalin to $\alpha 2\delta$ subunit of voltage dependent calcium channels at presynaptic membrane inhibits the release of excitatory neurotransmitters such as noradrenaline. Climbing behaviours associated with noradrenaline. Our goal was to investigate the effect of pregabalin on two different depression tests. We observed climbing, floating and swimming behaviours in forced swimming test (FST) and also applied sucrose preferance test (SPT).

Materials and methods: Fifty-six male Wistar Albino rats were divided into seven groups (control, vehicle, 5, 10, 30, 60, 100 mg/kg Pregabalin). According to FST protocol; as a pretest 15 min FST was conducted, 21 h later single doses pregabalin and vehicle were administered intraperitoneally and after 3 h following the injections 5 min FST was repeated. FST was recorded via camera and analyzed by two different researchers. SPT protocol was performed by placing two bottles contaning water and 1% sucrose solution in each cage.

Results: Sucrose in take percentage was above 65% among all groups. Climbing time decreased significantly in all pregabalin groups compared with control and vehicle except 5 mg/kg group (10 mg/kg ($P < 0.01$) and in 30, 60, 100 mg/kg ($P < 0.001$)). Floating time reduced significantly in all pregabalin groups compared with control and vehicle ($P < 0.05$). There was no change in swimming time among groups.

Conclusion: Significantly reducing in climbing time by Pregabalin administration suggests that pregabalin inhibits noradrenaline release. However, above a 65% sucrose solution consumptionin SPT of all groups proposed that this inhibition didn't cause a depression like behaviour.

PO.152

Correlation between vitamin D status and Wechsler Adult Intelligence Scale's comprehension subtest in patient with end-stage renal diseases

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Aim: Cognitive decline is an important problem for individuals, as well as for the community. Increasing evidence suggests that vitamin D may play a role in maintaining cognitive function and vitamin D deficiency may accelerate cognitive decline. The aim of this study was to evaluate the status of vitamin D in chronic renal failure (CRF) patients on peritoneal dialysis (PD) and to correlate the findings with cognitive functions.
Materials and methods: The study was performed in Inonu University Turgut Ozal Medical Center. Serum 25hydroxyvitamin D (25(OH)D) was measured and cognitive functions (Wechsler Adult Intelligence Scale's (WAIS) Comprehension Subtest) tested with 51 peritoneal dialysis patients and a control group consisting of 51 healthy individuals have similar conditions with patients. Individuals with other chronic diseases and smoking and alcohol habits which may impair cognitive functions were excluded from the study.
Results: Compared to each groups we found negative correlation between 25(OH)D3 levels and WAIS's Comprehension Subtest in patient and control group. The correlation between data was evaluated with the Spearman's test ($P = 0.597$, $r = -0.76$ and $P = 0.010$ $r = -0.356$ respectively).
Conclusions: In the literature, there is no consensus on the presence of an association between vitamin D levels and cognition. In this study vitamin D levels were measured as deficiency in both groups. Further studies are needed to investigate for increasing actual knowledge about this association.

PO.153

Modulation of spatiotemporal calcium dynamics in single astrocytes by neuronal activity

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Astrocytes play a number of important functions in the brain trough a generation of a repertoire of complex Ca^{2+} events. Although the astrocytic Ca^{2+} signalling has been intensively studied for a several last decades the principle of Ca^{2+} events integration in astrocytes during synaptic activity, however, remains unknown. Here we implemented an analysis of whole Ca^{2+} events (Wu et al., 2014 in single astrocytes in mice hippocampal slices and we found that spreads and durations of Ca^{2+} events follow power law distributions, a fingerprint of scale-free systems. The power law exponent (α) was decreased by activation of metabotropic glutamate receptors (mGluRs) either by specific receptor agonist, glutamate uncaging around astrocytic processes or by low frequency stimulation of glutamatergic fibers in hippocampal slices. Decrease in α indicated an increase in proportion of large Ca^{2+} events. Notably, mGluRs activation did not increase the frequency of whole Ca^{2+} events. This result suggests that neuronal activity does not trigger new Ca^{2+} events in astrocytes (detectable by our methods), but modulates the properties of existing ones. Pharmacological blockade of mGluRI leads to the decrease of the proportion of large Ca^{2+} events, suggesting that such Ca^{2+} dynamics might arise from intracellular inositol-3-phosphate diffusion triggered by mGluRs activation during synaptic transmission. Thus, our results provide a new perspective on how astrocyte responds to neuronal activity by changing its Ca^{2+} dynamics, which might further affect synaptic transmission and local network functioning.

PO.154

Effect of specific energy substrates on astrocytes calcium dynamic in the rat hippocampus

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Recent studies have demonstrated the important role of astrocytes in memory formation through delivery of lactate to neurons. During neuronal activity and in certain pathologies, the synthesis of lactate is activated in astrocytes. Because neuronal activity depends on energy substrate accessibility, this process serves as a regulatory mechanism for some neuronal functions. Lactate and pyruvate also become the main energy substrates in the brain when the glucose level is low. In addition, ketone bodies can serve as energy substrate in the neonatal brain. Using laser confocal microscopy we investigated how lactate, pyruvate and beta-desoxybutirate, thereafter specific energy substrates (SES), affect calcium dynamics in astrocytes in rat hippocampal slices at different stages of postnatal brain development: postnatal day (P) 5, 15 and 30. The astrocytes were stained with Oregon Green BAPTA AM (7.95 μM) and specific astrocytic marker

sulforhodamine 101 (200 nM). Application of each of SES significantly increased frequency of astrocytic calcium events at P5, but not P15 and P30. However, SES application in the presence of vesicular release blocker bafilomycin A1 (4 μM) decreased frequency of calcium events at P5. This suggests that at early development SES regulate astrocytic calcium activity though changes in vesicular release of neuro- or/and gliotransmitters. Because astrocytes play important role in the brain development (e.g. regulation of neuro- and gliogenesis, neuronal pathfinding, synaptogenesis) our funding points how these processes can be affected by availability of SES.

PO.155

Different sensitivity of brain to oxidative stress induced by methionine nutritional overload

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Introduction: Amino acid L-methionine is a precursor of homocysteine and methionine nutritional overload could result in hyperhomocysteinemia, a risk factor for number of neurodegenerative disorders via still not fully clarified mechanisms. Brain oxidative stress, as an imbalance between the formation and removal of reactive oxygen species could be one of them. The aim of this study was to investigate the effects of methionine-enriched diet on the oxidative stress in different rat brain regions.
Materials and methods: Wistar rats were randomly divided into control and experimental groups. During the 30 days the animals in the control group were given a standard diet and the experimental animal were given food enriched with methionine. Animals were sacrificed on 31st day and the lipid peroxidation and activity of antioxidant enzymes was determined in four brain regions: cortex, hippocampus, thalamus, and caudate nuclei.
Results: Lipid peroxidation was significantly increased in the cortex and nc. caudatus of experimental rats developing hyperhomocysteinemia, with no significant differences regarding it in the hippocampus and thalamus. Catalase activity was significantly increased in the cortex, hippocampus, and thalamus of experimental rats receiving high methionine diet. Content of glutathione was significantly increased in the thalamus and nc. caudatus, and in the hippocampus of experimental rats.
Discussion: The results of this study showed that methionine nutritional overload non-uniformly induced appearance of oxidative stress in the rat brain, where the cortex and nc. caudatus show greater sensitivity compared to the hippocampus and thalamus.

PO.156

Mechanism of hypothalamus glycine receptor involvement in regulation of sexual behavior

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Most of inhibition in the CNS is mediated by $GABA_A$- and glycine receptors. In rat hypothalamic medial preoptic area (mPOA) $GABA_A$-receptors are predominantly synaptically activated. The source glycine in mPOA is less clear. Here we hypothesized that glycine can be released by astrocytes though reverse of glycine transport. The latter may occur as result of glutamate uptake and sodium load of astrocytes.

To test this hypothesis, we studied mPOA neurons membrane potential and conduction using patch-clamp recordings in male rat acute brain slices. Local application of glutamate (500 μM) with multichannel perfusion system or high frequency stimulation of glutamatergic inputs to mPOA were used to trigger the taurine and/or glycine release from astrocytes. Blockade of glycine receptors with 20 μM strychnine led to elevation of neuronal excitability, suggesting glycine receptor-mediated neuronal inhibition during glutamate stimulation.

Because mPOA is a key brain structure controlling male sexual behavior in vertebrates, sexual performance of adult male rats was investigated upon blockade of glycine receptors. Bilateral microinjections of 20 μM strychnine into mPOA through chronically implanted intracranial cannulas significantly increased duration of postejaculatory interval, whereas 1 mM glycine decreased ejaculation latency, number of intromission and duration of the postejaculatory interval.

Our findings suggest that glycine-mediated glia-neuron interactions in mPOA can be a mechanism regulating sexual behavior.

PO.157

Transplantation of ephrin-B2 stimulated peripheral blood mononuclear cells from diabetic patients enhances post-stroke recovery in mice

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Objective: Clinical trials using cell therapy in stroke favor easy and ready to use autologous cell transplantation. Hence, mononuclear cells derived from bone marrow have been tested in feasibility clinical studies, but bone marrow cells harvesting is invasive. We investigated the therapeutic potential of peripheral blood mononuclear cells (PB-MNC) stimulated by ephrin-B2 (PB-MNC+), and collected from type-2 diabetic patients who are prone to a higher risk of stroke.

Material and methods: Adult male C57BL6/J mice underwent permanent middle cerebral artery occlusion (pMCAo) and received 24 h later intravenous injection of vehicle (PBS), unstimulated or stimulated PB-MNC (500 000 cells). Volume infarction and blood brain barrier (BBB) permeability were evaluated 3 days after stroke and neurological deficit, cell proliferation, angiogenesis, inflammation and neurogenesis 3 and 14 days after stroke.

Results: Compared to PBS mice, infarct volume was reduced by 60% at day 3 and functional recovery was enhanced at day 14 in PB-MNC+ treated mice ($P < 0.05$). Moreover, cell proliferation was increased early in both periinfarct and subventricular zones ($P < 0.05$ and $P < 0.01$) and microvessel density significantly increased at day 14. TGFb mRNA expression was up-regulated at day 3 in PB-MNC+ mice compared to PBS mice ($P < 0.05$). Cell therapy had no impact on BBB permeability or neurogenesis, but BDNF was significantly increased at day 14 compared to the other groups.

Conclusion: PB-MNC+ are able to reduce infarct volume and to stimulate regenerative processes after stroke. Mechanisms are currently under investigation. This approach should encourage developing quick and non-invasive bedside cell therapy strategies.

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Leptin plays a key role in the central control of hypercapnic ventilatory response

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Introduction: Obesity imposes mechanical and metabolic stresses affecting breathing. Most obese individuals maintain eucapnia but a subgroup develop chronic hypercapnia, described as the hypoventilation syndrome (OHS). Data of the literature suggest that leptin plays a role in maintaining adapted ventilation under obesity but mechanisms are not fully understood. Aims of the present work were to assess the role of leptin under hypercapnia and to determine the involved mechanisms.

Methods: Electrophysiological recording of *ex vivo* preparations from newborn wild type (WT) mice, containing the medulla oblongata with or without supramedullary regions, was performed under normopH and metabolic acidosis, in order to test the effect of bath exposure to leptin on respiratory frequency. Then ventilation *in vivo* under normocapnia and hypercapnia (3–5–8% CO_2) was analysed on leptin-deficient (ob/ob) and WT mice by plethysmography. Finally, we analyzed c-FOS expression concomitantly with the detection of brainstem respiratory structures in both mice models, under normocapnia and hypercapnia (8% CO_2).

Results: First, respiratory frequency was increased in normopH by leptin on all *ex vivo* preparations and increased by metabolic acidosis only when supramedullary regions were present. Second, the breathing pattern of ob/ob mice was altered in normocapnia and hypercapnia. Finally, absence of leptin in ob/ob mice changed the distribution of c-FOS activated cells by hypercapnia in respiratory areas. Part of these cells was serotoninergic.

Discussion: These data suggest that leptin exerts a facilitatory influence on the ventilatory response to hypercapnia through supra-medullary mechanisms but does not potentiate the respiratory response to metabolic acidosis by medullary mechanisms.

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Enhancement of the respiratory response to metabolic acidosis in newborn rat by a progestin: possible involvement of orexin neurons

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Introduction: Ondine's curse is a neuro-respiratory disease characterized by sleep-related life-threatening hypoventilation due to dysfunction of the CO_2/H^+ chemosensitive neurons of the retrotrapezoid nucleus/parafacial respiratory group. A clinical observation have shown on two women patients a recovery of CO_2/H^+ chemosensitivity concomitantly with oral contraception using desogestrel (Straus et al 2010). Recently, we have shown on *ex vivo* preparations of central nervous system that etonogestrel (active metabolite of desogestrel) induced a potentiation of the respiratory response to metabolic acidosis (MA: a model of hypercapnia) (Loiseau et al 2014). Our aim was to determine the encephalic subdivisions and structures involved in order to surround the action mechanisms of the progestin.
Methods: Under MA, etonogestrel's effect on respiratory frequency, was appreciated on *ex vivo* preparations from newborn rats containing either the medulla oblongata with or without pontine, mesencephalic and diencephalic regions. On preparations with diencephalon, etonogestrel's effect was examined under exposure with a specific antagonist of orexin receptors and appreciated in comparison to control preparations. In addition, immunohistological detection of c-FOS was performed concomitantly with detection of orexinergic neurons.
Results: Etonogestrel enhanced the acidosis-induced hyperventilation only when diencephalic structures were present. Under antagonization of the orexinergic signalization, this effect was abolished. Immunohistological quantification suggest that etonogestrel significantly increased *c-FOS* expression in orexin-containing neurons of the lateral hypothalamic area.
Conclusion: These data suggest that etonogestrel acts by activating the orexinergic signalization. Further experiments are required to highlight all the downstream structures involved and contribute to develop pharmacological treatment for CCHS.

PO.160

Direct-current stimulation of posterior tibial nerve modulates the Soleus H-reflex amplitude

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Introduction: Several studies demonstrated that transcranial direct current stimulation (tDCs) is a promising non-invasive tool able to modulate the excitability of several CNS structures. Its effect is usually facilitatory when using anodal polarity and inhibitory for the cathodal one. In most studies, DC stimulation was applied on cortical or spinal structures, while little is known about its effect on peripheral nerves fibres. This research aims at highlighting such effect.
Methods: In twenty subjects, electrical stimulation of the posterior tibial nerve (1 ms current pulses, 1 shock every 9 s) was used to elicit the H-reflex in the Soleus muscle. Once the H-reflex amplitude was stable for at least 15 min, DCs (either cathodal or anodal) was applied proximally to the same nerve for 10 min, looking for changes in reflex amplitude. Then, the H-reflex was measured for 30 further minutes, looking for after-effects.
Results: Cathodal DCs induced a significant increase of the H-reflex amplitude (about +35%) with respect to the control value. In this configuration the after-effect lasted about 25 min. Anodal DCs induced instead a significant decrease (about −25%) of the reflex amplitude. A significant after-effect was observed for just about 5 min.
Discussion: This study shows that DCs applied to a peripheral nerve is able to elicit neuromodulation. Its polarity dependence suggests a local change in the excitability of nerve fibres rather than a central modulation of the spinal reflex circuit. Moreover it is worth to note that the polarity dependence was opposite to what found for tDCS.

PO.161

The mouse isolated detrusor strip preparation as a useful model for exploring the paralyzing potency of botulinum neurotoxins at the urological level

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Introduction: Botulinum Neurotoxin type A (BoNT/A) has been clinically used for 15 years to treat several lower urinary tract (LUT) conditions as a second

line treatment when muscarinic therapy fails. While BoNT/A in these conditions likely affects multiple pathways and release of numerous neurotransmitters, like acetylcholine (Ach), adenosine-tri-phosphate (ATP), adrenaline, or substance P, efficacy depends in part on inhibition of presynaptic acetylcholine release from the parasympathetic system, producing a paralysis of the detrusor smooth muscle. Interestingly, only few preclinical models are available to assess the potency of recombinant BoNTs at the LUT level.

Materials and methods: Detrusor strips were prepared from mice bladders in organ baths and contractions were induced using electrical field stimulation (EFS). After assessing the effect of muscarinic and purinergic antagonists on this signal, we next compared the paralysis induced by BoNT/A and BoNT/B in this model in a concentration-dependent manner.

Results: Pharmacological characterization of the established model showed that neurogenic contractions were mainly driven by Ach and ATP, confirming data in the literature. While both botulinum toxin serotypes inhibited the neurogenic contractions in the mouse bladder detrusor smooth muscle, BoNT/B was significantly more potent than BoNT/A.

Discussion: This model could be a useful preclinical tool to explore the pathophysiology of bladder overactivity. It is interesting to note that the higher proportion of purinergic transmission in detrusor contractions in the described rodent model is a scenario seen in situations of neurodetrusor overactivity. Moreover, our model was able to discriminate between two different serotypes of neurotoxins.

PO.162

Molecular evaluation of obesity-related hypothalamic NPY and AgRP gene expressions in melatonin injected and pinealectomized Syrian hamsters (*Mesocricetus auratus*) housed in short and long photoperiod

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Hypothalamic region plays key role in the regulation of energy metabolism. Examining the role of hypothalamic neuropeptides and the relationships in the work of these physiological parameters/results in these basic studies are unsufficient. Because of a problem occuring during the provision of this communication, fat storage and obesity occur. Although such enviromental and hormonal interactional effects have been described, on the hand, in the literature the effects of enviromental factors and hormones on nutrition and body weight and their interactions with AgRP and NPY as peptidergic regulators is not yet fully clear. The effects of melatonin and pinealectomy in adult hamsters was examined on NPY and AgRP gene expressions depending on the photoperiod changes. Experiment was divided into two main groups as 16L and 8L photoperiods and each group had control, pinealectomy, melatonin and pinealectomy + melatonin sub-groups. Daily food consumption, body weight, serum leptin levels were examined. Animals at night and in the daytime periods were decapitated and tissues from the hypothalamus were collected for NPY and AgRP gene analyses. This study showed that melatonin increased the expression of NPY/AgRP genes but decreased leptin hormone levels. In the pinealectomised group we observed an increase in leptin hormone but a decrease in AgRP gene expression during the night period. On the other hand 16L/8L photoperiod hamsters exposed to pinealectomy, melatonin and pinealectomy + melatonin had a decrease in body weights. However, daily food consumption decreased only in 8L photoperiod. This study revealed that photoperiod and melatonin have extremely effective regulations on gene expressions.

PO.163

NMDA-dependent potassium accumulation in the synaptic cleft promotes glutamate spillover

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Potassium accumulates in the synaptic cleft during synaptic transmission, depolarizes presynaptic terminal and increases glutamate release probability (Shih et al., 2013). Here we investigated if such potassium accumulation can also affect electrogenic glutamate uptake in astrocytes. Glutamate transporter currents were recorded in CA1 *str. radiatum* passive astrocytes of hippocampal slices from C57BL/6J mice (P28-P35) in response to local glutamate uncaging or electrical stimulation of Schaffer collaterals. Increases in the extracellular potassium concentration from 2.5 mM (control) to 7.5 or 20 mM depolarized the astrocytes and significantly reduced uncaging induced transporter currents. Equivalent depolarization of the cell through patch pipette reduced the transporter current to the same extent, suggesting voltage dependent mechanism of potassium action. Repetitive synaptic stimulation (5 stimuli at 50 Hz) induced progressive increase in the decay time of the transporter currents, which was abolished by D-APV, NMDA receptor antagonist. This is consistent with pervious finding that NMDA receptors is a major source of potassium ions in the synaptic cleft. A detailed biophysical model also complemented experimental observations. Thus, NMDA receptor dependent accumulation of potassium during

repetitive synaptic activity can inhibit local glutamate uptake, potentially extending glutamate well-time in the synaptic cleft and boosting glutamate spillover effects.

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PO.164

Eye movements and asymmetric visuospatial perception

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Introduction: This research aims to investigate the reason of asymmetric visuospatial perception in healthy humans, the so-called 'pseudoneglect' phenomenon. The asymmetric visual perception could cause by asymmetrical distribution of spatial attention or representational asymmetry in the brain. We examined the distribution of spatial attention using a detection method for the eye's scanning time and area of the left-right half of lines in Landmark task.
Materials and methods: Twenty-one healthy participants performed a forced-choice judgment about the location of a transaction mark in relation to the veridical center of pretransected horizontal lines in the Landmark task. The same stimuli were presented in three different presentational conditons; left – right hemispatial and midsagittal. During the experiment, the subjects' data of eye movements were computed as two scores for the left/right half of correctly pretransected lines: mean scanning area and mean scanning time.
Results: The participants exhibited significant right judgment biases in the left hemispatial and midsagittal presentational conditions ($P < 0.001$). In the left hemispatial condition, mean scanning area of the left half of lines was found to be greater than the right half ($P < 0.05$, $P < 0.001$ respectively). In the right hemispatial condition, mean scanning area and mean scanning time scores of the right half of lines was found to be higher than the left half ($P < 0.001$ for both).
Discussion: The results indicate that pseudoneglect in the left hemispatial condition is related to asymmetric distribution of attention in space, while in the right hemispace both asymmetric attention and asymmetric representation have an effect.

PO.165

The effect of time-dependent social isolation rearing on nitric oxide generation in the brain

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Introduction: Recent findings indicate that brain nitric oxide (NO) and redox balance are involved in mental disorders. Nuclear factor kappaB (NFκB) is important factor activated during oxidative stress which regulates expression of NO-synthase (NOS). The aim of our study was to compare time-dependent effect of social isolation on behavioral and biochemical parameters in WKY rats.
Material and methods: At postnatal day 21 male WKY rats were randomly divided into two groups, rats reared singly (isolation reared, IR) and rats reared 3 per cage (socially reared, SR). The animals were sacrificed after 10 (13-weeks-old) or 29 (32-weeks-old) weeks of social isolation. The activity of NOS, expressions of neuronal NOS (nNOS), inducible NOS (iNOS) and NFκB proteins were determined in the brain. The behavioral tests, open-field test and startle reflex reactivity were also performed.
Results: NOS activity was significantly decreased in IR rats isolated for 29 weeks, but after 10 weeks of isolation there were no changes in NOS activity. The protein expression of nNOS and iNOS was decreased in both groups of IR rats. Additionally, the protein expression of NFκB was significantly increased in both groups of IR rats compared with SR rats. The changes in behavioral tests were not observed.
Discussion: Our results indicate that social isolation decreased protein expression of NOS isoforms. Prolongation of social isolation rearing showed also changes in NOS activity. Increased level of ROS and decreased NO production in the brain may play a role in the pathophysiology of mental disorders.

PO.166

CX45 and CX36 gap junctions are differently regulated by pH_i and volatile anesthetics

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Gap junction (GJ) channels formed of connexin (Cx) proteins provide a direct pathway for electrical and metabolic cell-to-cell communication. GJ channels are highly sensitive to intracellular pH (pH_i), which can vary substantially under ischemia. It is generally assumed that GJ conductance (g_j) can be reduced by acidification of intracellular milieu and GJ uncoupling agents, such as isoflurane, a volatile anesthetic, or hexanol, while it increases in alkaline conditions. However, the extent of modulation may depend on the Cx type. In this study, we focused on two neuronal Cxs, Cx36 and Cx45, exogenously expressed in HeLa cells.

Double whole-cell patch-clamp and fluorescence microscopy were used to measure g_j and pH_i. We demonstrate here that in contrast to Cx45, g_j of Cx36 GJ channels is almost insensitive to acidification and is strongly reduced by alkalization. Moreover, in contrast to other Cxs, g_j of Cx36 GJs was strongly stimulated by isoflurane and hexanol. Also, the sensitivity of Cx45 and Cx36 GJs to these uncoupling agents was dependent and independent on pH_i, respectively.

Protonation level of histidines and cysteines may vary under physiological conditions (pK_a is ~6 and 8, respectively). Therefore we hypothesized that under acidic conditions, g_j of Cx45 GJs decreased due to protonation of histidines, and under alkaline conditions, g_j of Cx36 GJs decreased due to deprotonation of cysteines. We demonstrate by site directed mutagenesis that replacement of histidines in the intracellular loop of Cx45 reduced its sensitivity to acidification, and that C264S mutation of Cx36 reversed its response to uncoupling agents.

PO.167

Olfactory deficits in individuals with autism spectrum disorder

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Introduction: Olfactory abnormalities has been reported in subjects with Autism Spectrum Disorders (ASD), a neurodevelopmental disorder characterized by social deficits. However, previous studies considered mainly olfactory threshold or other dimensions of olfactory function, usually in isolation or at least in a combination with olfactory threshold. Aim of this study is to examine odor identification and discrimination in ASD compared to typically developing (TD) controls.

Materials and methods: Olfactory identification and discrimination were studied, with the 'Sniffin' Sticks' test, in 30 participants: 15 with ASD (2 female and 13 male, mean age 19.13 years) and 15 controls (3 female and 13 male, mean age 21.73). Participants with ASD showed IQ of 103.2 ± 18.5. TD controls were volunteer university students matched for chronological age with ASD participants.

Results: Receiver operating characteristic (ROC) analysis indicated a difference between participants with ASD and TD controls in odour identification [area under the curve (AUC) = 0.709, 95% confidence interval (CI): 0.515–0.859, $P = 0.028$] and in odour discrimination (AUC=0.782, 95% CI: 0.594–0.911, $P = 0.002$).

Discussion: According to previous studies, our results confirmed an impairment in odour identification and discrimination in subjects with ASD, which suggests deficits in orbitofrontal cortex, in medial temporal lobe and in cerebello-thalamo-cortical circuits. Our findings support the importance of olfactory evaluations as a non-invasive tool to identify cortical dysfunction in ASD.

PO.168

Neuroprotective effect of $MgSO_4$ in a mouse model of neonatal cerebral lesion: comparison between male and female mice in short- and long-term studies

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Introduction: Preterm birth is the leading cause of cerebral palsy, a long-term acquired motor disability, often associated with cognitive deficits. We studied $MgSO_4$ as a protective strategy in a mouse model of neonatal cerebral lesion mimicking these neurological alterations. Studies were conducted in male and female mice to investigate the sex-dependency of MgSO4 effects.
Methods: Cortical lesion was performed in 5 days-old pups (P5) by injection of ibotenic acid. $MgSO_4$ was given intraperitoneally before the lesion. Sensorimotor tests were performed in young mice (P6–P10), and motor and memory tests in adult mice (P30-P40). Glutamate and GABA levels were measured by HPLC in P10 mice, and VGLUTs densities were quantified by immunoautoradiography in adults.
Results: Ibotenic acid injection induced sensorimotor impairments within 5-day post-injection in males and females. Glutamate level was increased in the prefrontal cortex of lesioned mice, together with GABA level in males. This was prevented by $MgSO_4$ pre-treatment. When adults, neonatally lesioned male mice showed motor impairments and both males and females displayed cognitive disabilities, prevented by $MgSO_4$ pre-treatment. VGLUT-1 level was increased in the perirhinal cortex of lesioned male mice with a prevention by $MgSO_4$. A correlation was found between VGLUT-1 level and behavioural scores in the novel object recognition test.
Discussion: $MgSO_4$ exerted a protective effect regarding sensorimotor alterations in young lesioned mice of both sexes, and sex-dependent behavioural alterations in adults. No proper effects of $MgSO_4$ were observed. $MgSO_4$ also prevented the increase in a presynaptic glutamatergic marker in lesioned males perirhinal cortex.

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Opposite effects of hypertension and physical exercise on cerebrovascular BDNF levels

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Introduction: Arguments exist for a control of vascular tone by BDNF through an increase of endothelial nitric oxide (NO) production. The present study explores the possibility that endothelial NO may in turn controls endothelial BDNF synthesis.
Material and methods: Physical exercise and hypertension were used as experimental approaches to modulate cerebrovascular NO production. BDNF protein and mRNA, endothelial NOS phosphorylated at serine 1177 (P-eNOS as an index of NO production) were measured in cerebral microvessels fractions that were prepared from the forebrain of four groups of rats ($n = 7$ each): SHR (spontaneously hypertensive rats), WKY rats (normotensive controls), EX (Wistar rats subjected to a daily (30 min) walking (18 m/min) activity on a treadmill for seven consecutive days, SED (sedentary Wistar control). BDNF protein levels were also measured in vascular fractions isolated from Wistar rats and incubated for 24 h with the NO donor glycerol trinitrate (10 μM).
Results: Cerebrovascular BDNF and P-eNOS were significantly lower in SHR than WKY and higher in EX than SED with a strong positive correlation between the two parameters ($r = 0.707$, $P < 0.001$). While BDNF mRNA was increased by exercise, BDNF gene expression was not modified by hypertension. *In vitro* exposure of cerebrovascular fractions to the NO donor significantly enhanced BDNF production.
Conclusion: These data revealed that cerebrovascular BDNF production is increased by physical training through activation of transcriptional mechanisms and decreased by hypertension through posttranscriptional mechanisms. In addition, our results provide arguments for NO as the link between endothelial function and BDNF levels in the cerebral endothelium.

PO.170

Contribution of video-electroencephalography in the study of non-epileptic psychogenic seizures

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Introduction: The non-epileptic psychogenic seizures (NEPS) are repetitive paroxysmal events related unconscious psychogenic process and not with excessive neuronal firing. They pose a differential diagnosis with authentic seizures.

The objective of this work was to study the clinical manifestations of NEPS in Senegal, using video-electroencephalography (video-EEG).

Patients and methods: A cross-sectional study was conducted in the department of functional studies of the nervous system of the Neurological Clinic of the University Hospital of Fann, over 19 months. The free and informed consent of participants was required.

Results: We recorded 22 cases of NEPS. The age group 16–20 years was the most affected, with a sex ratio of 4.5 for women. Epilepsy-NEPS association is encountered in 9.1% of patients and 10% with pure NEPS were wrongly put under antiepileptic treatment. Only 4.5% of our cohort were known as having psychogenic seizures and were aware.

Tonic manifestations were the most frequent, followed by automation, hypermotrices events, clonic, atony and tremors. The associated signs are dominated by closed eyes (86.4%). 95.5% of patients were unconscious during most times of crisis, with a postictal amnesia.

Conclusion: The severity of CPNE in Senegal can be considered average. It is important that the diagnosis is made to prevent excess antiepileptic treatment.

PO.171

Investigation of long-term depression in young and old hyperthyroid rats

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Purpose: The relationship between age-dependent cognitive decline and thyroid hormones, long-term synaptic depression is aimed to investigate in young and old euthyroid and hyperthyroid rats.

Methods: Young rats ranging in age from 60 to 90 days old and old rats ranging in age from 270 to 300 days old, were used. Thyroxine (0.2 mg/kg,) was administered for 20 days to young rats, and to old rats. LTD was induced by application of low frequency stimulation protocols at the perforant pathway- dentate gyrus synapses. The averages of the excitatory postsynaptic potential (EPSP) slopes and population spike (PS) amplitudes after LFS was used as a measure of the LTD magnitude.

Results: Serum T4 levels were higher than age matched controls after 3 weeks of treatment. The presence of a significant age effect ($P < 0.01$) showed that higher stimulus intensities are required to induce the same field potential in older rats compared to young rats. PS-LTD and EPSP-LTD revealed a significant age effect ($Fs_{1,34} = 10.63$ and 4.76, $Ps < 0.036$), a significant interaction between age and thyroid hormone state ($Fs_{1,34} = 7.08$ and 6.15, $Ps = 0.012$ and 0.017), but no significant thyroid state effect ($Ps > 0.27$). The magnitude of PS at last 5 min of recording was significantly lower in young hyperthyroid rats (74 ± 20% of baseline) than that of old hyperthyroid rats (240 ± 27%).

Conclusion: These results suggest that hyperthyroidism may decrease in the ability to make new memories in aging people; however, the precise underlying mechanism(s) remains to be elucidated.

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Effects of day-time and night-time food restriction on hypothalamic genes in adult mongolian gerbils (*Meriones unguiculatus*) housed in long and short photoperiods

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Introduction: Many temperate-zone animals use changes in photoperiod to time breeding. Shorter term cues, like food availability, are interested with photoperiod to adjust reproductive timing. We examined the effects of day or night food restriction on daily food intake, body weight, leptin, hormone values that were observed.

Material-method: Feeding in male and female gerbils was restricted to day time (DT) or night time (NT) for 30 days. Body weight and the amount of food intake were measured daily. Control animals had free access at all times. At the end of the experiment, animals were dissected and bloods were taken for leptin measurements in ELISA. Brains were removed and hypothalamic cuts were made and stored at −80°C for hypothalamic gene expressions (for further analyses of NPY and AgRP).

Results: In long photoperiod DT-restricted animals exhibited decreased NPY gene expression in dark phase. In short photoperiod NT-restricted animals exhibited decreased AgRP gene expression in dark phase. The leptin levels in ad lib-feeding and NT-

feeding male and females were similar. The leptin levels in DT restricted animal were higher in dark phase but lower in light phase. Also leptin level similar in DT restricted group compared with *ad libitum* group.

Conclusion: These results show that adult male and female gerbils are more sensitive to NT and DT food restriction and multiple potential environmental cues can be utilized to affect metabolic and hormonal status in adult Mongolian gerbil.

PO.173

Cortical voice processing in cochlear-implanted children

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Introduction: In those with prelingual deafness, the use of cochlear implants can restore both auditory input to the auditory cortex and the ability to acquire spoken language. Language development is strongly intertwined with voice perception. The aim of this electrophysiological study was to investigate human voice processing with cortical auditory evoked potentials (AEPs) in cochlear-implanted (CI) children.

Patients and method: Eight CI children, aged 4–12 years, with good auditory and language performance, were investigated with cortical AEPs and compared with 8 normal-hearing age-matched controls. The auditory stimuli were non-speech vocal sounds (laughing, sighing, coughing) and non-vocal sounds from the human environment (such as telephones, alarms, cars, bells) and nature (such as streams and wind). Independent component analysis was used to minimize the cochlear implant artifact in cortical AEPs.

Results: Fronto-temporal positivity to voice was found in normal-hearing children with a significant effect in the 140–240 ms latency range. In the CI children group, we found a positive response to voice in the 170–250 ms latency range with a more diffuse and anterior distribution than in the normal-hearing children.

Conclusion: Response to voice was recorded in CI children. The topography and latency of response to voice differed from that recorded in normal-hearing children. This finding argued for cortical voice processing reorganization in congenitally deaf children fitted with a cochlear implant.

PO.174

Precision of a pointing movement performed with either the dominant or non-dominant hand is linked to the timing of anticipatory postural adjustments

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Introduction: It is a common experience to feel motor awkwardness when performing a pointing movement with the non-preferred limb, which is known to be associated to less precise movements. Here we provide evidence that this last behaviour partly stems from changes in the temporal organization of the Anticipatory Postural Adjustments (APAs) in the non-preferred side.

Materials and methods: We investigated the effect of lateralization on APAs in Biceps Brachii, Triceps Brachii and Anterior Deltoid, which stabilize the arm when performing a pen-pointing movement (prime mover Flexor Carpi Radialis). Moreover, we analysed the elbow and wrist kinematics as well as the precision of the pointing movement.

Results: The mean kinematics of wrist movement and its latency, with respect to prime mover recruitment, were similar in the two sides, while APAs in Triceps Brachii, Biceps Brachii and Anterior Deltoid were less anticipated when movements were performed with the non-dominant (20–30 ms) versus dominant hand (60–70 ms). APAs in the non-dominant limb were associated with an altered fixation of the elbow, which showed a higher excursion, and with a more scattered pointing error (non-dominant: 16.3 ± 1.7 mm versus dominant: 10.1 ± 0.8 mm).

Discussion: By securing the dynamics of the more proximal joints, an appropriate timing of the intralimb APAs seems necessary for refining the voluntary movement precision. The linkage between APAs, elbow fixation and movement accuracy also agrees with the recent suggestion that APAs and prime mover recruitment are driven by a shared motor command, which strives to obtain an accurate pointing.

PO.175

Olfactory screening test in 115 Sardinian subjects

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Introduction: Olfactory function plays important role in human life and a decrease in olfaction induces daily life problems especially in food intake. The main components of olfactory function are odor threshold (OT), odor identification (OI) and odor discrimination (OD). The most important causes of olfactory deficits are infections of upper respiratory tract, sinonasal diseases, head trauma and allergic rhinitis. Aim of this study is to analyze olfactory performance, assessed by 'Sniffin' Sticks' test, in Sardinian subjects.
Materials and methods: Olfactory function was performed by the 'Sniffin' Sticks' test for OT, OI and OD in 115 participants (85 females and 30 males, mean age was 31.31 ± 10.62).
Results: The participants were assigned into three groups: normosmics, hyposmics and hyperosmics. Our results indicate that 88 subjects are normosmics, 20 hyposmics and 7 hyperosmics. Normosmics mean values were 8.52 ± 1.94, 13.39 ± 1.41 and 12.50 ± 1.74 for OT, OI and OD, respectively. Hyposmics mean values were 5.97 ± 1.37, 11.05 ± 1.43 and 9.54 ± 2.09, while hyperosmics mean values were 15.98 ± 0.04, 13.57 ± 1.61 and 13.57 ± 1.40 for OT, OI and OD, respectively. Mean values of threshold, identification and discrimination (TDI) score, which is the sum of these three test, was significantly different in hyposmics and hyperosmics respect to normosmics ($P < 0.0001$ and $P < 0.001$, respectively).
Discussion: Our results show the presence of normosmic, hyposmic and hyperosmic subjects in a selected sample of Sardinian people, similarly to data obtained in other countries.

PO.176

Glial aquaporin-4 expression in the mouse supraoptic and paraventricular nuclei and the median eminence in control and salt-loaded conditions

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To date, three water channel aquaporins (AQP) subtypes have been described in rodent brain, AQP1, AQP4 and AQP9. Among these AQPs, AQP4 was the most predominant in the brain and is mainly expressed in cerebral areas involved in central osmosensation and systemic osmoregulation. The present study was performed in order to examine the AQP4 distribution in supra-optic (SON) and paraventricular nuclei (PVN) and median eminence (ME) of hypothalamus in control and salt-loaded adult mice. Brain cryostat sections were treated by fluorescent double immunohistochemistry using a rabbit antibody against AQP4 water channel and an anti-arginine-vasopressin, anti-oxytocin and anti-glial fibrilary acidic protein antibodies as cell-markers. Our results showed that AQP4 water channels are expressed in glial processes surrounding both magnocellular neurons and vascular cells. Moreover, AQP4-immunofluorescence staining is highly expressed in salt-loaded mice hypothalamus compared to control mice. In accordance to previous study, heavy expression was found in highly vascularized areas and areas known to be involved in osmosensation. Taken together, these results suggest that AQP4 water channel could be involved in systemic osmoregulation and intrinsic osmosensitivity of the hypothalamus. The distinctive expression pattern and intensity of AQP4 staining suggest that this water channels allow variations of plasma osmotic pressure to be transferred from blood to neurons).

PO.177

Electrophysiological parameters of the learning process in subjects with chronic schizophrenia

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The purpose of the study was to evaluate the learning process that occurred during the electroexpectogram (EXG) paradigm in 30 chronic schizophrenia patients and 30 healthy subjects, aged 25–40 years. The EXG paradigm is an electrophysiological method, a modified and an expanded auditory CNV paradigm. Based on a biofeedback design, the occurrence of S2 tone in the paradigm depends on the amplitude of the CNV potential recorded from Cz. If CNV reaches a predefined threshold level, S2 tone turns off, which causes an extinction of the CNV potential after several consecutive trials. The computer recognizes this change and the S2 tone turns on again causing a consecutive increment in the CNV amplitude. As a result, an electrophysiological oscillatory process occurs in the subject's mind, and is graphically presented by a curve named electroexpectogram. The dynamics of expectancy and attention, represented by CNV amplitude, as parameters of associative learning taking place during the S1-S2-MR sequence of the EXG paradigm, can be observed in this manner. We performed one experiment with the EXG paradigm in which the threshold value of the CNV amplitude was 10 mV. Successful learning of biofeedback was accompanied by reduced oscillations of CNV amplitude, shortened duration of the oscillatory EXG cycles and faster motor reaction in healthy subjects, while this effect was diminished in patients with chronic schizophrenia. The results of the study suggest that patients with chronic schizophrenia show both disturbance in adjustment of their attention and a poor learning process during demanding cognitive tasks.

PO.178

The effects of emphaty level on electrodermal activity in medicine students

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Electrodermal activity (EDA) associated positively with emotional state and also known as a good indicator of sympathetic activity. Its measures acquire in both social and cognitive psychological paradigms. Nowadays behavioral studies have been explained neural mechanism of empathy by using EDA. Empathy is defined as the ability to understand and share the emotions of other individuals and essential for human social interactions and adaptive functioning.

Study is carried out with 43 male/female University students. The physiological recording was measured with MP30 System and equipment was attached to subjects. The experiment stimulus is consist of visual images which negative situation like cancer or traffic accident and following by verbal images. Subjects were asked to say their subjective ratings following each photograph. Throughout the experiment, hearth rate, and tonic/phasic EDA is recorded.

Statistical analyses were carried out using SPSS (11.5). The comparison between the groups is done with repeated measures analysis of variance (ANOVA). For EDA, visual and verbal responses is higher than basal stage ($P = 0.016$, $P = 0.001$) and for hearth rate visual and verbal responses is higher than basal stage ($P = 0.001$, $P = 0.001$).

The study has demonstrated that visual and verbal images provide substantial stimulation to generate an autonomic response which is confirmed by EDA. The present study examined the effects of stimulus types due to the subject's responses. According to our results, verbal image stimuli is much more arousal than visual image stimuli. As expected subjective ratings of empathy and arousal differentiated than basal situation.

PO.179

Pharmacological manipulations of the sensori-motor striatum induce a phenotype of epilepsia in the monkey

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The role of the basal ganglia in the pathophysiology of epilepsy remains a matter of debate. Although a line of evidence obtained in rodent models of epilepsy suggest that they may play a part, at this point no empirical evidence in human has been documented. The anatomo-functional organization of the sensori-motor cortico-subcortical loops suggests that the striatum could relay the cortical activity related to seizures. However, this question remains to be documented.

We performed microinjections of a $GABA_A$ antagonist (Bicuculline) and a muscarinic agonist (Oxotremorin-M) within the sensorimotor part of the striatum in three non-human primates. Video sessions, EEG and EMG were systematically assessed at the same time.

We found that Bicuculline and Oxotremorin-M injections induced partial motor seizures in the three monkeys. Motor seizures involved the face, the upper and lower limbs contralateral to the site of injections. Secondary generalized seizures could be occasionally observed depending on the volume of drug injection. Clinical seizures were clearly correlated to spike-waves on EEG. Sharp-wave activity preceeded clinical symptoms. Bicuculline injections had a faster but shorter effect regarding Oxotremorin injections whereas the latter had a delayed but prolonged effect.

Our preliminary results suggest that pharmacological manipulation of the sensori-motor striatum can trigger motor seizure in non-human primates. We hypothesized that striatal inhibition of the GABAergic system or reinforcement of the cholinergic system may induce an overactivity of this structure which in turn desinhibits the thalamo-cortical pathways responsible of a cortical overactivity.

Nutritional physiology

PO.180

Zizyphin modulates sugar and fat taste perception: evidence from behavioural and calcium signaling studies

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Introduction: Modulation of sweet-taste by Zizyphus species (sps) is widely known. An anti-sweet molecule, Zizyphin, isolated and purified from *Zizyphus* sps leaf extracts, has been shown to be responsible for its taste modulating properties. Recently, it has been convincingly shown that there exists a sixth Fat-taste modality in rodents and human. Keeping in view the earlier studies, it was thought worthwhile to explore for the first time, the sugar and fat taste modifying properties of Zizyphin through a series of behavioural and calcium signaling experiments.
Materials and methods: Previously reported procedures for the extraction and purification of Zizypihin were used. This was followed by a series of behavioral studies on rodents, mainly a two bottle preference test. Increases in free intracellular calcium concentrations $[Ca^{2+}]i$ were recorded in human fungiform cells by using Fura-2/AM probe.
Results: Behavioral studies showed that Zizyphin, isolated from *Zizyphus lotus*, was able to modulate preference for sweet and fat solutions. We also observed that Zizyphin triggered increases in $[Ca^{2+}]i$ taste bud cells. Besides, this agent seems to cross-talk with fat taste (CD36) and sweet (T1R2) receptors.
Discussion: This study is the first evidence of the modulation of sweet and fat taste modalities by Zizyphin at the cellular level in human taste bud cells. This study opens the door for the synthesis of Zizyphin analogues as taste modifiers with a potential for the treatment and prevention of obesity and related disorders.

PO.181

Cortical taste activity in response to sucrose and sweeteners solutions: a study using gustatory evoked potentials

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Introduction: Sweeteners are widespread in overweight or diabetic patients because of their high sweetening power and their light effect on glycaemia. Sweeteners effects on cortical activity, reflecting both cortical analysis and gustatory signalization pathway, are not well known. We observed previously that gustatory evoked potentials (GEPs) in response to sucrose varied in latency and/or in amplitude with sucrose concentration of the solution, the pleasantness of taste and the feeding status. The aim was to compare the gustatory cortical activity by recording GEPs in response to sucrose, aspartame and stevia solutions.
Methods: Twenty healthy non-smoker adult volunteers were included (mean age 22 ± 2.47 years). GEPs were obtained after stimulation of taste receptors with sucrose and sweeteners solutions. Three randomly assigned sessions (during 3 different days) were performed for each subject: one with sucrose (10 g/100 mL of Evian water), one with aspartame (0.05 g/100 mL) and one with stevia (0.033 g/100 mL). Concentrations have been chosen in order to induce the same sweetening power for each molecule.
Results: GEPs latency from primary gustatory cortex (Cz electrode) for sucrose, aspartame and stevia stimuli were respectively 149 ± 37 ms, 178 ± 42 ms and 203 ± 49 ms, showing a significant shorter GEPs latency in response to the sucrose solution ($P < 0.05$). The GEPs amplitude on Cz did not differ between the three stimuli.
Discussion: The differences observed in GEPs latency between sucrose and sweeteners stimuli could be explained by different gustatory receptors or neurological pathways which may be activated by these substances.

PO.182

Long-term (n-3) pufa supplementation improves lung histology of CftrΔF508 mice

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Introduction: Pro-inflammatory status of cystic fibrosis (CF) patients promotes pulmonary colonization with opportunist and pathogenic bacteria which is favored by a sticky mucus. Oral supplementation with (n-3) long chain polyunsaturated fatty acids (PUFAs) might correct this pro-inflammatory phenotype. The aim of this study was to demonstrate positive effects of a long-term diet enriched in (n-3) PUFAs on the lung histology of CftrΔF508 mice.
Materials and methods: Breeding CftrΔF508/+ mice received a control diet or a diet enriched in (n-3) PUFAs during 5 weeks before mating, gestation and lactation. After weaning, offsprings kept the same diet than their mother, until post-natal day 60. Effects of (n-3) PUFA supplementation on lung damages and inflammatory status were evaluated in homozygous CftrΔF508 mice and their wild-type littermates after acute lung inflammation induced by lipopolysaccharide from PAO1 (LPS, PBS as control) inhalation.
Results: While male and female CftrΔF508 mice exhibit growth delay and lung damages with collapsed alveoli, hyperplasia of bronchial epithelial cells and inflammatory cell infiltration, (n-3) PUFAs correct growth delay of male CftrΔF508 mice and decrease hyperplasia of bronchial epithelial cells for both males and females. Besides decreasing metaplasia of Club cells after LPS inhalation, (n-3) PUFAs prevent the overproduction of mucous plugs, promote the recruitment of inflammatory cells and increase pro-inflammatory cytokine production after LPS challenge.
Discussion: Long-term (n-3) PUFA supplementation shows benefits on body mass and bronchial abnomalities of CftrΔF508 mice and improves inflammatory response in lung after LPS-induced lung inflammation.

PO.183

The effects of HMG-COA reductase inhibition on trace elements in early-phase sepsis in rats

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Introduction: Trace elements are essential for direct antioxidant activity as well as function as cofactors for a variety of antioxidant enzymes in tissue during systemic inflammation. In this study we aimed to investiagte the effect of simvastatin on the level of

trace elements in liver tissue in Wistar albino rats with LPS-induced sepsis.
Material and methods: Adult male rats were divided into four groups, control, LPS (20 mg/kg, i.p.), simvastatin (20 mg/kg, o.p.), and simvastatin + LPS. Four hours after LPS injection, rats were sacrified and liver tissue samples were taken to measure selenium, zinc, iron, manganese, magnesium, calcium, copper and potassium element levels and also investigate histologic changes. Element levels were measured using inductively coupled plasma optical emission spectroscopy. Results were evaluated using one way analysis of variance.
Results: In liver tissue, selenium, copper, and manganese elements levels decreased, also calcium increased in all experimental groups compared with levels in the controls ($P < 0.01$). During the light microscopic examination, hepatocyte cell membrane and sinusoid structure were seen to be damaged in the LPS group. In the simvastatin + LPS group, hepatocytes and sinusoidal cord structure in periportal areas were partially improved; in some areas, the structure was even close to the classic liver lobule.
Discussion: LPS and simvastatin caused significantly changes in the oxidant/antioxidant system. Also, simvastatin was shown to improve damage in the liver tissue of endotoxemic rats.

PO.184

Carob leaves polyphenols trigger intrinsic apoptosis pathway and induce cell cycle arrest in colon cancer cells

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Introduction: Colorectal cancer (CRC) is the third most common cause of cancer deaths and its chemoprevention is a major concern for improving public health. The present study was therefore designed to investigate the mechanisms involved in the pro-apoptotic effects of polyphenol-rich extract from *Ceratonia siliqua* L. leaves (CLP) on colon cancer cells.
Materials and methods: Human HCT-116 and murine CT-26 colon cancer cells were used for experiments. Cell viability was determined by Trypan blue assay. Cell cycle progression was evaluated with propidium iodide staining. Whereas, apoptosis assay was performed by 7-AAD and Annexin-V staining. Caspase 3/7 activity was measured by FLICA assay while caspase-9 and PARP cleavage was determined by western blot analysis.
Results: Our results demonstrated that CLP decreased in a dose-dependent manner the viability of HCT-116 and CT-26 with an IC50 around 20 μg/mL. We noticed that CLP induced cell cycle arrest in G2/M phase. Furthermore, western blotting assay showed that the pro-apoptotic effects of CLP were caspase-9 and PARP-dependent. Accordingly, caspase-3/7 were activated in response to CLP treatment.
Discussion/Conclusion: These data suggest that CLP exert anti-cancer activities on colon cancer cells via two pathways; 1. inducing cell cycle arrest in G2/M phase and 2. Triggering caspase-9 cleavage which forms with cytochrome *c* a multienzyme complex, Apaf 1, that activates caspases-3 leading to PARP cleavage and ultimately apoptosis death of CRC cells.

PO.185

Inhibitory effect of apple cider vinegar on digestive enzymes in normal and STZ-diabetic rats

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Introduction: Apple cider vinegar (*ACV*) is an ancient folk remedy and it is common for patients with diabetes because of its positive effect on blood glucose and insulin sensitivity. The purpose of the present study is to investigate the possible inhibitory effects of *ACV* on some carbohydrate metabolising enzymes in the intestine and the livers in diabetic rats.
Materials and methods: The animals were fasted overnight and diabetes mellitus were induced by an intraperitoneal injection of prepared streptozotocin (STZ). *ACV* was administrated orally during 4 weeks.
Results: The present findings indicated that *ACV* significantly decreased intestinal maltase, sucrase and lactase, and hepatic glucokinase (GK) activities with led to a significant decrease in blood glucose rate and an increase in hepatic glycogen levels. In addition to that, significant increase in hepatic phosphofructokinase (PFK) and glucose 6 phosphate dehydrogenase (G6PDH) was observed. Moreover, the treatment with *ACV* potentially inhibited key enzymes of lipid metabolism and absorption such as lipase activity in small intestine with led to a notable decrease in serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), and triglycerides (TG) rates and an increase in high density-lipoprotein-cholesterol (HDL-c) levels. *AVC* was also observed to protect the liver-kidney functions efficiently, with were evidenced by the

significant decrease in the serum aspartate and alanine transaminases (AST and ALT) activities and the level of total and direct bilirubin, creatinine and urea.
Discussion: The present findings showed that *AVC* significant improves glucose and lipid homeostasis in diabetes by delaying carbohydrate and lipid digestion and absorption.

PO.186

Screening of insulin resistance in subjects at risk for type 2 diabetes

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Introduction and purpose of the study: It is important to detect insulin resistance in a population at risk for type 2 diabetes, recruted from among the obese, women with gestational diabetes history and related first degree diabetics, may be candidates for primary prevention program.

In our prospective study, the HOMA-IR index (Homeostatic Model Assessment-Insulinresistance) was used.
Material and methods: Our work focused on 139 subjects aged between 35 and 45 years with at least one risk factor: overweight or obesity (BMI 26–32), gestational diabetes in the past 10 years and the presence of at less directly related to a diabetic. 112 subjects with no risk factors and other metabolic diseases were controls.

Blood glucose was assayed by enzymatic method and insulin method électroimmunoluminescence 'ECLIA'. Calculating the HOMA index was performed according to the formula: glucose (mM) × insulin (mU/L)/22.5. A value of HOMA >2.44 indicates insulin resistance.

The software Statistical Package of Social Sciences was used for statistical analysis of results.
Results: It was observed cross elevated HOMA-IR in 54% of subjects at risk (HOMA-IR = 2.66 ± 0.19 versus 2.16 ± 0.21 in controls).

A HOMA-IR index increased was found in a few witnesses or less than 1% of the workforce of this group.
Conclusion: Our study shows the usefulness of calculating the HOMA-IR index in screening for insulin resistance in subjects at risk, suggesting interest in the practical implementation of medical care dedicated to this category of applicants to type 2 diabetes.
Key words: HOMA-IR – Insulin resistance – type 2 diabetes.

PO.187

Tunisian grape seed extracts decrease LPS-induced inflammation in murine macrophages

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There is an increasing interest in natural products for their anti-inflammatory proprieties. Polyphenols are known to exert antioxidant and anti-inflammatory activities. The aim of this study was to investigate the anti-inflammatory effects of three Tunisian grape seed (Syrah, Merlot and Carignan) polyphenolic extracts on Macrophages stimulated by lipopolysaccharide (LPS).
Methods: RAW 264.7 macrophage cells were cultured and activated by lipopolysaccharide (LPS), and then treated with various concentrations of grape seed extracts. The expression and the secretion profiles of pro-inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and nitrite oxide (NO) were determinated by using enzyme-linked immunosorbent assay (ELISA), reverse transcriptase polymerase chain reaction (RT-PCR) and western blot analysis.
Results: Our results revealed that polyphenolic extracts from three seed varieties inhibited, in a dose dependent manner, NO production. Interestingly, TNF-α and IL-6 were decreased in LPS-activated macrophages, treated with polyphenolic extracts in ELISA. Moreover, we observed decreases in mRNA expression of TNF-α and IL-6 which were correlated with the down-regulation of the NF-kB signaling pathway.

The present data demonstrate that grape seed extracts are major anti-inflammatory agent and can be used in the treatment of inflammatory diseases.

PO.188

Effect of a high fat diet in Wistar rats and therapeutic approach

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Introduction: Anvilea radiata, Ammodaucus leucotricus and Berberis hispanica are plants used in traditional medicine. Indeed the root bark of the latter is

used in the treatment of various diseases: cancer, inflammation, diabetes.

Material and methods: For this study, 24 Wistar rats were divided into 5 groups: control, subject to hyper fatty diet (30% cooked sheep strainer) for 3 months and three batch submitted to high fat diet and treated daily by gavage with Anvillea radiata, Ammodaucus leucotricus and Berberis hispanica extracts (300 mg/kg body weight) for 2 weeks. We have evaluated some serum biochemical parameters (glycemia, triglyceridemia, cholesterolemia, HDL-c, LDL-c) and liver glycogen and total lipids.

We have also analyzed the electrophoretic profile of serum lipoproteins and measuring lipid peroxidation rate of liver, muscle and adipose visceral tissue by malondialdehyde (MDA).

Results: The animals subjected to high fat diet showed an exacerbation of lipids and lipid peroxidation in particular. The three treatment induced a significant reduction of blood lipid levels, LDL-c, liver total lipids. We have also unregistered a decrease of the MDA rates of liver, muscle and adipose tissue. The analyze of the electrophoretic profile of lipoproteins showed an improvement with an increase of HDL and a moderate decrease of LDL.

Discussion: The three plants have a positive effect on dyslipidemia and can help to improve lipids parameters. Furthermore, the decrease in blood glucose and tissular MDA have indicated the hypoglycaemic effect of these treatments and their involvment in the modulation of the oxidative stress.

PO.189

Effectiveness effects of three medicinal plants in diabetic pregnancy

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Background: Diabetes remains chronic disease which needs permanent high cost treatments with modern synthetic drugs not accessible for financially poor populations. Thus, populations in Africa and south Asia mostly rely on herbal concoctions for their primarily health care, but so far scientific studies supporting the use of plants in traditional medicine remain poor. The present study reveals evidence of anti-hyperglycemic effects of three medicinal plants commonly used in Africa in diabetic pregnancy: *Picralima nitida* (seeds), *Nauclea latifolia* (root and stem) and *Oxytenanthera abyssinica* (leaves).

Methods: Diabetic pregnant rats were treated with plant extracts selected by their antioxidant activities. Vitamin C concentrations, fatty acid compositions and phytochemical analysis of plants extracts were determined. Effects of selected plant extracts on human T cell proliferation were also analysed.

Results: Plant extracts exhibited substantial antioxidant activities probably related to their content in polyphenols. The highest antioxidant capacity was observed in *Picralima nitida*. Ethanolic and butanolic extracts of *Picralima nitida*, butanolic extract of *Nauclea latifolia* and ethanolic extract of *Oxytenanthera abyssinica* significantly decreased hyperglycemia in diabetic pregnant rats. Butanolic extract of *Picralima* also appeared to be the most potent immunosuppressor although all of the extracts exerted immunosuppressive actions on T cell proliferation probably due to their linolenic acid (C18:3n-3) and/or alkaloids content. Nevertheless, all plants seemed to be good sources of saturated and monounsaturated fatty acids.

Conclusion: Having antioxidant, anti-hyperglycemic and immunosuppressive activities, these plants could be good candidates in the treatment of diabetes and diabetic pregnancy.

PO.190

Dietary habits and vitamin D deficiency of school age children in rural of Morocco

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Introduction: Micronutrient and vitamin deficiencies, especially vitamin A and D, are a major public health problem in Morocco. Very few foods naturally contain vitamin D and foods that are fortified with vitamin D can't satisfy human vitamin D requirement.

Objective: The aim of this study is to determine vitamin D status and dietary habits of schoolchildren in a rural region of Morocco.

Methods: In an observational study, 191 school children aged 7–9 years selected from three primary schools. Weight, height, age and sex were recorded. Fasting blood samples were taken to assess vitamin D as serum [25(OH)D] concentration and a food frequency to determine food habits.

Results: Vitamin D deficiency was prevalent in schoolchildren; 65.8% of subjects had a 25 OHD <75 nM and a median equal to 73.1 nM (Q1 = 65.9 nM and Q3 = 89.6 nM) Dietary habits of deficient children showed that 21.6% consumed eggs, 3.4% fish, 5.2% yogurt and 0.9% cheese at less than one time/day.

Conclusion: This study showed that among the school children in Morocco, the prevalence of vitamin D deficiency is very higher and their diet is very poor

in foods rich in vitamin D who can't satisfy children's needs.

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Polyphenol-rich extract from solanum nigrum induced apoptosis and cell cycle arrest in U2OS human osteosarcoma cell line

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Introduction: Several anti-cancer drugs induce cell cycle arrest and/or cell death. Natural bioactive compounds, including polyphenols, have been shown to inhibit carcinogenesis and angiogenesis. The aim of the present study was to investigate the anticancer activity of polyphenol-rich extract from *Solanum nigrum* (SN) on U2OS human osteosarcoma cell line.
Material & methods: Cytotoxic effect of Polyphenol-rich extract from SN on U2OS human osteosarcoma cell line was determined using the ViaCount Assay (Guava Technologies). Cell cycle arrest was assessed by Propidium Iodide (PI) staining. Cell death was detected by measurement of apoptosis and necrosis (i.e. Annexine V/ 7AAD staining).
Results: Our results have shown that polyphenol-rich extract from SN dose dependently diminished U2OS proliferation. Flow-cytometric analysis showed that the polyphenol-rich extract arrested the cell cycle progression at G2/M phase and induced cell death by apoptosis.
Discussion: The anti-proliferative and apoptotic effects of polyphenol-rich extract from SN on U2OS human osteosarcoma cell line could be mediated via cell cycle arrest in G2/M phase. No anti-osteosarcoma effects of SN have been reported; nevertheless, it has been shown that extracts from SN induced apoptosis in AU565 breast cancer cells, at concentrations? 25 μg/mL (Huang et al., 2010). Also, Lee *et al*. (2004) have reported that SN inhibited 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion in HCT-116 human colon cancer cells. *In vivo* experiments showed that SN whole plant extract arrested transplanted uterine cervical carcinoma (U14) growth in G0/G1 phase.

PO.192

Impact of hyperhomocysteinemia on pancreas of sand rat, psammomys obesus

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Introduction: Hyperhomocysteinemia, defined by elevated plasma homocysteine (Hcy) level, is associated with many diseases affecting various organs. (heart, vessels, kidney, liver, etc.). The aim of the study was to investigate whether Hhcy has an effect on the pancreatic extracellular matrix of sand rats (*Psammomys obesus*). The sand rat, diurnal rodent which lives in the south east of the Algerian Sahara is an excellent animal model for research (atherosclerosis, diabetes, obesity, etc.).
Material and methods: The sand rats were divided into two groups: a control group received their natural halophile plant (Suaeda mollis, Chenopodiacae family) and a experimental group received the same diet and methionine at the rate of 150 mg/kg of body weight/day during 6 months. Blood samples obtained from retro-orbital plexus were used for the estimation for homocysteine by Fluorescence Polarization Immunoassay (Abbott AxSYM system). Histological, histochemical and histomorphometric changes was observed by light microscope.
Results: Methionine supplementation caused a hyperhomocysteinemia. An accumulation of fibrillars collagens and glycoproteins is observed in islets of Langerhans, intertitial tissu, around blood vessels and channels excretors showing a fibrosis. A phenotypic modulation of endothelial cells, a disorganization of the vascular wall and a steatosis micro- and macrovvesicular are also observed in the pancreas of hyperhomocysteinemic Psammomys obesus.
Conclusion: Hyperhomocysteinemia leads remodeling of the pancreatic extracellular matrix of sand rats.

PO.193

Effects of pesticides (Mancozeb) on *in vitro* proliferative function of human lymphocytes in the presence of vitamin C

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Pesticides are used in intensive agriculture worldwide. An important target organ of pesticide exposure is the immune system and they had significant immunomodulatory properties with oxidative stress induction at high concentrations. The aim of this study is to determinate the effects of vitamin C on lymphocyte function including proliferation and intracellular redox status in presence of Mancozebe (fungicide).

Materials and methods: Peripheral blood lymphocytes were isolated using differential centrifugation on a density gradient of Histopaque. They were cultured with mitogen concanavalin A, vitamin C (10 μM), and with the pesticide Mancozeb (50 μM). Proliferation (MTT assay), intracellular superoxide anion, hydroperoxides, carbonyl proteins, reduced glutathione and superoxide dismutase activities were determined.

Mancozeb induced a significant inhibition of Con A-stimulated human lymphocyte proliferation. The presence of vitamin C in the medium potentiated the stimulatory effects of con A upon lymphocyte proliferation. Vitamin C protected lymphocytes in the presence of pesticides. Pesticides induced an oxidative stress reflected by a reduction in glutathione content and an increase in superoxide anion, hydroperoxide and carbonyl protein contents in lymphocytes. Antioxidant enzyme activities were also altered by pesticides.

Vitamin C restored all these abnormalities. In fact, in the presence of vitamin C, glutathione was increased while superoxide anion, hydroperoxide and carbonyl protein contents were decreased in lymphocytes exposed to pesticides. Vitamin C also stimulated enzyme activities.

PO.194

In vitro effects of vitamins and polyphenols on hepatocyte function

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Introduction: Liver is an organ implicated in oxidative metabolism, and disturbances in its function can cause several diseases. The aim of our work was to determine *in vitro* effects of vitamins (A, C, E) and polyphenols (chlorogenic acid) on hepatocyte function (proliferation, LDH, redox markers) in the presence of a free radical generator (H_2O_2-$FeSO_4$).

Material and methods: Hepatocytes were isolated from adult rat livers. They were cultured in appropriate medium with vitamins (A, C and E, 50 μM), and with chlorogenic acid (10 μM) in the presence or absence of H_2O_2-$FeSO_4$ (100 μM) during 48H. Proliferation (MTT assay), LDH activity and intracellular redox parameters were determined.

Results: Our results showed that the free radicals generator induced a decrease in hepatocyte proliferation and an increase in LDH release with a high oxidative stress (increased MDA and carbonyl proteins and decreased GSH). In the presence of vitamins (A, C, E) and also chlorogenic acid, hepatocyte proliferation and redox status were restored.

Conclusion: In conclusion, vitamin and polyphenol supplementation could improve liver function especially in the presence of high oxidative stress.

PO.195

Oxidative stress induced by high fat diet on heart biochemical parameters of pre-pubertal female and male rabbits

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Introduction: We compare the impact of high-fat diet on oxidative stress in male and female rabbits.

Material and methods: Experiments were carried on 24 pre-pubertal rabbits, randomized into 4 groups ($n = 6$), male and female controls (MC, FC), and High Fat Diet groups (MHFD, FHFD). HFD (1 g peanut butter + 0.5 g fat) was administered during 3 months. At the end, heart weight, levels of TG, cholesterol, TBARS, uric acid (UA), Ascorbic acid (AA), antioxidant activity (AOA) were measured, in plasma and cardiac tissue.

Results: Cholesterolemia showed a small raise in both MHFD and FHFD versus controls. LDL-C increased among MHFD and FHFD versus Controls ($P < 0.0001$); HDL-C were similar between female and male. There was a small raise of TG among FHFD versus MHFD ($P < 0.05$). In the heart, total lipids raised for both MHFD and FHFD versus controls ($P < 0.05$).

HFD increased significantly plasmatic TBARS among MHFD and FHFD versus controls ($P < 0.001$, $P < 0.01$), while in heart, it increased TBARS only among MHFD versus MC ($P < 0.001$). Plasmatic levels of UA, AOA and AA decreased significantly among MHFD versus MC ($P < 0.001$) and

significantly among FHFD versus FC. In heart, UA decreased significantly most among MHFD versus MC ($P < 0.001$) than among FHFD versus FC ($P < 0.01$). Cardiac AA level was similar between female and male. A significant increase of heart weight was noted among FHFD versus MHFD.
Discussion: High Fat Diet is responsible for oxidative stress in plasma (Yalçin, 1989; Devi, 2014) and heart of pre-pubertal rabbits, with excessive effect on male.

PO.196

Anti-inflammatory effect of grape seed and skin extract (GSSE) in the pancreas and Min-6 cell line

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Introduction: Obesity is associated with high production of adipokines and inflammatory factors like TNFα, IL-6, IL-1β. In the present work, we studied the potential anti-inflammatory effect of grape seed and skin extracts (GSSE).
Materials and methods: We conducted our study on rats divided into four groups. First two groups of rats were fed a standard diet (SD), and the other two groups were maintained on a high fat diet (HFD). Rats fed the SD and HFD received intraperitoneal injections of either 10% ethanol or 4 g/kg of GSSE daily for 45 days. We also conducted a study on a pancreatic cell line, Min-6, which were incubated with different concentrations of GSSE. The pro-inflammatory cytokines measured by using real-time RT-qPCR.
Results: HFD fed rats progressively gained weight which was significantly higher than that in control animals. Treatment with GSSE inhibited HFD-induced weight gain. Moreover, GSSE downregulated the expression of TNFα, IL-1β and IL-6 mRNA in pancreas both in HFD and SD fed animals, and also in Min-6 cells.
Discussion: Our results suggest the beneficial anti-inflammatory effects of GSSE on the pancreas and Min-6 cells. These observations suggest that GSSE might be used in the treatment of pathologies like obesity, associated with mild and sustained inflammation.

PO.197

Effect of Stevia rebaudiana bertoni on glyceamia of psammomys obesus forced fed hypercaloric diet

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Introduction: Sweetening properties found in the Stevia rebaudiana bertoni can have an impact on nutritional problems caused by too much sugar consumption worldwide. The purpose of this work is to check its effect on body weight gain and glyceamia.
Materials and methods: The study involved 20 males of *Psammomys obesus* equally divided into 4 groups for a period of 4 months. Group 1: control receiving natural diet (Chenopodiaceae), group 2: Chenopodiaceae + 4 mg/kg/bw of Stevia simultaneously (preventive), group 3: standard diet and 4 mg/kg/bw of Stevia successively (curative) and group 4: standard diet (diabetics).
Results: Stevia is rich in flavonoids (39.74 ± 0.35 mg EQ/g) and polyphenols (51.83 ± 0.99 mg EAG/g). Biochemical assays, after 4 months of testing, indicate normal blood sugar levels for the control group (1.33 ± 0.29 g/L) compared to preventive (1.32 ± 0.15 g/L), curative (1.27 ± 0.04 g/L) and diabetic ones (2.35 ± 0.10 g/L). The same events were observed for triglycerides. Histological sections of the liver clearly show the beneficial and curative effects on liver cells proved by the absence of hepatosteatosis (NASH).

Furthermore, we noted a 41 g weight gain in diabetic lot and only 9 and 15 g only for rats of curative and preventive batches successively.
Conclusion: Stevia, a natural sweetener, can be an alternative to the consumption of sugar for the obese and diabetic.
Keywords: Stevia – glycemia – psammomys obesus – antioxidant

PO.198

Effect of Stevia rebaudiana bertoni on glyceamia of psammomys obesus forced fed hypercaloric diet

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Furthermore, we noted a 41 g weight gain in diabetic lot and only 9 and 15 g only for rats of curative and preventive batches successively.

Conclusion: Stevia, a natural sweetener, can be an alternative to the consumption of sugar for the obese and diabetic.

PO.199

Dietetic and tyrosinemia hepato-renal

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Methabolism hereditary diseases (MHD) comes usually from enzyma deficit of one of numerous methabolic pathways, dyrived carbohydrates, proteins, fats or intercellular traffic. Amongst protein poisonning deseases, we'll focuss on Tyrosinemia type1 (TH1) known as hepatorenal.

Objective: Evaluation of nutritional state of children with type 1 tyrosinemia.

Patients and methods: Twenty patients, children (both sex), NTBC treatment, supplemented or not by a 'protein mixture'. Children have to undertake hypoprotidic diet. Their antropometric state is defined by weight and height. Effected biochimical examinations are tyrosine and succinylacetone dosage. Food research concerned patients type of diet, followed by the way they use their protein. Nature of parrental marriage as well as socioeconomical situation are also studied.

1) Tyrosine dosage: Protein mixture consumption: important drop of tyrosine rate within patients consuming mixture treatment at the opposit of the ones who do not consume it, ($P < 0.001$). The rate moves from 204 to 18 mg/L for patients who regularly take their diet, otherwise no fluctuations. Drop of rate ($P < 0.001$), before and after the diet. Progressive drop 141 mg/L: emergency diet, 70 mg/L: semi-emergency and 20 mg/L: cruising speed.
2) Succinylacetone rate before and after the intake of NTBC drops ($P < 0.001$). Treatment regularly followed: stabilization at 0 μM, for thr authers, perturbation.
3) Supplementation protein mixture: Height and weight growth staturo-ponderale close to standrard; Distrupted in nonsupplemented, stays of the standard.

The gain in weight is not influnced by nutritional supplementation. Disease severity can be strangled by restrictive diet of phenylalanine, associated to NTBC medical treatment.

PO.200

Implication of hyperhomocysteinemia and oxidative stress on non alcoolic hepatic steatosis

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Introduction: The oxidativet stress is associated with many pathologies like atherosclerosis and type 2 diabetes. It supports the development of insulinoresistance in tissues implied in the energy metabolism, like the liver.

Our objective is to evaluate the oxidizing stress effect induced by an experimental hyperhomocysteinemia on the hepatic lipids composition.

Material and methods: The animal model is the local domestic male rabbit. The experiment consists to induce a hyperhomocystéinémie by administration of a standard diet supplemented with 1, 5 gr of DL-methionine per day during 2 months, the control received standard diet in the same conditions. At the end of experiment, we evaluate the level of homocysteinemia, the TBARS as well as the hepatic total lipids. This study was completed with histological analysis.

Results: We showed that the hyperhomocysteinemia registered with methionine supplemented diet (31.35 ± 5.55 μM versus 8.99 ± 0.93 μM in control) induced an oxidative stress in the liver with increase of the TBARS level (0.38 ± 0.06 μM versus 0.30 ± 0.03 μM in control) and a rise of total lipids content (88.47 ± 8.43 mg/g versus 57.67 ± 5.76 mg/g of tissue). The histological study showed the existence of hepatic steatosis.

Discussion: Our work underlines the importance of the methionine oxidative stress-induced in the development of the hepatic steatosis.

PO.201

Dietary patterns, nutritional knowledge and anemia among school children in Morocco

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Inadequate dietary patterns can lead to several deficiencies including iron deficiency. The objective of the present study was to investigate the association between dietary patterns, nutritional knowledge and anemia among school children in Morocco.

253 children were chosen randomly from five public schools. Anthropometric measurments were taken using standardized methods, whereas haemoglobin was assessed using a hemocue instrument.

Dietary patterns and nutritional knowledge were evaluated using a structured questionnaire. dietary intake was evaluated using 24 h recall.

The median age was 9.58 (8.83–10.33) years; mean *z*-score BMI (-0.15 ± 1.31) kg/m^2.

The proportion of anemic children was 28.8%; 11.8% of these had mild and 17.1% had moderate anemia. Dietary patterns of children showed that 51.5% reported skipping breakfast, 71.2% never consumed milk and 50.0% eating fast foods at least three times per week.

The unadjusted odds ratio (OR) showed that children who never consumed milk had a three-fold increased odds of being anemic [OR = 3.4, 95% confidence interval (CI) = 1.6–7.8; $P = 0.001$], whereas those who consumed fast foods at least three times per week had an 0.2 increased risk [OR = 0.2, 95% confidence interval (CI) = 0.1–0.5; $P = 0.001$]. Anemia among children was also found to be associated with limited nutrition knowledge. The proportion of children with less nutrition knowledge was 73.2%. These children were four times more likely to be anemic [OR = 4.7, 95% confidence interval (CI) = 1.9–11.2; $P = 0.001$].

Poor dietary habits and nutritional knowledge were associated with anemia. Appropriate nutrition interventions are needed to promote healthy dietary habits among school children.

PO.202

The association between a low physical activity and body composition indexes in young Moroccan students

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We aimed to assess the association between a low physical activity and body-composition indexes in young Moroccan students.

One hundred forty university students, participated in a cross-sectional study (female sex 67.1%; mean age 21.3 years).

Anthropometric measurements were acquired according to standardized procedures. Body composition namely body mass index, fat percentage and degree of obesity were obtained by portable bioelectrical impedance analyser TANITA BC 420-MA. The physical activity was assessed by the Arabic International Physical Activity Questionnaire (IPAQ). The association between a low physical activity and body-composition indexes was assessed using Chi-square test.

A low physical activity was significantly associated to female sex ($P = 0.001$); a higher body mass index ($P = 0.001$) and to a high fat percentage ($P = 0.001$).

Low physical activity seems to be related to obesity, especially in female sex in university students. Sensitization programs should be encouraged to improve the lifestyle of university student.

PO.203

Glycemic control is adjusted by increased insulin secretion during growth of lean and MSG-obese rats

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Blood insulin levels change during growth and their control is consonant to metabolism changes. Reduced insulin secretion has been reported in pancreatic islets from neonatal, post-weaning and young rats as a response to high glucose stimulation, when compared to islets of adult rats. A decline in insulin secretory response from pancreatic islets with aging has been observed. Aging is a risk factor in the developpment

of degenerative diseases with high prevalence worldwide, such as obesity and non-insulin-dependant diabetes (NIDDM). Glucose-induced insulin secretion is impaired in obese and diabetic patients. The mechanism underlying the islet adaptation to insulin resistance, or rather, how beta cell may experience a reduction in insulin sensitivity, is not yet known. However, whether alteration in insulin sensitivity and pancreatic beta cell function exist and progress in the easrly stage of growth, from the post-weaning phase through adult life, are still unknown. Administrations of monosodium L-glutamate (MSG) to rodents promote the death of neurons in hypothalamic areas. Adult MSG-treated rats exhibits increased adiposity, hyperinsulinemia and insulin resistance. In spite of hyperinsulinemia and insulin resistance, adult MSG-rats are normoglycemic. It is suprising that adult 90-day-old MSG-obese rats do not develop hyperglycemia and diabetes. While pancreatic islets from these obese rats oversecreted insulin stimulated by high glucose concentration. The aim of current research was to investigate whether. (i) growth affects the glucose-stimulated insulin secretion from pancreatic islets and peripheral insulin resistance and (ii) onset of MSG-obesity speeds up damage to the functioning of beta cells and to insulin peripheral action in rats.

PO.204

Effect of method extraction on phytochemical composition and antiradical capacity of fresh extract from leaves and bulbs of *Allium ursinum*

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Introduction: The aim of this study consists to study variability of biochemical composition and antiradical activity of fresh bulbs and leaves of *Allium ursinum* according to the extraction method.
Material and methods: Extractions of fresh bulbs and leaves were carried out by three methods: infusion, decoction and maceration to obtain the different extracts. Quantitative analysis of total polyphenols, flavonoids and tannins were investigated by spectrophotometric methods and the organic composition was carried out by GC/MS. Antioxidant activity was evaluated by anti radical activity against synthetic radical DPPH.
Results: Total polyphenol, flavonoid and condensed tannin contents and anti radical activity of different fresh extracts show an important variability depending on extraction method and organ. In fact, the leaves are richer in phenolic compounds than bulbs. Decoction of fresh leaves and fresh bulbs allows giving the richest extract in total polyphenols (4.81 mg GAE/g FL and 1.18 mg GAE/g FB, respectively) and flavonoids (0.82 mg CE/g FL and 0.1 mg CE/g FB, respectively). However, infusion method gives the richest extract in condensed tannins (2.42 mg CE/g FL from leaves and 1.69 mg CE/ FP from bulbs). Furthermore, organic composition depends also on extraction method.

The highest percentage of DPPH inhibition was attributed to the extract of fresh bulbs obtained by infusion (66.61%).
Conclusion: Decoction seems to be the best method of extraction of phenolic compounds. In addition, fresh bulbs and leaves of wild garlic represent an interesting source of bioactive molecules.

PO.205

Lifestyle and dietary habits of school children 11–14 years old in Morocco

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Introduction: Lifestyle factors in childhood have a profound impact on health during mid- and late-adulthood. Healthy eating habits of children which are the basis for the health status of adolescents and adults, should be monitored to ensure children's correct physical and psychological development. The purpose is to examine the dietary habits of the Moroccan school children aged 11–14-year-old.
Methods: Information on body weight, height, fasting glucose, blood pressure and diet were collected on sample of school children
Results: 107 children (mean age = 11.67 ± 0.67 years; 54.3% boys; mean *z*-score BMI = −0.2171 ± 1.4330; mean systolic BP = 105.6 ± 12.97 mmHg, diastolic BP = 69.12 ± 9.28 mmHg) participated in this study. 1.86% of children had abnormal glucose levels. The prevalence of overweight and of obesity was respectively (19.6%, 6.48%).63.5% reported taking breakfast, 5.60% consuming vegetable and fruit ≥4 time/day, 57.94% reported taking fast-food ≥1 times/week. 84.11% spend more than an h/day watching TV, 57.94% eat in front of TV/Computer.
Disscusion: This study has provided more evidence to enhance our understanding on some of the factors that influence children's weight. Unfortunately, children in Morocco are leading unhealthy lifestyles, noticeable in their poor nutrition habits. There are a high percentage of children that do not have breakfast. Children are abandoning the 'mediterranean Diet' in favour of

industrial products and fatty foods. Furthermore, a high proportion of children eat in front of TV/Computer, since television viewing appears to be linked to obesity. The only key of a healthy adult life is good nutrition, starting from childhood.

PO.206

Gastric bypass specifically alters liver metabolism as compared to sleeve gastrectomy in subjects matched for postoperative weight

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Introduction: Some studies suggest that Roux en Y gastric bypass (RYGBP) has a specific effect on glucose metabolism as compared to sleeve gastrectomy (SG), which is not only explained by a greater weight loss. However, few studies have explored whether other metabolic parameters are specifically modified by GBP.

Methods: Eighty-one subjects that underwent SG were matched with 81 subjects that underwent RYGBP, for gender (11 men/80 women), age (42 ± 11 versus 43 ± 10 years) and 1-year postoperative weight (87.8 ± 17.2 versus 87.5 ± 17.2 kg). Metabolic parameters were prospectively recorded before and 1 year after surgery.

Results: Before surgery, BMI (45.3 ± 5.9 versus 44.5 ± 7.7 kg/m²) and metabolic parameters were not significantly different between both groups. After surgery, although BMI was similar (32.8 ± 5.8 versus 32.3 ± 6.5 kg/m²), C-reactive protein (2.87 ± 2.78 versus 3.97 ± 3.05 mg/L, $P = 0.021$), ferritin (71 ± 60 versus 116 ± 101 μg/L, $P = 0.001$) and LDL-cholesterol (2.65 ± 0.71 versus 3.33 ± 0.99 mM, $P < 0.001$) were lower in RYGBP as compared to SG, whereas alkaline phosphatase (90.3 ± 28.8 versus 73.8 ± 22.5 UI/L, $P < 0.001$) and transaminases (ASAT 21.1 ± 8.3 versus 18.2 ± 7.4 UI/L, $P = 0.020$ and ASAT 28.9 ± 13.6 versus 21.0 ± 6.6, $P < 0.001$) were higher. In contrast, the parameters of metabolic syndrome (including blood pressure, blood glucose, triglycerides and HDL-cholesterol) and insulin resistance (serum insulin and HOMA-IR index) were not different in the two groups after surgery.

Conclusion: When matched for 1-year postoperative weight, the parameters of metabolic syndrome improved to the same extent after SG and RYGBP. In contrast, GBP induces specific effects on liver metabolism, independently of weight loss.

PO.207

Protective effects of polyphenol-rich infusions from carob '*Ceratonia siliqua*' leaves and cladodes '*Opuntia ficus-indica*' against inflammation associated with diet-induced obesity and DSS-induced colitis

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Introduction: Carob '*Ceratonia siliqua*' and cladodes '*Opuntia ficus-indica*' have been widely used in traditional medicine for their content in polyphenols which exhibit anti-inflammatory and anti-obesity activities. Hence, we investigated the effects of polyphenol-rich infusions from carob leaves and cladode on inflammation associated with obesity and DSS-induced ulcerative colitis in Swiss mice.

Material & methods: Swiss male mice were subjected to control or high fat diet. At the 8th week, animals received or not 1% infusion of either carob leaves or cladodes for 4 weeks. Acute ulcerative colitis was induced by feeding mice with 2% DSS solution dissolved in drinking water over last 7 days. After sacrifice, pro-inflammatory cytokines levels in plasma and their mRNA expression in adipose tissue were determined.

Results: Our results showed that cladodes and carob leaf infusions significantly reduced body weight in obese mice which was correlated to a decrease in adipose tissue weight. Thus, infusions significantly diminished levels of IL-6 and TNF-alpha in plasma and mRNA expression of TNF-alpha and IL-6 in adipose tissue.

Discussion: The protective effect of cladodes and carob leaf infusions against inflammation associated with obesity and ulcerative colitis may be partly due to the antioxidant and anti-inflammatory properties of their polyphenols. Indeed, polyphenols from pomegranate peel alleviates tissue inflammation in obese mice by decreasing the mRNA expression of IL-6 in the colon and IL-1β in the visceral adipose tissue.

PO.208

Oral sensitivity to oleic acid and food behaviors in athletes

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The purpose of this study was to test the hypothesis that fat oral-intensity perception may be associated with level of physical activity, food preferences and consumption of fat-rich foods.

Fifty-two top athletes from different discipline (weightlifting, kayaking, athletics…) of both sexes (27 male, 25 female) underwent anthropometric measurement, assessment of body composition, and completed a 4-day diet record and physical activity diary. In addition, they were screened for oral sensitivity to oleic acid (0.375, 0.75, 1.5, 3, 6, 12 mM) using triplicate sensory evaluations for each concentration. According to the threshold of fat perception, subjects were allocated to three groups G1 (0.375–3 mM), G2 (6–12 mM), G3 > (12 mM). Further, food habits and behaviors (food habits and behaviors questionnaire) were also established.

Male athletes; BMI, 25.3 ± 4.4 kg/m², age, 18.88 ± 1.53 years, BF%, 12.52 ± 9.2, TEE, 3763.1 ± 460 kcal. Female athletes; BMI, 24.17 ± 4.54 kg/m², age, 16.11 ± 1.93 years, BF% 21.70 ± 7.4, TEE, 3106 ± 310.05 cal. 85%. (n = 44) of subjects were classified as hyposensitive and non-sensitive (G2, G3). These subjects differed from those who were classified as hypersensitive (G1). Hyposensitive and nonsensitive subjects have a higher training volume, and total energy expenditure. They consumed significantly more energy, fat, saturated fat, fatty foods,, compared to hypersensitive subjects (all $P < 0.05$).

An inability to perceive low concentrations of fatty acids in foods was associated with higher training volume and greater consumption of fatty foods. Our results suggest the probability of criminalization of physical activity in the regulation of orosensory fat taste perception and therefore fat intake.

PO.209

Antioxidant and antidiabetic activities of some Tunisian traditional medicinal plants

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Introduction: Diabetes mellitus is a global health challenge and medicinal plants continue to play an important role in the management of this disease. Three Tunisian plants with a reputation of usefulness in treating diabetes were examined for α-amylase inhibition.
Materials and methods: Antioxidant activity of the methanolic extract of three species (*Rosa canina, Ajuga iva* and *Artemisia herba alba*) was determined. The α-amylase inhibition assay was also evaluated.
Results: Extract of *Ajuga iva* show the highest total Phenolic levels (34.363 mg EAG/g MS). *Artemisia herba* alba *and Ajuga iva showed the highest* flavonoid levels, respectively (25.98 and 18.099 mg ER/g.) The free radical scavenging activity was found to be prominent in extracts of *Artemisia herba alba* against DPPH with an IC50 of 0.04 mg/mL and against ABTS with an IC50 of 032 (mg/mL).

Extracts of *Artemisia herba* alba and *Ajuga iva* showed an appreciable ferric ion reducing activities with an IC50 of 1.300 mg/mL. The highest inhibitory activity against α-amylase was exerted by Rosa canina with 100% inhibition followed by *Artemisia herba alba* with 68%.
Discussion/Conclusion: Many studies have associated the antioxidant and α-amylase inhibitory activity of plants with their anti-diabetic activity. Our results support the traditional use of the analyzed species in the management of diabetes. Further investigation will be required to identify the compounds responsible for their promising anti-diabetic activity.

PO.210

Sage tea drinking improves lipid profile and glucose levels in Tunisian diabetic patients

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Sage (*Salvia officinalis* L.) is among the plants which are known to be beneficial for the diabetic patients, and previous studies suggested that some of its extracts have hypoglycemic effects to the normal

animals and the diabetics. In the present study, we tried to verify the antidiabetic effects of an infusion (tea) of common sage, which is the most common form of this consummate plant.

Twenty healthy volunteers (age 40–65) and twenty type 2 diabetic patients participated in this study after having signed a form of consent. The tea of sage was systematically prepared by adding 300 mL of boiling water to 4 g of the drying plant material and allowing to infuse during 5 min.

Four weeks of treatment with tea of sage had no effect on the glycemia. An improvement of lipid profile was observed with decrease of the LDL cholesterol, decrease of the rate of total cholesterol as well as the increase of the plasmatic levels of HDL cholesterol during the study and 2 weeks after the treatment.

No unwanted effect was indicated. The results suggest that the sage can be effective and without danger in the treatment of diabetes and the hyperlipidemia.

Other Allied Disciplines

PO.211

Hypoxia induces endoplasmic reticulum stress in alveolar epithelial cells: potential implication in idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is characterized by lung tissue remodelling probably due to repeated aggressions of alveolar epithelial cells (AECs). AECs undergo apoptosis, and acquire a fibroblast-like phenotype through epithelial-to-mesenchymal transition (EMT). Induction of endoplasmic reticulum (ER) stress has been evidenced in AECs of IPF patients. This ER stress could be favored by alveolar hypoxia. Here, we try to decipher the relationship between hypoxic signalling and the induction of ER stress.

Rat primary AECs were cultured in normoxia or hypoxia (1.5% O_2) for 4–48 h. Activation of the unfolded protein response (UPR) pathway was studied by western blotting and RT-qPCR, apoptosis by the cleavage of the caspases 3 and 12, and EMT by the induction of specific transcription factors. Next, the implication of ER stress in response to hypoxia was studied *in vivo* in rat exposed 16–72 h to 8% O_2.

In vitro, a 4 h exposition led to the activation of ER stress markers ATF4, ATF6, XBP-1, and the pro-apoptotic transcription factor CHOP. Moreover, after a 24 h exposition, we observe the cleavage of caspases 3 and 12, and the transcriptional induction of TEM markers: Snail, Twist, Zeb, CTGF, TGF-b1. Interestingly treatment of hypoxic AECs with pharmacological inhibitors of ER stress (sodium phenylbutyrate or Salubrinal) prevented these effects. *In vivo* we observed similar effects, with an increase in the UPR pathway (ATF4, ATF6, CHOP), apoptotic (Caspase 3, 12) and TEM markers.

Our results suggest that modulation of hypoxia-induced ER stress in AECs could be a pharmacological approach to limit fibrogenesis.

PO.212

Partial B1-blockade does not worsen the effects of sepsis on ventilation, hepatic and renal clearance

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β1-blocker therapy may control the heart rate and attenuate the deleterious effects of β-adrenergic receptor stimulation in septic shock. However, β1-blockers are not traditionally used in this condition and may worsen organ functions. The aim of this research was to explore the effects of Atenolol on lung, liver and kidney in a rat model of endotoxemia.

Sepsis was induced by intraperitoneal administration of lipopolysaccharide (LPS, 5 mg/kg). Partial β1-blockade was realized through intraperitoneal injection of Atenolol (6 mg/kg) 6 h after LPS administration. The indocyanine green method was used to study the hepatic clearance and the inulin method to measure glomerular filtration rate. The samplings were collected on separate animals 22 h after the induction of sepsis. Ventilation (inspiratory and expiratory times, refractory period, tidal volume and frequency) was analyzed with whole-body unrestrained plethysmograph before first injection and 22 h later.

Signs of sepsis were observed 6 h after LPS administration. Urine output and clearance of indocyanin green or inulin were significantly decreased in LPS group compared to control group whereas minute ventilation was increased. Atenolol reduced inulin clearance in control-treated animals versus untreated. Partial β1-blockade limited the effects of LPS on minute ventilation and urine output. Administration of Atenolol partly restored the hepatic and renal clearance in LPS-treated group versus untreated.

Partial β1-blockade does not worsen the effects of sepsis on different organ functions. Partial B1-blockade seems to be even beneficial in our model.

PO.213

Morphofunctional changes in the testes of juvenile rats after administration of gold nanoparticles

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Introduction: Gold nanoparticles are widely used in modern medicine and technology. They can be used as drug carriers, anti-tumor agents and biosensors. While gold itself is a safe element, nanoparticles have unique physical and chemical properties that can influence possible outcomes of nano-gold therapy. Toxicological studies are mainly focused on *in vitro* investigation, while our knowledge about i*n vivo* toxicity of nanoparticles, especially reproductive toxicity, is limited. Therefore, the aim of our study was to estimate any hazardous effects of gold nanoparticles on the reproductive system of juvenile rats.

Materials and methods: Twenty-four male albino rats were divided into three groups (8 each). Animals from these groups received intraperitoneal injections of saline (control), sodium polyphosphate (vehicle control) and gold nanoparticles (1 mg/kg bodyweight) for 10 days respectively. Next, animals were decapitated; their left testicle was taken and routinely processed for histological examination. We used seminiferous tubules diameter, height of seminiferous epithelium, cross-sectional area of Sertoli and Leydig cells as morphological markers of testicular function.

Results: Any morphological parameter in the group that received injections of sodium polyphosphate did not differ significantly from control values. There were also no histopathological changes. In contrary, administration of gold nanoparticles resulted in a decrease in cross-sectional area of Leydig cells ($P = 0.02$), while other parameters changed nonsignificantly. We also observed early signs of spermatocytes degeneration, which may indicate testosterone homeostasis impairment.

Discussion: Our results demonstrated that gold nanoparticles affect the endocrine function of testes without significant quantitative influence on the spermatogenic potential.

PO.214

Effects of cadmium, lead and mercury on progesterone synthesis in cultured bovine luteal cells

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The aim of this study is to examine the effects of these metals on progesterone synthesis in bovine luteal cells.

Luteal tissue was dissociated into cells in oxygenated culture medium containing bovine serum albumin, collagenase, DNase and antibiotic. Cells were incubated without treatment for first 24 h. Then the luteal cells were incubated in medium containing heavy metals (0.1, 0.25, 0.5 and 1 μM cadmium chloride; 0.1, 1, 10 and 50 μM lead acetate trihydrate; 1, 2.5, 5 and 10 μM mercury chloride) for an additional 96 h. Used medium was collected after each 48 h and stored at -20°C. Progesterone level in medium was determined by using radioimmunoassay method.

All the doses of cadmium and the three doses of lead (1, 10 and 50 μM) and along with three doses of the mercury (2.5, 5 and 10 μM) were significantly decreased the progesterone levels ($P < 0.01$). Additionally progesterone synthesis was decreased by 55% on the 5th day of 1 μM cadmium chloride incubation. Treatment of cells with 50 μM lead acetate trihydrate resulted in 43 and 61% inhibition in progesterone levels on day 3 and day 5 respectively. Steroid synthesis was decreased by 42 and 54% on the 3rd and 5th day of mercury chloride treatment.

Conclusively, progesterone synthesis of luteal cells is negatively affected by the exposure of even lower doses of cadmium (0.1 μM), lead (1 μM) and mercury (2.5 μM).

PO.215

Contribution to the phytochemical study and antioxidant activity of phenolic compound of *Cistus salvifolius* L. a cistaceae in the region of tlemcen west north of Algeria

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Plants synthesize a diverse spectrum of antioxidant phenolic compounds as secondary products, which prevent oxidative damage in planta but also confer protective effects on humans when the plants are consumed as food. The relationship between polyphenols-richedfood consumption and reduced possibility of being affected by some disease, has attracted increasing interest from consumers, food manufacturers and nutritional scientist.

Cistus salvifolius is a wild plant belonging to the *Cistaceae* family (also known as rock- rose) which is a large family of perennial shrub (of almost 200 species) that grow mostly in the Mediterranean semi- arid ecosystem. This species is much appreciated in the perfume industry and has been used since ancient time in traditional folk medicine as anti inflammatory, antiulcerogenic, wound healing, anttimicrobial, cytotoxic and vasodilator remedies. The antioxidant

properties of the phenolic extract of leaves of *C. salvifolius* were investigated. The amounts of total phenols, total flavonoïd and condensed tannin in the aqueous acétone extract were determined spectrometrically.

From the analyses, the phenolic extract of leaves *showed* the highest antioxidant power, using DPPH, FRAP and ß- carotene method. Finally, a relationship was observed between the antioxidant activity potential and the different phenolics compounds levels of the extract. These results demonstrate that phenolic compounds of *Cistus salvifolius* leaves have excellent antioxidant activities and thus they have great potential as sources for natural health products.

PO.216

Late-night cinema watching with friends does not affect cortisol awakening response and heart rate variability in the next morning

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Effect of watching a screen late in the night on the hypothalamo-pituitary-adrenal axis (HPA) and autonomous nervous system (ANS) activity in the next morning is not known. Aim of the current study was, therefore, to measure effects of late-night cinema watching on sleep quality, cortisol awakening response (CAR) as an indicator of HPA and heart rate variability (HRV) as an indicator of ANS activity.

Medical students (n = 22, 20–26 year-old) were followed for two consecutive days: a control day followed by a cinema watching day. In each day, sleep dairies were filled; salivary samples were taken at 0, 15, 30 and 60 min post-awakening for measurement of CAR; and electrocardiogram was recorded for 5 min for determination of HRV. A film lasting 140 min, 'Fast and Furious 7', was watched altogether at the last 09:00 pm session.

Late-night cinema watching did not affect CAR (mean, area under the curve) and sleep parameters (time, duration, disturbed sleep, awakening problems) and time- and frequency-domain parameters of HRV ($P > 0.05$).

The results suggest that late-night cinema watching does not affect HPA and ANS activities in the next morning if the person is accustomed to sleeping late in the night. Additionally, it might be speculated that watching a film with a groups of friends is an entertaining activity counteracting the negative effects of late-night sleeping.

PO.217

The assesment of radiation therapy-resistant tumor response to the treatment with beta-glucan

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Introduction: Radiation therapy is one of the main treatment methods of cancer, but development of resistance to radiation therapy limits its treatment outcome. The aim of the study was to assess radiation therapy-resistant tumor response to the treatment modulation by beta-glucan, known as powerful immune stimulant and antagonist to malignant tumors.
Methods: C57BL/6 female (8–10 weeks of age) radiotherapy-resistant Lewis lung carcinoma (LLC) tumor bearing mice were used. Mice were treated with radiotherapy or/and beta-glucan. Single radiotherapy (dose 10 Gy) or fractionated radiotherapy (2Gyx 5 days) have been applied. Tumor growth by measuring of tumor volume and mice survival were evaluated. Expression of the genes Bbc3, P21, Ccng1, ID1, Dcn, THBS2, influencing development of resistance, was determined by RT-PCR.
Results: The lowest tumor volumes were in the group of mice treated with beta-glucan alone ($P < 0.05$). Tumor growth was suppressed more when fractionated radiotherapy with beta-glucan was applied than this with single dose radiotherapy ($P < 0.05$). The best survival was achieved in the group treated with beta – glucan. The expression of the genes increased after the radiation therapy similar as in 3D cell culture. Co-administration of beta-glucan doesn't affect the expression level of the genes.
Discussion: The tumor matrix may have a big influence to the development of the resistance to radiotherapy.

PO.218

Relationship among serum lipids, fibrinolytic enzymes and factor VII in women during menopause

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Background: Hypercoagulability and reduced fibrinolytic capacity, often seen in menopausal women, are associated with hypertriglyceridemia. The mutual correlation among serum lipids, fibrinolytic enzymes:

tissue type plasminogen activator (t-PA), plasminogen activator inhibitor type 1 (PAI-1), and factor VII in women during menopause was studied.

Material and methods: Study comprised a total number of 76 women divided into two groups: group of women in perimenopause ($n = 36$) and group of women in postmenopause ($n = 40$). Lipid level (HDL-CH, LDL-CH, TG, total cholesterol) was determined with colorimetric-spectrophotometric method, fibrinolytic enzymes were determined using immunoenzyme sandwich method and factor VII of coagulation with the method of deficiency plasma. Correlation analysis (Pearson's coefficient) was used for assessing the relationships between the examined parameters.

Results: Fybrinolytic activator (t-PA) was in poor negative correlation with fibrinolytic inhibitor ($r = -0.18$), factor VII of coagulation ($r = -0.28$), total cholesterol ($r = -0.17$) and triglycerides ($r = -0.35$), but in weak positive correlation with HDL-CH ($r = 0.73$) as well. There was a positive correlation between PAI-1 on one hand and factor VII ($r = 0.18$), triglycerides ($r = 0.245$) and total cholesterol ($r = 0.14$) on the other hand, but there was also a weak negative correlation between PAI-1 and HDL-CH ($r = -0.048$).

Conclusions: These data suggest that serum lipids, particularly triglycerides have a close relationship with thrombogenesis as evidenced by activated f. VII in the extrinsic coagulation system and also by elevated PAI-1 activities in fibrinolysis.

PO.219

Sulodexide pretreatment affects vascular responses and liver mitochondrial function in diabetic rats

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Diabetes mellitus (DM) is associated with endothelial dysfunction and induces liver mitochondrial changes. Treatment with sulodexide (SLX), glycosaminoglycan, has been previously reported to be beneficial in various pathological conditions.

Fifteen-weeks old Wistar rats were randomized into four groups: untreated control (C), treated control (C+SLX), diabetic (DM) and treated diabetic (DM+SLX). SLX was administered i.p. at the dose 100 IU/kg daily for 5 weeks. Diabetes was induced with single i.v. dose of streptozotocin 45 mg/kg. Vascular responses of isolated femoral arteries to norepinephrine (NE) and acetylcholine (ACH) were measured using Mulvany-Halpern myograph in these conditions and in the presence of diclofenac. The parameters of oxidative phosphorylation were evaluated in liver mitochondria using voltamperometric method on oxygraph Gilson.

We found significantly increased NE-induced contractions (2.03 ± 0.21 versus 5.11 ± 0.27 mN/mm) and decreased ACH-induced relaxations (80.06 ± 4.39 versus $63.24 \pm 6.10\%$) in C versus DM rats. Application of diclofenac significantly decreased NE-induced contractions in all groups of rats. SLX treatment significantly decreased sensitivity to NE and ACH-induced relaxations were not significantly enhanced in C versus DM rats. Oxygen consumption rate in state 3 [QO_2 (S_3)], state 4 [QO_2 (S_4)], oxidative phosphorylation rate (OPR) and respiratory control ratio (RCR) were decreased in the mitochondria of DM rats. Mitochondria of C rats were not affected with SLX treatment. Administration of SLX in DM was associated with increase of RCR, while other parameters were not affected.

Sulodexide treatment may be associated with protective effects on vascular and liver mitochondrial functions in diabetes.

PO.220

Investigation of the effects of pulsed electromagnetic fields and calcium-channel blockers on osteogenesis

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Introduction: Calcium channel blockers may affect many metabolic processes, including bone metabolism. Electromagnetic field applications have also affect bone formation. The aim of the present study was to investigate whether amlodipine, a calcium channel blocker, interferes with healing of rat calvarial bone defects in compare with pulsed electromagnetic fields.

Materials and methods: Forty eight male Wistar rats were divided into six groups: control, pulsed electromagnetic field (PEMF), Amlodipine, platelet rich plasma (PRP), PRP + PEMF, Amlodipine + PEMF. A critical-sized surgical defect was created in the calvaria of all groups. Amlodipine at a dose of 0.04 mg/rat/day administrated orally for 4 weeks. PEMF were applied for 3.5 h/day with a faraday cage. PRP were

implemented on the defective area. The animals were sacrificed 28 days after the creation of the bone defect. At the end of the experiment, bone mineral density (BMD g/cm^2) of calvaria using dual energy X-ray absorptiometry (DXA) and serum concentrations of nitric oxide (NO) were measured.
Results: BMD levels were decreased at amlodipine treated group while increasing at PRP and PRP + PEMF groups. In addition, serum concentrations of NO were increased at PEMF, amlodipine and amlodipine + PEMF treated groups while decreasing at PRP and PRP + PEMF groups.
Discussion: This study showed that amlodipine treatment decreases BMD. Amlodipine might exert its effect by blocking Ca^{++} transport into cells. PRP and PEMF was found to prevent the deleterious effect of amlodipine in rats with bone defects.

PO.221

Learning physiology – between linear and modular curriculum

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Introduction: In order to evaluate the impact of the modular teaching system, an integrated modular course, *Biosignals-Clinical Applications*, was initiated.
Methods and results: Thirty students enrolled in the 1st year of studies were selected. The modules are: Neuron, Striated muscle, Myocardium, Clinical exploration and was conceived as a 4 week programme. The theme was chosen so that to cover subjects taught in the first year of studies: Physiology, Cellular and Molecular Biology, Biophysics and Medical Informatics. Information regarding pathological changes is also given, strictly linked to the theory taught in the first year. There are four modules comprising 20 h of academic lectures and 12 h of practical applications. Besides the novelty of learning in a modular way, the students benefit also of different methods of teaching, which are not applied in the regular lectures, such as the use of SMART Notebook programme and of the Smart Response PE interactive response system.
Conclusion: This way of teaching has the potential to improve the quality of both teaching and learning processes. But the great gain of this particular experience for the students is that they are now able to compare the modular against the traditional education, as they study the same subjects in both ways.

PO.222

Fine-scale population genetic structure in western France: consequences in gene mapping

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Background: Genetic mapping is a major tool for establishing the molecular bases of human phenotypes. The genetic structure of human populations varies throughout the world, being influenced by migration, admixture, natural selection and genetic drift. Human population structure has first been investigated at broad, continental, scales. Currently researchers focus on finer scales and here we are examining genetic structure in a Western France Atlantic Coast region. Characterising such genetic variation can provide insight into demographical history and informs research on disease association studies, especially on rare recent variants.
Methods: We genotyped 456 individuals sampled from *Finistère* to *Vendée*, with at least three of their grandparents born within 15 km, using Axiom CEU Chip. We applied Principal Components analysis and related methods to investigate the genetic structure.
Results: Principal Components analysis revealed a high correlation between geographical position and components (P-value < 2e-16). Many independent methods support the hypothesis that Loire River is a genetic barrier. The two groups of individuals, from north or south of Loire, are well differentiated along PC1 axis. The first split of hierarchical clustering, and the one based on identity-by-descent segments are between north and south of Loire.
Conclusion: We here report both evidence for isolation by distance and existence of a genetic barrier, the Loire River. This fine-scale population structure may have consequence in association analyses, especially for rare variants which tend to be geographically clustered. These results support the need for a genetically matched panel of controls in gene mapping analyses in French population.

PO.223

Cytokine level dynamics in serum after corneal UVA-riboflavin crosslinking in patients with keratoconus

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Aim: Corneal collagen crosslinking (CXL) with UVA irradiation 370 nm and 0.1% riboflavin is used for treatment of keratoconus. As a result of CXL treatments, an increase biomechanical stiffness of the cornea, arrest the progression of the disease. To investigate the expression of cytokines in serum of patients with keratoconus after CXL treatment.
Methods: The study enrolled 25 patients with keratoconus I-II by Amsler classification – 10 females, 15 males. Control – 14 healthy subjects. The mean age was 32 years. CXL treatments were performed with deepitelization of cornea (UVA irradiation 3 mW/cm^2, 30 mins, with 0.1% riboflavin / 20% dextran). The levels of TNF-a, IL-1b and IF-a in serum of patients with keratoconus has been evaluated in 3, 7, 14 days after CXL using enzyme-linked immunoassay.
Results: Increasing of level of IL-1β (6.12 $\pm$ 1.5 pg/mL) compared with normals (1.5 $\pm$ 0.3 pg/mL), at normal range of TNF-a and IF-a was observed in serum of the patients with keratoconus. The postoperative period showed normalization of IL-1β in serum (3 days – 2.94 $\pm$ 0.8 pg/mL; 14 days – 2.4 $\pm$ 0.3 pg/mL). TNF-a in keratoconus 2.92 $\pm$ 0.5 pg/mL, 3 days after CXL – 4.34 $\pm$ 1.0 pg/mL, 14 days after CXL – 3.5 $\pm$ 1.1 pg/mL and IF-a, respectively, 5.72 $\pm$ 1.6 pg/mL versus 5.96 $\pm$ 1.2 pg/mL and 3.3 $\pm$ 0.4 pg/mL.
Conclusions: The pathological process in keratoconus was accompanied by increasing of level of IL-1β in serum, at normal range of TNF-a and IF-a. After CXL normalization was observed of IL-1β in serum and the absence of significant systemic inflammatory response.

PO.224

Intracellular protein C inhibitor: mechanism of internalization and possible intracellular functions

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Protein C inhibitor (PCI) is a secreted serine protease inhibitor with broad protease reactivity. Binding of heparin and certain phospholipids modulates the inhibitory activity of PCI. In man PCI has a wide tissue distribution; in mice PCI is expressed almost exclusively in the reproductive tract. It is therefore not surprising that the only phenotype of PCI-knockout mice is infertility of homozygous males. PCI can cross the cellular plasma membrane and pure phospholipid membranes. In current studies we are analyzing the mechanism of PCI internalization and its intracellular role. We could show that testisin, a glycosylphosphatidylinositol-anchored serine protease, which co-localizes with PCI in the testis, cleaves PCI not only at its reactive site but also at a site close to its N-terminus. This N-terminal cleavage releases a peptide rich in basic amino acids. N-terminally cleaved PCI as well as a truncated PCI-mutant lacking the N-terminus cannot be internalized by cells, whereas the N-terminal peptide itself functions as a cell-penetrating peptide. Testisin and/or other proteases might therefore regulate the cellular uptake of PCI. We have also shown that PCI translocates to the nucleus and that it contains a functional nuclear localization signal. PCI is mainly found in the nuclear envelope fraction, where it interacts with cathepsin L. Enrichment of cathepsin L in the nucleus has been observed in cancer cells, and several substrates of nuclear cathepsin L, e.g. histone H3 and 53BP1, have recently been identified. Internalized, nuclear PCI may be involved in the regulation of epigenetic modifications and/or cell cycle progression.

PO.225

Chitosan induces liver injuries regression in an experimental model of toxic hepatitis

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Liver fibrosis is a pathological condition induced by many drugs, hepatic viruses or toxic substances used in industry. Potentially, it is a reversible condition. There is a continuous search for substances that might have the ability to induce the regression of liver injuries. The aim of the study was to assess the ability of the chitosan to induce regression of liver injuries in an experimental model of thioacetamide induced liver fibrosis. Twenty-four male Wistar rats have been randomly assigned to three groups. Group I was the control group. Groups II and III received intraperitoneally 200 mg/kg thioacetamide three times/week, for 10 weeks. Group III received intraperitoneally 3 mg/kg chitosan three times/week, four more weeks. Blood samples from the retroorbitary sinus and liver samples have been obtained. Liver function markers, oxidative

stress markers (malondialdehyde, reduced glutathione) and hialuronic acid levels were assessed from blood and liver tissue. Histopathological examination was performed in order to assess the extension of the liver injuries. Thioacetamide administration induced a significant increase of liver enzymes, of malondialdehyde in plasma and in liver tissue and of hialuronic acid in liver tissue ($P = 0.05$). Chitosan administration lead to a significant decrease of transaminases, of lipid peroxidation, stimulated glutathione synthesis and decreased hialuronic acid levels to values similar to those of the control group ($P = 0.05$). Biochemical results have been supported by the histopathology findings. Chitosan administration has potential hepatoprotective effects, and in the used dose, it induces the regression of the experimental liver injuries.

PO.226

Referent values of oxidative stress markers related to sex, age and place of living

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Oxidative stress (OS) may be a cause of different chronic diseases, accelerating aging, etc. The aim of the study was to determine the referent values of OS markers related to sex, age and place of living.

From 65 healthy subjects: 24 were men and 41 women; 45 from city and 20 from Village; 18 at age of 20–40 years, 28 at 41–60 years, and 19 at 61–80 years. For determination of free radicals (FR), thiol groups (SH), total antioxidative status (TAS) and LDL, colorimetric assay by Diacron was used. Biomedica gruppe for LDL-ox antibodies, fluorimetric method for lipid peroxidation (MDA); and for antioxidative enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathion reductase (GR), and glutathion 6 phosphat dehysrogenase (G-6-PD), colorimetric assays by Randox were used.

No significant difference was shown referent values of our laboratory and referent laboratory for all parameters. Men showed increased value of OS by FR, 290 UCarr and MDA 3.8 μM/L compared to women ($P < 0.05$). People from city showed higher values of OS by FR, 320 UCarr and MDA, 3.98 μM/L than people from villages ($P < 0.01$) and the oldest group showed highest OS: FR, 347 UCarr and MDA, 4.2 μM/L. No significant difference was found for SH, TAS, and LDL and any of antioxidative enzymes in all groups.

Determination of these parameters may discover the body defense against FR which may be used as a prevention and even determination of some chronic disease and accelerated aging process.

PO.227

Calpain inhibition protects against inflamm-aging

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Introduction: Intracellular calpains are ubiquitous pro-inflammatory proteases activated by calcium whose activity is controlled by calpastatin, their ubiquitous inhibitor. We have previously shown that transgenic mice over-expressing calpastatin (CalpTG) are protected against vascular remodelling and angiotensin II-dependent inflammation. Since angiotensin II mediates aging-related vascular lesions, we hypothesized that CalpTG mice would be protected against aging.
Material and methods: We analyzed kidney, heart, aorta, brain and skin from 2-months and 2-years-old CalpTG and control mice housed together and performed transcriptomic analysis (RNA-seq) of kidney tissue in the four groups. In a second step, we analysed inflammatory response in the kidney of aged CalpTG and control mice, and in both *in vivo* (monosodium urate peritonitis, MSU) and *in vitro* (bone marrow-derived macrophages in culture) models of inflammation.
Results: At 2 years, control mice exhibited aging-related lesions, especially in kidney, arteries, brain and skin. By contrast, CalpTG mice had preserved renal function and histology and less vascular remodelling. Markers of senescence (p21) were decreased and telomere length was preserved in the kidney and the skin of aged calpTG mice. RNA-Seq differential analysis evidenced that inflammatory and fibrotic pathways were reduced in aged CalpTG mice, especially cytokine-related NF-κB and NLRP3 inflammasome activation. CalpTG mice had reduced macrophage infiltration with aging and CalpTG mice produced less IL-1α and IL-1β *in vivo* in response to MSU. *In vitro*, macrophages from CalpTG mice produced less IL-1α in response to activators of inflammasome.
Discussion: Calpain inhibition decreases inflammatory process, especially IL1-a production and thereby inflamm-aging-related lesions.

PO.228

Metabolic processes influence on tumor proliferation *in vitro*

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Background: Most cancer cells produce energy by increasing aerobic glycolysis even in the presence of oxygen. This phenomenon is often referred as the Warburg effect. Dichloroacetate (DCA) was observed to reverse the Warburg effect by inhibiting pyruvate dehydrogenase kinase and indirectly activating the gate keeping enzyme pyruvate dehydrogenase. DCA shifts aerobic glycolysis towards mitochondrial glucose oxidation in cancer cells forcing them to attain apoptosis. Metformin (Met), an oral anti-diabetic agent, has been shown to have a strong anti-proliferative effect in many breast cancer cell lines. The aim of this study was to investigate the anticancer effect of DCA and metformin in breast cancer *in vitro*.

Methods: MCF-7 breast cancer cells were treated with DCA (5 mM; 10 mM), Met (5 mM; 10 mM), or their combinations. Apoptosis was measured after 24 and 48 h using flow cytometry with Annexin V-FITC/PI staining.

Results: Increased apoptosis of MCF-7 cells was observed in both DCA and Met treated cells compared to control. After 24 h the highest apoptotic rate was observed with 10 mM Met (51.04 ± 9% apoptotic cells) and 10 mM DCA (50.69 ± 9.15% apoptotic cells). There was no significant synergistic effect observed after 24 h. Interestingly, after 48 h the number of apoptotic cells varied between 49 and 59% in all concentrations, however the combination of 10 mM Met and 10 mM DCA showed the highest number of apoptotic cells (59.37%).

Conclusion: Dichloroacetate and metformin effectively sensitizes MCF-7 cell line to apoptosis and gives a good ground for expanding further investigations in breast cancer treatment.

PO.229

Vestibular graviception during early postnatal development builds basis of motor, cognitive and emotional adult behaviours in mice

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The vestibular system is early functional in individual's life: anatomical structures are almost completely developed at birth and maturation continues during postnatal period. Recent works strongly supported a role of this system in bodily self-consciousness and space cognition at adulthood. However only few data have reported whether the vestibular system influences the development during childhood. Indeed, we hypothesized that Earth's gravity might, through the vestibular system, build from the early stage of development different aspects of cognition, emotion and social interaction.

We longitudinally evaluated performances of an original Het mouse model (B6Ei.GL-Nox3het/J) selectively devoid of otoconia within the inner ear (i.e. without gravitational vestibular perception) for the first time. Behavioural performances were assessed by a set of motor, cognitive and emotional evaluations from birth to adulthood. Compared to controls, we globally observed a developmental delay with a maximum at P7 in homozygote (HO) Het mice highlighting the crucial role of gravity perception during this period. These observations are similar to symptoms observed in vestibulo-deficient children. At adulthood HO Het mice behaviours oscillate between autism spectrum disorders and hyperactivity-like symptoms such as stereotyped behaviour and repetitive tasks. Moreover HO mice appeared less anxious compared to heterozygote while social interaction seemed impaired. We demonstrated for the first time that gravity vestibular perception is involved in cognitive, emotional and social development. Our findings support that early screening of the vestibular function at childhood as well as early therapeutic care and psycho-behavioural follow-up have to be taken into account by clinicians.

Sleep Physiology

PO.230

Overnight rostral fluid shift in group 1 pulmonary arterial hypertension

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High prevalence of both obstructive and central sleep apnea has been reported in group 1 pulmonary arterial hypertension (PAH). Central and obstructive sleep apnea can be promoted by overnight fluid shift from the legs to the lungs and the upper airway, respectively, in fluid retaining states. PAH is also characterized by fluid retention but fluid shift in PAH has not been previously evaluated.

To detect the occurrence of fluid shift in PAH.

We developed a questionnaire to test the frequency of symptoms caused by fluid accumulation in lower body at the end of the day and in the upper body during night and in the morning. Sleep quality was evaluated by Pittsburgh Sleep Quality Index (PSQI), daytime sleepiness by Epworth Sleepiness Scale (ESS), fatigue by Pichot's Fatigue Scale (PFS).

A total of 82 stable PAH patients and 55 control subjects matched for gender, sex and body mass index were included in this study. The total score of fluid shift was higher in PAH than in control group, in particular orthopnea (0.6 ± 0.9 versus 0.2 ± 0.6, $P = 0.004$), sleep with 2 pillows (0.6 ± 1.1 versus 0.2 ± 0.6, $P = 0.029$) and obstructed nose (1.2 ± 1.1 versus 0.6 ± 0.8, $P < 0.001$) were more frequent in PAH than in control group. PFS was higher in PAH than in control group ($P < 0.001$). There was no difference in the PSQI and ESS regarding the two groups.

Conclusions: A clinically detectable overnight fluid shift is present in PAH which could explain the high prevalence of sleep apnea in PAH.

PO.231

Sleep and sleep disordered breathing in a cohort of pre-LTX patients

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Poor sleep quality is reported by candidates and recipients of lung transplantation (LTX) patients, but sleep pathologies are often unrecognized. High prevalence of sleep disordered breathing (SDB) in end-stage chronic respiratory diseases is reported.

Aim: To describe sleep metrics and evaluate SDB-prevalence and characteristics in a french monocentric cohort of pre-LTX patients.

Methods: Prospective, observational and analytical study of sleep and SDB in pre-LTX patients by polysomnography. Anthropometric, clinical, and pharmacological data were collected. Comparizons were performed by *t*-test and Chi2, significant $P < 0.5$.

Results: Fifty-one pre-LTX patients had a polysomnography (01/2013–12/2014), 21 patients had COPD, 27 lung fibrosis, three bronchietasis or asthma. Mean age (±SD) was 52.3 ± 9.6 years; BMI 24.8 ± 5.8 kg/M^2. Mean TST was 378 ± 80 min; sleep efficiency $85 \pm 13\%$; sleep stage N3 108 ± 51 min; REM sleep $17 \pm 10\%$; aurousal-awakening index 17 ± 10 /h. Apnea-hypopnea index (AHI) was 9.1 ± 11.0 /h. No patient had a history of SDB but moderate-severe SDB (AHI ≥15/h) was found in 16% patients. SDB was obstructive. Prevalence of SDB was significantly higher in fibrosis (22%) compared to COPD (9%), maybe explained by significantly higher BMI and higher proportion of male.

Conclusions: Objective sleep quality in pre-LTX transplant patients is poor with low TST, low sleep efficiency, and decreased REM sleep. SDB is less prevalent in our cohort than in previous, but still higher than in the general population, with significantly higher prevalence in fibrosis than in COPD.

PO.232

Sleep disordered breathing in Silver-Russell syndrome patients: a new outcome

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Introduction: Imprinting disease such as Prader-Willi syndrome are associated with sleep disordered breathing (SDB). No data are available in Silver-Russell syndrome (SRS) – an imprinted disease associating low birth weight, failure to grow, difficulties to eat and dysmorphic features – although patients describe excessive daytime sleepiness and snoring.

Materials and methods: We retrospectively analyzed 61 sleep recordings in 40 patients with genetically proven SRS (methylation anomalies in 11p15 region or maternal unidisomy of chromosome 7): 21 prior, 39 during and 1 after GH therapy. Twelve patients

had sleep evaluation prior and after GH therapy initiation.

Results: mean apnea-hypopnea index (AHI) was 3.4 (0–12.4), with a mean central AHI of 0.5 (0–2.4). SDB was identified in 73.8% ($n = 45$) of the recordings and was severe in 4.9%. SDB was present in 86.4% of patients before GH therapy and was severe in 13.6%. In 12 patients with sleep recording before and after GH therapy initiation, AHI worsened in 5, and reached mild impairment. Mean GH dose was 0.23 mg/kg/week (0.09–0.37), with mean plasmatic IGF1 levels 1.7SDS (−1.9 to 6.6).

Discussion: Most patients with SRS present SDB with obstructive profile. As airways narrowness could explain SDB, we recommend systematic Ear-Nose-Throat evaluation in SRS patients and polysomnography in case of anomaly, preferably before GH therapy initiation.

PO.233

Impact of sleep quality on weaning duration in intensive care unit patients

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Rationale: In mechanically ventilated patients, mortality increases with weaning duration. It is then crucial to identify factors that lengthen weaning duration. Sleep deprivation has been shown to interfere with inspiratory endurance. Sleep alterations are particularly frequent in intensive care units and could delay weaning.

Our objective was to evaluate the impact of sleep quality on weaning.

Methods: All patients mechanically ventilated (>24 h) who failed the first spontaneous breathing trial were proposed to be involved in the study. Patients with ongoing sedation, neuromuscular disease, known neuropsychiatric diseases were excluded. An 18-h polysomnography was performed the night following the spontaneous breathing trial failure.

Results: Twenty-five patients were included in the study.

Fifty-seven percent patients had a weaning duration longer than 3 days and 43% had a weaning duration lasting less than 3 days. Age, PaO_2/FiO_2 ratio, total sleep time and number of arousals were not different in patients with long weaning and short weaning.

Patients with prolonged weaning had less REM sleep duration compared to patients with short weaning [0 min (0–4) versus 33 min (9–53), $P = 0.03$]. Absence of EEG reactivity (a feature of atypical sleep) was significantly more frequent in patients with long weaning. Atypical sleep or/and pathological wake tended to be more frequent in patients with long weaning (8/12 versus 2/9, $P = 0.08$).

Conclusion: A low amount of REM sleep and an altered EEG reactivity, both features of atypical sleep, could be associated with prolonged weaning. Atypical sleep, rather than fragmentation, could be the target to be improved to shorten weaning.

PO.234

High prevalence of obstructive sleep apnea (OSA) in patients with incident idiopathic pulmonary fibrosis (IPF)

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Introduction: We wanted to evaluate: (i) the prevalence of OSA in incident IPF; (ii) possible correlations between apnea-hypopnea index (AHI) and demographic, physiologic and chest HRCT data; (iii) whether OSA was associated with cardiovascular comorbidities.

Material and methods: This prospective study was conducted in five French Universitary Pulmonary Departments. Patients underwent nocturnal polysomnography, PFTs and HRCT. We registered smoking status, cardiovascular and metabolic comorbidities; their frequencies were compared between patients.

Results: Forty-five patients completed the study (age: 68.8 ± 8.7 years, BMI: 28 ± 3.5 kg/m^2, M/F: 38/7, FVC: 72.8 ± 20.2%, DLCO: 45.1 ± 18.9%). Forty (88.8%) had OSA: 12 (26.6%) mild OSA (AHI, 5–15 events/h), 10 (22.2%) moderate OSA (15–30 events/h), and 18 (40%) severe OSA (≥30 events/h). AHI did not correlate with age, BMI, FVC, DLCO, extension of pulmonary fibrosis. All patients with severe OSA suffered from at least one cardiovascular comorbidity, versus only 40.7% of patients with AHI < 30 events/h ($P = 0.0002$). Coronary artery disease (CAD) was more frequent in patients with severe OSA (61.1% versus 18.5%, $P = 0.009$). Bivariate analysis showed that the association between CAD and severe OSA was independent of demographic characteristics, cardiovascular risk factors and comorbidities [OR for

CAD in severe OSA: 6.91 (1.78–26.82)]. Moderate-to-severe coronary artery calcifications on HRCT scans were more frequently detected in severe OSA (85.7% versus 30%; $P = 0.002$).

Discussion: OSA is highly prevalent in a French population of patients with incident IPF. AHI isn't correlated with demographic or physiologic characteristics. In this population, severe OSA is strongly associated with the presence of cardiovascular comorbidities, particularly CAD.

PO.235

Telomere shortening in middle-aged men with sleep-disordered breathing

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Introduction: Sleep disorders may lead to stress-induced premature aging and telomere shortening. We asked whether obstructive sleep apnea syndrome causing intermittent hypoxemia episodes was associated with telomere shortening independently from the comorbidities associated with this syndrome.

Materials and methods: Cross-sectional study in 161prospectivelly enrolled untreated middle-aged men free of known comorbidities related or unrelated to sleep apnea. Each participant underwent full standard overnight polysomnography. Patients with apnea sleep syndrome were naïve of treatment.

Results: By univariate analysis, telomere shortening was associated with older age, apnea-hypopnea index, oxygen desaturation index, waist circumference, and fat mass. After adjustment for age, only apnea-hypopnea index and oxygen desaturation index were significantly related to telomere shortening. Mean telomere length ratio was 0.70 ± 0.37 in the participants without sleep apnea, compared to 0.61 ± 0.22 and 0.53 ± 0.16 in those with mild-to-moderate and severe sleep apnea, respectively ($P = 0.01$). By multivariate analysis, apnea-hypopnea index and oxygen desaturation index was the only factor independently associated with telomere length. Arterial stiffness assessed by carotid-femoral pulse wave velocity correlated negatively with telomere length.

Discussion: Intermittent hypoxemia due to obstructive sleep apnea syndrome is a major contributor to telomere shortening in middle-aged males. Oxidative stress may explain this finding.

PO.236

Effects of pre-existing sleep quality on emotional reactivity

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While much is known about the effects of stress on sleep quality, few studies investigated how pre-existing sleep patterns determine emotional reactivity to experimentally induced real-life stressful events [1]. We report on pilot data conducted to this latest aim.

Sleep quality and emotional reactivity outcomes were tested for multiple correlations from an experiment ran during two consecutive days and nights at the sleep laboratory. Participants performed one different condition each day in a driving simulator, including a neutral scenario (NEU) and a stressful scenario (STR). STR included multiple stressful traffic challenges and random neutral events, while NEU only included neutral events on the same road.

Participants' pre-sessions sleep quality and emotional profiles were determined through actimetry, standardized questionnaires and a semi-structured medical interview. Heart rate variability (HRV) is taken as an objective marker of the physiological state related to emotional arousal. HRV outcomes were computed from 5-min rest measures recorded before and after driving sessions. The subjective stress experienced during driving was recorded using visual analogic scales.

We will show how individuals' pre-existing sleep quality is related to emotional reactivity to the stressful scenario. Results may aid to give more insight into prevention of chronic sleep problems after stressful events and aid to identify those at risk of developing chronic insomnia.

PO.237

Obstructive sleep apnoea in severe asthma modulates airway inflammation and remodelling

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Obstructive sleep apnoea (OSA) is frequently observed in severe asthma but causal link between the two diseases remains hypothetical. The role of OSA related- systemic and airway neutrophilic inflammation in asthma bronchial inflammation or remodelling has not been investigated. The aim of this study was to compare hallmarks of inflammation in induced sputum and features of airway remodelling in bronchial biopsies in a population of severe adult asthmatics.

Methods: An overnight polygraphy was performed in 55 patients referred for difficult-to-treat asthma. We compared sputum analysis, reticular basement membrane (RBM) thickness, smooth muscle area and vascular density in bronchial biopsies.

Results: We investigated 55 patients, whom 27 (49%) had OSA diagnosed on night polygraphy. Despite moderate increase in AHI (mean AHI 14.2 ± 1.6/h), a higher percentage of neutrophils and a lower percentage of macrophages, associated with elevated levels of IL-8 and MMP-9, was observed in the sputum in OSA patients. The RBM was significantly thinner in patients with OSA (5.9 ± 0.4 versus 7.8 ± 0.4 μm, $P < 0.05$). A negative correlation was found between RBM thickness and OSA severity assessed by the AHI. OSA patients compared with non-OSA patients did not differ in terms of age, sex, BMI, lung function, asthma control test or treatment. The proportion of patients with hypertension, cerebrovascular disease and diabetes was significantly higher in OSA patients.

Conclusion: Mild OSA in severe asthma patients is associated with an increased proportion of neutrophils in sputum and changes in airway remodelling.

PO.238

Effect of intermittent hypoxia during mice gestation on pups' growth and ventilation

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Introduction: Sleep apnea is rare in women except during pregnancy, with weight gain, oedema, change in CRF and hormonal changes. The consequences of gestational OSA are still poorly known. We hypothesized that intermittent hypoxia (IH) induces neurodevelopmental abnormalities of cardiorespiratory control and learning and that these abnormalities are related to inflammation.

Methods: IH was applied in pregnant mice (5% FiO_2) 10 h a day during either the last 10 days of gestation or the last 15 days, control mice are exposed to room air. The HI model was characterized by monitoring SpO_2 and by weighing the mothers. Cytokine assays were performed in maternal serum and tissue samples at the end of IH. The evaluation of the pups consisted of weighing, measuring cardio respiratory parameters at basal state and during hypoxic and hypercapnic challenges. Cognitive functions were evaluated at 8 days post-natal age (P8-9) by Fox battery tests and at P30 by the object recognition test.

Results: The IH induced oxygen desaturations and delayed weight gain in mother but neither serum nor tissue inflammation. In the IH-pups, we found transient respiratory anomalies (P5) with increase in basal normoxic ventilation (Ve, Ttot), delayed reponse to hypercapnic challenge (P12), a growth retardation up to 30 days of life but no abnormalities of cognitive function. No difference was noted between groups with the length of IH exposure.

Discussion: Apart the unexpected lack of inflammation these results are consistent with the current literature and suggest a modest but consistent impact on pups of gestational IH.

PO.239

The effect of dynamic stretching on short-term maximal performance and perceived exertion after a total sleep loss night in athletes

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Previous study has reported a significant reduction in shor-term maximal performance (Souissi et al., 2003) after a total-sleep-deprivation-night (TSD). However, no-previous study has investigated how to contract this reduction of performance after TSD. It has been shown that dynamic-stretching (DS) could improve short-term maximal performances (Chtourou et al., 2013). Therefore, we aimed to investigate the effect of DS after a TSD on short-term maximal performances.

In a randomized order, 15 athletes performed four tests sessions: normal-sleep-night (NSN) and TSD with no-stretching (NS) or DS. During each session, after NS or DS, participants performed the 5-m shuttle run test and the total distance covered during this test was recorded (D5R). The rating-of-perceived-exertion (RPE) was recorded after each test.

The results showed a significant reduction in D5R after TSD in comparison with NSN after NS and DS ($P < 0.05$). D5R increased after DS in comparison with NS after TSD and NSN ($P < 0.05$). The reduction of D5R after TSD was significantly lower after DS in comparison with NS. There was no significant effect of TSD on RPE after NSN and TSD.

The main findings of the present study was that DS performed after a TSD night could contract the reduction of performance typically observed after this condition. This is the first study that examined the relation between DS and TSD. However, the findings of the present study couldn't be explained by a reduced fatigue during DS as we didn't observe a significant effect on RPE.

EYPS Abstracts
Renal Physiology

YCO.001

Modulation of aldosterone-sensitive enac activity in the renal cortical collecting duct by protein kinase D

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Aldosterone is one of the key regulators of Na^+ conservation through its actions on the epithelial sodium channel (ENaC). We have previously reported a role for the protein kinase D isoform PKD1 in regulating renal Na^+ reabsorption. Here we report a novel mechanism by which aldosterone can regulate the subcellular trafficking of ENaC subunits through the activation of protein kinase D2 (PKD2) in CCD cells.

Aldosterone (10 nM) produced a rapid phosphorylation of PKD2 (within 10 min) and subcellular redistribution of PKD2 from the apical membrane into the cytosol in M1-CCD cells. The activation of PKD2 correlated with an increased abundance and stability of ENaC subunits at the apical membrane, an increase in the phosphorylation of the E3 ubiquitin ligase Nedd4-2 and a 3-fold stimulation of the amiloride-sensitive short-circuit current (I_{SC}). Suppression of PKD2 expression in M1-CCD cells using shRNA increased ENaC abundance at the apical membrane and produced an 8-fold stimulation of the I_{SC}. Conversely, aldosterone treatment resulted in a paradoxical 4-fold decrease in I_{SC} in the M1-CCD PKD2 knock-down cells.

In conclusion, we propose that PKD2 has two opposing actions on ENaC activity. PKD2 activation by aldosterone stimulates ENaC possibly by de-ubiquitination via PKD2-dependent phosphorylation of Nedd4-2. Knocking down PKD2 releases a tonic inhibitory effect of PKD2 on ENaC and exposes a previously unknown pleiotropic effect of aldosterone on ENaC membrane stability and channel conductance.

YCO.002

Effects of FGF23 and Klotho on adult rat cardiomyocytes in culture

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Introdution: The bone derived hormone fibroblast growth factor 23 (FGF23) and its co-receptor Klotho represent a novel endocrine axis regulating mineral metabolism in health and disease. In chronic kidney disease (CKD), serum levels of FGF23 rise and in contrast, tissue expression of Klotho decreases. Numerous studies identify elevated FGF23 as a predictor of adverse clinical outcome. In particular, elevated FGF23 has recently been associated with greater risks of major cardiovascular events and mortality. However, there have been very few studies that have attempted to address the direct effects of FGF23 on myocardium. Moreover whether Klotho is involved in FGF23- mediated actions on cardiomyocytes is still unclear. In this context, we investigate the role of FGF23 and Klotho in adult rat ventricular myocytes (ARVMs).

Materials and methods: We used video-edge-detection, epifluorescent microscopy and an Ionoptix® system, performed in isolated cardiomyocytes subjected to FGF23 or Klotho alone, or in association.
Results: We showed that FGF23 increases cell size, contractiliy and calcium transients in ARVMs, and induces arrhythmia in the presence of Isoprenaline. In addition Klotho prevents FGF23 effects on ARVMs. Indeed, ARVMs subjected to Klotho showed marked protection from FGF23-induced hypertrophic and pro-arrhytmiques responses.
Discussion: Altogether these data provide a direct evidence of the role FGF23 in adult cardiomyocytes and suggest that Klotho may have a beneficial effect in preventing adverse cardiovascular outcomes in patients with or without CKD.

YCO.003

Epithelial common gamma chain confers B and T cell-independent resistance to glomerulonephritis

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Introduction: The renal lesions developed during experimental anti-glomerular basement membrane glomerulonephritis (anti-GBM-GN), an equivalent to human immune-mediated glomerulonephritis, have been traditionally attributed to perturbations of T cell functions.
Material and methods: We studied the role of adaptive immunity and IL15 receptors in murine kidney by using an anti-GBM model in $Rag2^{-/-}$, $Rag2^{-/-}Il2rg^{-/-}$, $Rag2^{-/-}IL\text{-}2rb^{-/-}$ and $Rag2^{-/-}$Il15$^{-/-}$ mice.
Results: In this work, we observed that $Rag2^{-/-}$, or even $Rag2^{-/-}Il2rg^{-/-}$ or $Rag2^{-/-}Il2rb^{-/-}$, mice devoid of T/B/NK cells develop severe anti-GBM-GN. Compared to $Rag2^{-/-}$, $Rag2^{-/-}Il2rg^{-/-}$ or $Rag2^{-/-}$Il2rb$^{-/-}$ mice harbor an additional deletion of either the common gamma chain (γC) or the interleukin-2 receptor β subunit (IL-2Rβ) respectively, impairing IL-15 signaling in particular. Using bone marrow transplantation replenishment in $Rag2^{-/-}Il2rg^{-/-}$, and observing $Rag2^{-/-}Il15^{-/-}$ anti-GBM-GN, we demonstrated an important role for the renal epithelial-expressed IL-15 receptor (γC/IL-2Rβ) during anti-GBM-GN. As $Rag2^{-/-}$Il2rb$^{-/-}$ anti-GBM-GN lesions appear extremely severe compared to $Rag2^{-/-}Il2rg^{-/-}$, we analyzed the nature of the IL-15 renal response in primary renal cultured cells prepared from either wild-type, γC or IL-2Rβ knockout animals. As opposed to current lymphocyte data, we found that despite the absence of γC, IL-15 fully induces downstream JAK1/3 but not SYK. None of these IL-15 downstream events were observed in the absence of IL-2Rβ.
Conclusion: In conclusion, anti-GBM-GN lesions appear in the absence of lymphocytes and are dependent on unsuspected intrinsic renally-expressed γC receptor responses distinct from current data on lymphocytes.

YCO.004

Comparison of muscle-derived stem/progenitor cells and bone marrow mesenchymal stem cells for the treatment of acute kidney injury

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Introduction: The skeletal muscle-derived stem/progenitor cells (MDSPCs) have been thoroughly investigated in preclinical studies. However, the therapeutic potential of MDSPCs for acute kidney injury (AKI) has only been evaluated by our research group. We aimed to compare MDSPCs with bone marrow mesenchymal stem cells (BM-MSCs) and to evaluate their feasibility for the treatment of AKI.
Material and methods: Rats were randomly assigned to one of four groups: healthy controls, AKI group, AKI treated with MDSPCs, AKI treated with BM-MSCs. AKI was induced by gentamicin (80 mg/kg/day; i.p.) for 7 consecutive days. PKH-26-labeled MDSPCs and BM-MSCs (1×10^6 cells/animal) were injected intravenously 24 h after the last gentamicin injection. Physiological and histological kidney parameters were determined on day 0, 8, 14, 21, 28, 35 (6 animals per time point).
Results: Both, MDSPCs and BM-MSCs accelerated functional kidney recovery and regeneration, as

reflected by significantly lower serum creatinine levels and renal injury scoring, higher urinary creatinine and GFR levels ($P < 0.05$) compared with the non-treated AKI group. PKH-26 labelled MDSPCs and BM-MSCs were present in the renal cortex on day 9, day 21 and day 35, indicating the capacity of both cell types to migrate and populate the renal tissue. There was no significant difference in any parameters between MDSPCs and BM-MSCs at any time point ($P > 0.05$).
Discussion: Both, MDSPCs and BM-MSCs are capable of mediating functional and histological kidney recovery after AKI. MDSPCs were found equivalent to BM-MSCs, therefore can be considered as a potential candidate for the treatment of AKI.

Neurophysiology

YCO.005

Recombinant tissue plasminogen activator enhances microparticle release from mouse brain-derived endothelial cells

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Thrombolysis with recombinant tissue plasminogen activator (rt-PA) is currently the only approved pharmacological strategy for acute ischemic stroke. However, rt-PA exhibits vascular toxicity mainly due to endothelial damage. In order to investigate the mechanisms underlying rt-PA-induced endothelial alterations, we assessed the role of rt-PA in the generation of endothelial microparticles (EMPs), which are emerging biological markers and effectors of endothelial dysfunction.

The mouse brain-derived endothelial cell line bEnd.3 was used. Cells were treated with rt-PA at 20, 40 or 80 μg/mL for 15 or 24 h, and EMPs were quantified in the culture media using Annexin-V staining coupled with flow cytometry. Rt-PA enhanced EMP release from bEnd.3 cells with a maximal increase with the 40-μg/mL dose for 24 h (+78% compared to controls). Using tranexamic acid and trasylol we demonstrated that plasmin was involved in rt-PA-induced EMP release. The p38-MAPK inhibitor SB203580 and the poly (ADP-ribose)polymerase (PARP) inhibitor PJ34 also reduced rt-PA-induced EMP production, suggesting that p38-MAPK and PARP are downstream intracellular effectors of rt-PA. Rt-PA also altered the morphology of bEnd.3 cells that were furthermore no longer confluent. Plasmin was implicated in these changes, unlike p38-MAPK and PARP.

This study demonstrates that rt-PA induces the production of microparticles by cerebral endothelial cells, through plasmin, p38-MAPK and PARP pathways. Determining the phenotype of these EMPs could be of particular interest to clarify their role on the endothelium in ischemic conditions.

YCO.006

Adult onset hyperthyroidism impairs spatial learning and attenuates long-term potentiation: possible involvement of mitogen-activated protein kinase signaling pathway

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Purpose: Given the evidence that MAPK/ERK activation is part of non-genomic action of thyroid hormone, we investigated possible consequences of hyperthyroidism for cognitive functions of adult rats.
Methods: To this end two-aged rats were treated with L-thyroxine or with serum physiologic. Thyroxine (0.2 mg/kg, i.p.) was administered for 20 days to rats starting at postnatal day 40. Twenty rats *in each group were exposed to Morris water maze testing for measuring their performance in a hidden platform spatial task*. LTP was induced by application high frequency stimulation protocols at perforant pathway-dentate gyrus synapses. The averages of excitatory postsynaptic potential (EPSP) slopes and population spike (PS) amplitudes between 55 and 60 min after HFS was used as a measure of LTP magnitude. In a separate set of these rats, expression and phosphorylated levels of p38-MAPK, and its two downstream effectors, Elk-1 and CREB, were evaluated by quantitative reverse transcriptase polymerase chain reaction and Western blot.
Results: Hyperthyroid rats had delayed acquisition of learning compared to their wild type counterparts as shown by increased escape latency and distance moved on the last two trials of daily training in water maze. In addition, it is interest that hyperthyroid rats showed no difference during probe trial. A simple *t*-test for magnitude of LTP revealed a significant difference between euthyroid and hyperthyroid rats ($P = 0.003$). Western blot analysis of hippocampus

showed that hyperthyroidism increased phosphorylated p38-MAPK levels in naïve hyperthyroid rats.
Conclusion: The present study provides *in-vivo* evidence for action of L-thyroxine leading to increased phosphorylation of p38-MAPK, is responsible for impaired acquisition during actual learning in rats with hyperthyroidism.

Endocrinology

YCO.007

Do HNF-4 alpha and HNF-4 gamma have opposite effects in the susceptibility to type 2 diabetes?

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Introduction: Transcription factors, HNF-4α and HNF-4γ belong to nuclear receptor superfamily. In intestine, our team showed that HNF-4α controls epithelium homeostasis and HNF-4γ impacts the glucose homeostasis through a decreased incretin effect. Furthermore, HNF-4α and HNF-4γ have opposite effect on abundance and differentiation of enteroendocrine cells producing incretin hormones. We thus hypothesized that HNF-4α and HNF-4γ have opposite effects in susceptibility to diet-induced type 2 diabetes (T2D).
Materials and methods: We used 2 mouse models: 1-a constitutive and total HNF-4γ gene invalidation and 2-an inducible and intestine specific HNF-4α gene invalidation, both were fed with a high-fat/high-fructose diet for 6 weeks. Oral glucose tolerance tests, insulin and plasma enterohormones concentration measurements were performed to evaluate the T2D susceptibility. GLP-1 cells were quantified by immunohistochemistry. The transcription factors expression involved in the differentiation of enteroendocrine cells and enterohormones expression were studied by RT-qPCR.
Results: We show that the HNF-4γ invalidation protects mice from weight gain and glucose intolerance, induced by 6 weeks of diet rich in fat and fructose. The high-fat/high-fructose diet does not further modify the impact of HNF-4γ invalidation on the abundance of GLP-1 cells and the transcription factors expression involved in enteroendocrine lineage. Surprisingly, HNF-4α invalidation also protects from body weight gain and prevents the glucose intolerance through an increased insulinosensitivity.
Discussion: We demonstrate for the first time that HNF-4α and HNF-4γ increase the susceptibility to T2D by two different mechanisms. Targeting the enteroendocrine cell differentiation through nuclear receptors HNF-4 might be a new therapeutic approach in DT2. This abstract has been retracted.

Integrative Biology

YCO.008

A C-terminal truncating mutation in PIK3R1 impairs growth, development and insulin sensitivity in mouse liver, white adipose tissue and skeletal muscles

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Introduction: *Pik3r1* encodes three regulatory subunits (p85α, p55α and p50α) of the class IA phosphatidylinositide-3-kinase, and is critical in regulating growth and metabolism. Knockout of either p85α or p55α and p50α resulted in an insulin sensitivity improvement; however, recent human genetic studies have shown that a truncated mutation in PIK3R1 (p.Tyr657*) results in severe insulin resistance and lipodystrophy. We have generated a truncated Pik3r1 knock-in murine model to dissect its pathophysiological roles.
Materials and methods: The body weight and composition of the mice were measured longitudinally for 8 weeks. Insulin and glucose tolerance of the mice were assessed, and the physiological roles of the mutation in liver, white adipose tissues (WAT) and skeletal muscles were studied using a hyperinsulinemic euglycemic clamp and *ex vivo* muscle incubation. Insulin signalling was investigated by Western Blot with liver, WAT and skeletal muscles from mice administrated with insulin subcutaneously.
Results: Compared to wild type mice (WT), heterozygous mice (het) had a reduced fat gain, and were glucose intolerant and insulin resistant. Het had a reduced hepatic glucose production suppression, WAT lipolysis and skeletal muscle deoxyglucose uptake under insulin stimulation. The Western Blot data indicated impaired Akt signalling in these tissues.
Discussion: Our data suggest that insulin signalling in liver, WAT and skeletal muscles are impaired by truncation of Pik3r1. Mechanistically, reduced Akt signalling in these tissues could perhaps partially account for insulin resistance. We next plan to investigate if the non-canonical roles of Pi3kr1 may play a role in the insulin resistance.

Cardiovascular Physiology

YCO.009

Clathrin is the main retrograde trafficking pathway of the atria-specific KV1.5 channel

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Introduction: Trafficking of ion channels in cardiomyocytes is a highly dynamic process which is determinant for shaping action potential. In this study, we focused on the dynamic of Kv1.5 channel which carry the atria-specific potassium outward current.
Materials and methods: We first investigated the involvement of the cytoskeleton in Kv1.5 channel delivery to the plasma membrane. Staining dyes for tubulin or actin were used in live cells and eGFP-Kv1.5 trafficking was followed by Total Internal Reflection Fluorescence microscopy (TIRFm).
Results: Interestingly, Kv1.5 channels seemed to track along microtubules in the membrane plane and not associated with actin cytoskeleton. The endocytosis pathway for Kv1.5 channels in the atrial myocyte was investigated using high resolution 3D deconvolution microscopy. Kv1.5 channels were associated with clathrin vesicles but not with caveolin in atrial myocytes. Blockade of the clathrin pathway using hypertonic media or SiRNA induced a two fold increase in IKur (whole-cell patch-clamp) and an accumulation of Kv1.5 channels at the sarcolemma as shown by biotinylation assay. Clathrin blockade led to a moderate global increase of eGFP-Kv1.5 fluorescence (33%) in the membrane plane as observed by TIRFm. However, particle analysis showed a drastic accumulation of eGFP-Kv1.5 channels into big clusters (2.8 μm^2 compared to 0.12 μm^2 before treatment) and reduced mobility (before: 0.08 μm/s/ after: 0.03 μm/s).
Conclusion: The clathrin pathway is the main internalization route for Kv1.5 channels in atrial myocytes. The blockade of this pathway modifies Kv1.5 channels dynamic at the plasma membrane.

YCO.010

EnNaC-dependent endothelial nanomechanics are disturbed within endothelial dysfunction

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The mechanical properties of endothelial cells (ECs) strongly influence endothelial function as they control the production of the vasodilator nitric oxide (NO). Recently, the endothelial Na^+ channel (EnNaC) could be identified as modulator of the mechanical properties of ECs, in that its membrane abundance determines endothelial stiffness. One factor increasing EnNaC membrane abundance and thus EC stiffness is the mineralocorticoid aldosterone. In contrast, mineralocorticoid receptor antagonism (spironolactone) and functional EnNaC inhibition (amiloride/benzamil) induce cortical softening. In a clinical trial we found an abolished softening after amiloride exposure in ~50% of *ex vivo* preparations from human vessels. This observation is associated with two predictors of cardiovascular risk. Since the EnNaC function can be defined as marker for endothelial dysfunction, it is postulated that the regulation of EnNaC-dependent endothelial plasticity is disturbed within endothelial dysfunction. To test this, ECs derived from a mouse model for endothelial dysfunction (ApoE/LDLR−/−) were employed and compared to wildtype (WT) EC. *Ex vivo* patches of aortic rings were fixed onto glass-dishes with the ECs facing upwards and studied with an Atomic Force Microscope (AFM) to detect the endothelial stiffness. We found the basal stiffness increased in the ApoE/LDLR−/− ECs by ~10% compared to WT. Benzamil (1 μM) and spironolactone (100 nM), however, reduced the stiffness in WT (~38% and ~30% respectively), but not in ApoE/LDLR−/−. We conclude that EnNaC contributes to the phenotype and function of the vascular endothelium and that a loss of an adequate endothelial response to functional EnNaC inhibition potentially reflects endothelial dysfunction and vascular damage.

Exercise Physiology

YCO.011

Acute cardiovascular responses to prolonged eccentric cycling

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For the same mechanical power, a lower energy expenditure is required during eccentric (ECC) than concentric (CON) exercise. ECC exercises are thus of interest for populations with reduced capacities. However, little is known about specific ECC cardiovascular adjustments, especially for exercise lasting 30–45 min as used for rehabilitation purpose.

Ten healthy men (30 ± 6 years) performed two 45-min CON and ECC cycling sessions. During the first 5-min, power was adjusted to elicit similar heart rate (HR), and was maintained thereafter. HR, systolic (SAP) and diastolic (DAP) arterial pressures, cardiac output (CO), stroke volume (SV), VO_2, and number (Nseq) and length (Lseq) of baroreflex sequences were recorded. A Two Way Repeated Measures ANOVA was performed: times (5, 15, 30, 45 min) x exercise modality (CON/ECC).

Power output was 82 ± 16W and 210 ± 40W for CON and ECC, respectively. SAP, CO and VO_2 were similar during ECC and CON. A slow HR drift was observed during ECC (significantly higher at 45 min versus CON). SV was lower while DAP was higher during ECC throughout the exercise. Nseq and Lseq were lower during ECC at 30 and 45 min.

We observed indices of increased peripheral resistances during ECC versus CON cycling that decrease SV, increase HR and impaired baroreflex activity. Thus, despite similar HR at the beginning of exercise, our results highlighted increased constraint on the cardiovascular system during ECC, suggesting that caution should be taken when prescribing ECC for rehabilitation purpose.

Respiratory Physiology

YCO.012

Role for IL-1R1/MYD88 signalling in development and progression of pulmonary hypertension

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Objective: Pulmonary arterial hypertension (PAH) results from complex vessel remodeling involving pulmonary-artery smooth muscle cell (PA-SMC) proliferation and inflammatory processes. Interleukin-1ß (IL-1ß) binds to its receptor IL-1R1, thereby recruiting the molecular adaptor MyD88 (which allows signal transduction via IL1R1 and toll-like receptors) and inducing the synthesis of IL-1, IL-6, and TNFα via NF-kB activation. We investigated the role for the IL1R1/MyD88 pathway in the pathogenesis of PH.

Approach and results: Marked IL-1R1 and MyD88 expression, with predominant immunostaining of PA-SMCs, was found in lungs from patients with idiopathic PAH, mice with hypoxia-induced PH, and SM22-5-HTT+ mice developing spontaneous PH. The elevations in lung IL-1ß, IL-1R1, MyD88, IL-6, and TNFα preceded PH in hypoxic mice. IL1R1$^{-/-}$ mice, MyD88$^{-/-}$ mice, and control mice given the IL1R1 antagonist anakinra were similarly protected against the development of hypoxic PH and recruitment of perivascular macrophages. Anakinra treatment partially reversed PH in SM22-5-HTT+ mice. The potent IL-1ß-mediated growth-promoting activity on mouse PA-SMCs was abolished by anakinra and absent in PA-SMCs from IL1R1$^{-/-}$ and MyD88$^{-/-}$ mice. Mice with MyD88 gene deletion confined to the myeloid lineage (M.lys-Cre MyD88$^{fl/fl}$ mice) showed less severe PH compared to controls but more pronounced PH compared to MyD88$^{-/-}$ mice, suggesting IL-1ß-mediated effects on PA-SMCs and macrophages. Accordingly, the growth-promoting effect of media conditioned by M1 or M2 macrophages from M.lys-Cre MyD88$^{fl/fl}$ mice was attenuated.

Conclusions: Pulmonary vessel remodeling and inflammation during PH development require IL1R1/MyD88 signaling. Pharmacological interventions targeting the IL-1b/IL1R1 pathway may hold promise for treating human PAH.

YCO.013

Imaging dynamic lung strain and specific elastance by K-edge subtraction computed tomography

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Introduction: Artificial ventilation induces mechanical stresses that are potentially injurious to the lung tissue. We describe a new technique to quantitatively image regional lung Strain and Specific Elastance that uses radiation generated by a synchrotron source.
Materials and methods: We used K-edge subtraction synchrotron CT imaging during the washin of stable Xe gas, in 3 healthy anaesthetized rabbits at 2 axial image levels, to image regional specific ventilation (sV') at a PEEP of 0 (ZEEP) and 9 cmH_2O. Dynamic Strain within a given image voxel was computed based on sV', as the percent tidal volume change per voxel gas volume. Specific elastance was given by: (Plateau pressure – end expiratory pressure)/Strain.
Results: While mean dynamic strain significantly decreased with PEEP (14.8 ± 4.6 versus $17.3 \pm 7.7\%$, $P = 0.001$), specific elastance increased (1.63 ± 0.23 versus 0.67 ± 0.21 $cmH_2O/\%$, $P = 0.002$). High specific-elastance regions with poor ventilation in one animal on ZEEP were reversed with PEEP (Figure not uploaded).
Discussion: Our data demonstrate the feasibility of imaging regional dynamic strain and specific elastance, using synchrotron K-Edge subtraction CT. This method can be useful for exploring the consequences of changes in mechanical ventilation settings on regional lung function in preclinical models of disease.

Respiratory Physiology

YPO.001

Increased ventilatory variability and complexity in patients with hyperventilation disorder

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Introduction: It has been hypothesized that hyperventilation disorders could be characterized by an abnormal ventilatory control leading to enhanced variability of resting ventilation.
Materials and methods: The non-normal distribution of tidal volume [VT] was described by the negative slope characterizing augmented breaths formed by the relationship between the probability density distribution of VT and VT on a log-log scale. The objectives of this study were to describe the variability of resting ventilation [coefficient of variation (CV) of VT and slope], the stability in respiratory control (loop, controller and plant gains characterizing ventilatory-chemoresponsiveness interactions) and the chaotic-like dynamics (embedding dimension, Kappa values characterizing complexity) of resting ventilation in patients with a well-defined dysfunctional breathing pattern characterized by air hunger and constantly decreased $PaCO_2$ during a cardio-pulmonary exercise test.
Results: Compared to 14 healthy subjects with similar anthropometrics, 23 patients with hyperventilation were characterized by increased variability of resting tidal ventilation [CV of VT median (interquartile): 26% (19–35) versus 36% (28–48), $P = 0.020$; slope: −6.63 (−7.65; −5.36) versus −3.88 (−5.91; −2.66), $P = 0.004$] that was not related to increased chemical drive [loop gain: 0.051 (0.039–0.221) versus 0.044 (0.012–0.087), $P = 0.149$] but that was related to an increased ventilatory complexity (Kappa values,

$P < 0.05$). Plant gain was decreased in patients and correlated with complexity (with Kappa 5 – degree 5: Rho = −0.48, $P = 0.006$).

Conclusion: Well-defined patients suffering from hyperventilation disorder are characterized by increased variability of their resting ventilation due to increased ventilatory complexity with stable ventilatory-chemoresponsiveness interactions.

YPO.002

Correlation of diffusing capacity of the lung for carbon monoxide (DLCO) components Dm and Vc with high resolution computed tomography (HRCT) and their prognostic impact in idiopathic pulmonary fibrosis (IPF)

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Introduction: DLCO is constantly decreased in IPF. The mechanisms are usually complex, involving impairment of the transfer through alveolar-capillary membrane and/or loss of capillary bed. Combined DLCO/DLNO allows dividing lung transfer into membrane diffusing capacity (Dm) and capillary volume (Vc).

Material and methods: We included 55 patients with incident IPF in this prospective, monocentric study. They performed PFTs with DLCO/DLNO, echocardiography and HRCT. The pulmonary artery diameter/body surface area (PA/BSA) ratio was measured. These parameters were correlated and their prognostic impact on transplantation-free survival was assessed.

Results: Mean age at inclusion was 65 ± 11.7 years. Functional parameters were: FVC 77.2 ± 17.69%; DLCO 43.8 ± 15.88%; DLNO 39.0 ± 16.2%; Dm 53.3 ± 21.88%; Vc 42.1 ± 17.6% of theoretical values. Dm correlated to FVC, desaturation during 6MWT, HRCT fibrosis score, PA/BSA ($r \geq |0.45|$). Vc had a similar profile, but neither Vc nor PA/BSA correlated to sPAP. Median transplantation-free survival was 53.5 months. HRCT fibrosis score, emphysema score and PA/BSA were all predictive of survival using univariate Cox regression analysis. Among functional parameters, Dm was a better prognostic factor than DLCO or desaturation during 6MWT. In a step-by-step multivariate model, the remaining factors were HRCT fibrosis score [HR = 1.71 (1.25–2.37)], PA/BSA [HR = 1.19 (1.04–1.35)] and sPAP [HR = 1.27 (1.05–1.53)]. In an alternative model, fibrosis score could be replaced by Dm.

Discussion: Dm and Vc are both decreased in incident IPF, and Dm is the most powerful functional prognostic indicator. Another original finding is the strong prognostic value of PA/BSA, independent of fibrosis score and sPAP, suggesting that it takes into account various mechanisms.

YPO.003

Genetic inactivation of the phospholipase A2 receptor (PLA2R1) protects against lung cell senescence in chronic obstructive pulmonary disease (COPD)

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Telomere dysfunction and exaggerated lung cell senescence now appear as major pathological processes leading to lung alterations in COPD. However, the mechanisms underlying lung cell senescence in COPD remain unclear. Recent studies suggest that cell senescence may occur in response to activation of the phospholipaseA2 receptor (PLA2R1) signaling pathway.

We assessed the link between PLA2R1 activation and cellular senescence in lung specimens, derived cultured pulmonary-endothelial cells (P-ECs) and pulmonary artery smooth muscle cells (PA-SMCs) from patients with COPD and control. Cells were infected by retroviral vectors encoding PLA2R1, shRNA PLA2R1, shRNA p53 and control vectors.

Increased PLA2R1 protein levels were found in lungs with prominent immunostaining in pulmonary vessels from patients with COPD. Increased PLA2R1 mRNA and protein levels were also measured in cultured PA-SMC and P-ECs from patients with COPD compared to controls, which exhibited an early onset of cell senescence as assessed by a decreased number of population doubling and an increase in β-gal-positive cells. PLA2R1 knockdown in P-ECs or PA-SMCs delayed the onset of cell senescence to a similar degree as the cells infected by shRNA p53, it was also associated with significant decreases in inflammatory cytokines released by senescent cells. Constitutive expression of PLA2R1 in PA-SMCs induced cell senescence, whatever the cells were from patient with COPD or from controls.

Lung PLA2R1 overexpression in patient with COPD and ability of PLA2R1 knockdown to delay replicative cell senescence support the concept that this signalling pathway is strongly involved in the lung cell senescence process in COPD.

YPO.004

Repetitive transcranial magnetic stimulation elicits long lasting phrenic motoneuron excitability increase in a rat model of respiratory neuroplasticity

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Introduction: Repetitive transcranial magnetic stimulation (rTMS) is a cortical neuromodulation technique that has already proven its clinical effectiveness. However, the underlying mechanisms are poorly understood, thus emphasizing the need to develop pre-clinical animal models. We have recently demonstrated the feasibility of using TMS to evaluate diaphragmatic corticomotor pathways by recording specific motor evoked potentials (MEPdia) in normal rats and in a pre-clinical rat model of neurological respiratory deficit (Vinit et al., 2014, 2016). We evaluated the evolution of the phrenic motoneuron excitability (MEPdia) following an acute frequency rTMS protocol applied to the cortex of our animal model.
Materials and methods: In an anesthetized adult rat preparation, we applied a repetitive high frequency rTMS protocol (9 trains of 100 biphasic pulses at 10 Hz, with 30s intervals) and evaluated the phrenic motoneuron excitability by recording MEPdia before and up to 60 min after the rTMS session.
Results: Acute high frequency rTMS protocol induces a robust and long lasting increase in phrenic motoneuron excitability (39.9 ± 6% increase from baseline compared to time control animals 1.7 ± 4.4%) at 60 min post rTMS protocol. Interestingly, pretreatment with Clonazepam ($GABA_A$ agonist) before rTMS blocks this increase in respiratory excitability.
Discussion: rTMS protocol can induce a long-lasting respiratory excitability increase in normal animal, based on GABAergic receptors repression. Additional experiments are needed to investigate whether chronic daily rTMS session might extend this acute effect, and might be used to treat some neurological respiratory deficits.

YPO.005

Respiratory response during maximal exercise testing in children with single ventricle compared to healthy population

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Introduction: Cardio-pulmonary exercise test is recommended in the follow-up of patients with chronic heart failure. However few data are available in the pediatric cardiac population, especially for single ventricle. The aim of this study was to evaluate the VO_{2max} and ventilatory response among children with single ventricles during a maximal exercise testing compared with controls.
Method: Fifty-eight children with single ventricles and total cavo-pulmonary connexion (TCPC) (11.1 ± 2.6 years old) and 302 healthy children (12.4 ± 3.2 years old) performed a maximal cardio-pulmonary exercise test; they were divided in three sub-groups based on age (6–9 years/old, 10–13 years/old and 14–17 years/old).
Results: Compared with healthy children, VO_{2max} was lower (32 ± 7 versus 43 ± 7 mL/kg/min, $P < 0.005$) in children with single ventricles. At maximal exercise, the calculated ratio of dead space volume over tidal volume (VD/VT) was higher (23 ± 9 versus 19 ± 7%, $P < 0.005$). During exercise, the VE/VC02 slope was higher (36.6 ± 5 versus 29.6 ± 4, $P < 0.005$) and the oxygen uptake efficiency slope (OUES) was lower (1161 versus 1858, $P < 0.005$). Moreover, differences observed increased with age ($P < 0.005$). Finally we observed a correlation between VO_{2max} and OUES ($P < 0.005$)
Conclusion: Infants with single ventricles and TCPC present a lower VO_{2max} and an impaired ventilatory response to exercise, that deteriorate with age. Ventilatory response during exercise could be a useful variable to take into consideration: indeed, as opposed to VO_{2max}, this variable does not need to be measured at maximal exercise performance.

YPO.006

Impact of acidification in ASL on bacterial killing capacity in cystic fibrosis airways

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Introduction: Respiratory failure determines prognosis in Cystic Fibrosis (CF). CF patients are colonized in childhood with *Staphylococcus aureus*.

It has been shown in pig CF model that pH influence bacterial killing capacity by acting on Airway Surface Liquid (ASL) antimicrobial peptides activity (Pezzulo *et al.* Nature, 2012).

We hypothesize that HCO_3^- transport is defective in CF respiratory epithelium which in turn alters bacterial killing efficiency.

Methods: pH was measured using a microelectrode in WT and F508del bronchial cells lines and in bronchial primary cells after apical addition of 50 μL ringer (25 mM HCO_3^-, pH = 7.4).

To evaluate bacterial killing capacity, we infected epithelia apically with *S. aureus* CIP 76.25 and collected ASL after 1 h30 incubation to count survival bacteria.

Results: A significant difference in ASL pH was found between WT and CF cell lines (WT: 7.83 $\pm$ 0.06 versus CF: 7.69 $\pm$ 0.03; ($P = 0.036$, $n = 7$). The same pattern of data was observed in primary cells (WT: 7.69 $\pm$ 0.02 versus CF: 7.58 $\pm$ 0.03; ($P = 0.007$, $n = 6$).

A significantly higher bacterial killing capacity was observed in WT than in CF CFBE and in primary cells. After infecting epithelia with an inoculum of 3000 CFU/mL, we collected about 4400 versus 76000 CFU/mL in WT primary cells ASL versus CF ($P = 0.036$, $n = 6$).

Discussion: F508del epithelium presents defect in ASL pH regulation and in bacterial killing capacity.

We plan to investigate further the mechanism of HCO_3^- transport in airways, especially CFTR role and to test the association between defect in ASL pH regulation and bacterial killing capacity defect in airways.

YPO.007

Curosurf enriched with selective NF-KB inhibitor IKK-NBD peptide improves lung function more effectively than budesonide in meconium induced acute lung injury

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Introduction: Meconium aspiration syndrome (MAS) is associated with triggering of inflammatory and oxidative pathways which cause inter alia surfactant inactivation and thus respiratory failure. Comparing addition of corticosteroid and alternative selective inhibitor of NF-kB, a key factor of inflammation, into the exogenous surfactant (Curosurf) therapy verified a treatment potential of these two combinations in experimental MAS therapy.

Methods: New Zealand white rabbits with meconium-induced respiratory failure were divided according to the therapy to: non-treated (Mec group), surfactant-treated (Surf group) and treated with combination of surfactant and budesonide (Pulmicort) (Surf+BUD group) or surfactant and selective inhibitor of NF-κB (IKKγ NEMO Binding Domain Inhibitor) (Surf+IKK-NBD group). Blood gases and ventilation parameters, i.e. PaO_2/FiO_2, oxygenation index (OI), $PaCO_2$, O_2 saturation ($SatO_2$), ventilation efficiency index (VEI), were observed before and 30 min after meconium instillation and 30 min, 1, 2, 3, 4 and 5 h after the treatment.

Results: Surfactant treatment improved OI and $SatO_2$ compared to untreated animals immediately after administration. Surf+BUD increased the therapy effectiveness in PaO_2/FiO_2, $PaCO_2$ and VEI compared to Mec group. Surf+IKK-NBD combination improved the therapy significantly in PaO_2/FiO_2, OI compared to Surf group and in VEI compared to Surf+BUD group.

Discussion: The addition of IKK-NBD to surfactant therapy rapidly improved ventilation parameters, probably due to suppressing inflammation and preventing surfactant inactivation. Thereby, selective NF-kB inhibitor may be a useful alternative for potential improvement in the MAS therapy.

YPO.008

Ventilatory oscillations in healthy humans at exercise in hypoxia: a mathematical model

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Introduction: Periodic breathing during exercise in hypoxia was recently observed and described. Spectral analyses of ventilation (VE) signals evidenced a 11-second periodic pattern. Further analyses showed that exercise and greater cardiac output (Qc) shorten period and increase magnitude of VE oscillations in hypoxia (versus rest and normoxia). Total respiratory cycle time (Ttot) was the main factor correlated with changes in VE period.

Materials and methods: A mathematical model was built around pulmonary gas exchange and chemoreflex regulation systems, connected by the cardiovascular system: VE is the sum of a basal component and peripheral and central chemoreflex modulations. The model includes environmental, physiological and systemic parameters: inhaled fraction of O_2 (FIO_2), circulation delays from lung to peripheral and central chemoreceptors ($DeltaTO_2$ and $DeltaTCO_2$ respectively), lung capacity (TLC), peripheral and central gains (Gp and Gc respectively).

Results: Physiological observations were confirmed by various simulations, with a 11-s period. Greater $DeltaTO_2$ and FIO_2 lengthened VE period whereas they had adverse effects on magnitude. A sensitivity analysis allowed us to identify the important factors responsible for VE oscillations. Gp, FIO_2, $DeltaTO_2$ and $DeltaTCO_2$ had a significant impact on VE magnitude.

Discussion/conclusion: Our model closely matched with the observed experimental data in various protocols and studies, confirming the existence of an internal oscillator in ventilation control. It demonstrated the crucial role of circulatory delays and gains of chemoreceptors in the homeodynamic behavior of the respiratory control system when exposed to brisk changes in physiological or environmental constraints.

YPO.009

Dynamic mechanical interactions between neighboring airspaces determine cyclic opening and closure in injured lung

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Rationale: Positive pressure ventilation exposes the lung to mechanical stresses that can exacerbate injury. The exact mechanism of this pathological process remains elusive.

Objectives: To quantify the extent and distribution of recruitment/derecruitment (R/D) at acinar length scales over short time frames using phase-contrast synchrotron tomography imaging and produce a mechanistic computational model compatible with the observed spatial and temporal distributions of R/D.

Methods: Experiments were performed in anaesthetized rabbits ventilated in pressure controlled mode with 6 mL/kg tidal volume. The lung was imaged nine times at ~1.5 min intervals, consecutively at positive end-expiratory pressure (PEEP) 12, 9, 6, 3 and 0 cmH_2O before and after injury. The extent and spatial distribution of R/D was analysed by registering and subtracting subsequent volumetric images. In a 3D right lung branching structure we implemented a mechanistic model in which each unit has individual pressures and speeds of opening and closing.

Results: R/D occurred in neighboring alveoli over short time scales despite stable pressure controlled ventilation. The computational model accurately reproduced this behavior only when structural deformations were considered as dynamic phenomena and when parenchymal interdependence between neighboring acini was included.

Conclusions: Our data show that cyclic R/D of neighboring airspaces can occur as a result of dynamic opening/closure of airways and acini, provided that mechanical interactions exist between neighboring terminal lung units. These findings give further insight into the microscopic behavior of the injured lung and provide a means of testing protective-ventilation strategies to prevent R/D and subsequent lung damage.

YPO.010

Breathing-related postural disturbance in patients with left hemidiaphragm weakness

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Introduction: Unilateral diaphragmatic contractions induced by phrenic stimulation in healthy subjects result in medial-lateral acceleration of the center of gravity (Hamaoui et al. 2014). We hypothesized that this would also occur during volitional breathing in patients suffering from unilateral diaphragm dysfunction.
Methods: Six patients with unilateral left hemi-diaphragmatic dysfunction (65 ± 13 years, 174 ± 18 cm, 76 ± 11 kg) participated in this study. Center of gravity (CG) acceleration was recorded using a force plate and respiratory kinematics using thoracic and abdominal belts. Patients performed sniff maneuvers in theseated and standing postures.
Results: While the patients were seated, abdominal sniff maneuvers consistently produced a forward and left acceleration of the CG. This was not detectable in the standing posture.
Discussion: The results suggest that a dysfunction of the left hemi-diaphragm induces a postural disturbance characterized by an additional leftward medial-lateral component. Consequences on gait remain to be assessed. Ongoing experiments will extent this study to dysfunctions of the right hemi-diaphragm.

YPO.011

Effects of repetitive magnetic cervical stimulation of phrenic roots on diaphragmatic function in healthy volunteers

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Diaphragmatic inactivity might decrease diaphragmatic trophicity and strength. Transcutaneous magnetic stimulation of phrenic nerves is commonly used to assess diaphragmatic function but has never been used repeatedly to maintain diaphragmatic activity. This preliminary study was aimed at determining the consequences of a session of repeated cervical magnetic stimulations (CMS) on peripheral and central fatigue of the diaphragm.

Fifteen healthy volunteers were involved with one placebo and one effective experimental sessions in a random order. During these sessions, phrenic roots were stimulated by single twitches every 8 s over 1 h. Diaphragmatic function was assessed before (T0), immediately (T1), and 30 min (T2) after each session using CMS induced Transdiaphragmatic pressure (Pditw), diaphragm EMG signal in response to CMS and to transcranial magnetic stimulation (TMS) and maximal voluntary inspiratory transdiaphragmatic pressure (Pdimax). Tolerance was evaluated by an analog scale quantifying discomfort. The effect of each session was considered as positive (if Pditw increased more than 11% versus baseline), negative (if Pditw decreased more than 11%) or neutral .

Four subjects 'effective session', happened to be negative. However, only one subject had a negative effecton both T1 and T2 corraleted with a decrease of others parameters. For other subjects, the negative effect was transient and not correlated with any other change parameter. Three subjects described a discomfort. The 'placebo' session was also followed by transient negative effect in two subjects.

None of these 15 healthy volunteers had any sign of peripheral or central fatigue but one. The safety and interest of such 'training' session on diaphragmatic function should now be assessed in ventilated patients.

YPO.012

Targeting the mtor signaling pathway to inhibit lung cell senescence in chronic obstructive pulmonary disease (COPD)

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Cell senescence, defined as a stable cell-cycle arrest combined with stereotyped phenotypic changes, might play a causal role in COPD. We previously reported that senescent cells are increased in lungs from patients with COPD and express a robust senescence-associated secretory phenotype (SASP). The aim of this study was to investigate whether lung cell senescence in COPD was related to overactivation of the mTOR signaling pathway and whether targeting this pathway would inhibit cell senescence and/or suppress the SASP in COPD. Strong activation of the mTOR signaling pathway was found in lungs from patients

with COPD compared with controls, with an activation of mTOR complex 1 (mTORC1) substrates, and of mTORC2 substrates, together with an increase in p21 and p16 protein levels. Similar activation of the mTORC1 and mTORC2 substrates were found in cultured PA-SMCs and P-ECs from patients with COPD compared with controls. Cultured P-ECs or PA-SMCs from patients with COPD exhibited an early onset of cell senescence as assessed by a decrease in the number of population doubling (PDLs). Treatment of the cells from patients with COPD with rapamycin (10 nM) normalized the number of PDLs to that seen in controls and decreased the number of beta-gal-positive cells. Cultured PA-SMCs from SM22-TSC1−/− mice, which exhibited strong mTORC1 activation in PA-SMCs, were characterized by an early onset of cell senescence compared with control mice. These results show that the increased propensity of lung cells to senescence in COPD is related to over-activation of the mTOR signaling pathway and can be suppressed by low doses of rapamycin.

YPO.013

Effect of chronic intermittent hypoxia (IH) in a murine model of bleomycin-induced pulmonary fibrosis

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Introduction: Obstructive sleep apnea (OSA) is common in idiopathic pulmonary fibrosis (IPF). The impact of repeated episodes of hypoxia-reoxygenation is unknown. We evaluated whether chronic IH would modulate the severity of bleomycin-induced pulmonary fibrosis in mice.

Material and methods: C57Bl6J mice received intratracheal bleomycin (Bleo, 3.5UI/g) or saline solution (SS), and were exposed to IH (40 cycles/h; FiO_2 nadir: 6%; 8 h/day) or air (IA) until sacrifice at day 4, 8 or 21. In each of the 4 groups (SS-IA, SS-IH, Bleo-IA, Bleo-IH), we evaluated: overall survival, lung inflammation and oxidative stress, lung cell apoptosis, pulmonary fibrosis.

Results: Survival was 100% at day 21 for SS-IA and SS-IH mice, 70% for Bleo-IA, 52% for Bleo-IH ($P = 0.015$). At day 4, Bleo-IH mice showed more severe neutrophilic alveolitis ($P < 0.001$), increased oxidative stress in broncho-alveolar lavage fluid ($P = 0.07$) and lower lung protein expression of antioxidant enzymes ($P < 0.05$). At day 8, wet-to-dry lung weight ratio was increased in the Bleo-IH group ($P = 0.014$). Immunohistochemistry revealed greater apoptosis in the lung of Bleo-IH mice, with higher percentage of nuclei positive for caspase-3 staining than in Bleo-IA mice (66.8 ± 0.10 versus $40.1 \pm 0.05\%$; $P = 0.006$). At day 21, pulmonary fibrosis was more severe in Bleo-IH mice, as assessed by lung collagen contents ($P = 0.02$) and histological analysis.

Discussion: Exposure to chronic IH increases mortality, lung inflammation and lung fibrosis in bleomycin-treated mice, through an increase in lung oxidative stress. This work raises the question of the potential worsening impact of OSA in IPF.

YPO.014

Progesteronergic system plays a key role during hypoxic but not hypercapnic ventilatory response, regardless of the presence of erythropoietin

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Introduction: Erythropoietin (Epo) is synthetized by neurons and astrocytes and plays a key role in the ventilatory response to hypoxia (HVR). Furthermore, Epo interacts with female sexual hormones in HVR. However, few studies focused on the role of Epo and these hormones on ventilatory response to hypercapnia (HcVR).

Material and methods: To study the impact of Epo and sexual hormones on HcVR and HVR, we used WT and Epo-deficient female mice (Epo-TAg^h). Ventilatory parameters were evaluated using plethysmography in hypercapnic (4% CO_2) or in hypoxic (8% O_2) conditions, with or without progesterone antagonist drug RU486 (Mifeprestone). Metabolic response to hypoxia and hypercapnia was also evaluated by indirect calorimetry.

Results: Epo-TAg^h displayed higher minute ventilation in normoxia when compared to WT mice. Furthermore, WT mice have a higher HcVR in the luteal phase of the ovarian cycle whereas HcVR of Epo-TAg^h was not affected by hormonal cycles. HVR was normal in WT mice but Epo-TAg^h mice did not respond to acute hypoxia. RU486 treatment had no effect on HcVR but increased HVR in WT and restored HVR in Epo-TAg^h mice.

Discussion: The difference in HcVR in Epo-TAg^h female mice supports the hypothesis that interaction between Epo and sexual hormones is necessary for normal CO_2 chemosensitivity. Furthermore, Epo is essential for normal HVR. Finally, experiments with

RU486 suggest that progesteronergic system have no influence on CO_2 chemosensitivity but could have an inhibitory effect on HVR regardless of the presence of Epo.

Renal Physiology

YPO.015

Hepatic fibroblast growth factor 23 production in autosomal dominant polycystic kidney disease

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FGF23 is a central regulator of phosphate homeostasis that promotes urinary phosphate excretion. This hormone has recently emerged as a biomarker predicting death and left ventricular hypertrophy in patients with reduced renal function. Patients with autosomal dominant polycystic kidney disease (ADPKD) have been reported to display high FGF23 concentration but the mechanisms involved and the consequences of this elevation on phosphate homeostasis and cardiac function remain unclear.

Using different analytic approach of a cohort of 320 patients with autosomal dominant polycystic kidney diseases and 485 control subjects who underwent FGF23, mineral metabolism hormones and GFR measurement at our institution between 2007 and 2013, we show that ADPKD patients display higher FGF23 plasma concentrations than matched controls but do not present renal phosphate leakage. Unexpectedly, we found a positive correlation between FGF23 plasma concentration and liver volume, an indicator of the severity of cystic liver disease in this population. We further show that FGF23 hepatic mRNA expression is increased in ADPKD patients and trace the increase in FGF23 expression to pericystic hepatocytes. Finally, a retrospective analysis of echocardiographic finding in 63 ADPKD patients shows that high FGF23 concentration correlates positively with E/A ratio, an indicator of diastolic dysfunction but not with left ventricular mass or ejection fraction.

This study reveals that an ectopic production of FGF23 by pericystic hepatocytes contributes to FGF23 elevation in ADPKD and that, in this population high FGF23 correlates with a reduction in left ventricle compliance.

YPO.016

Albuminuria and microcirculation in patients with sickle cell disease: evidence for an impaired myogenic tone and a preserved no biovailability

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Introduction: Sickle cell associated nephropathy (SCAN) is encountered in up to 80% of homozygous sickle cell disease (SCD) patients and characterized by a microangiopathy responsible for albuminuria and/or hyperfiltration before chronic renal failure. Though cardiac output increase, glomerulomegaly and peritubular capillaries rarefaction are well known striking features, the pathophysiology of SCAN remains mostly unraveled.

Patients and methods: We conducted a prospective study in 21 patients with SCD nephropathy (mGFR >60 mL/min/1.73 m^2) and in 26 healthy volunteers. We examined skin blood flow (SkBF) using laser-Doppler fluxmetry and analyzed spectral signals to investigate endothelial, sympathetic, and myogenic activities under basal, post occlusion, hyperthermia and acetylcholine stimulation.

Results: Median age was 31 years [24–44] in SCD patients and 23 years [21–24] in healthy volunteers. Despite no SkBF difference between the two groups (at basal and under stimulation), spectral analysis showed a lesser decrease in myogenic activity in SCD patients compared to volunteers following occlusion ($P = 0.01$) with a similar endothelial activity, noteworthy, under acetylcholine stimulation. Comparison of SCD patients according to albuminuria (urine albumin to creatinine ratio (ACR) above or below median value of 24.7 mg/mmol), showed a decreased basal myogenic tone in high versus low albumin group ($P = 0.02$) with no difference for endothelial or sympathetic activities. Moreover, albuminuria was inversely correlated with myogenic activity under basal and acetylcholine stimulation (rho = −0.45, $P = 0.04$; rho = −0.53, $P = 0.01$ respectively).

Conclusion: Our results suggest that in SCD patients with an early SCAN, microangiopathy could be due to an impaired myogenic tone with surprisingly no obvious endothelial NO bioavailability decrease.

YPO.017

Discoidin domain receptor 1 is a key mediator of acute kidney disease

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Introduction: DDR1 is a non-integrin collagen receptor with tyrosine-kinase activity.

We have demonstrated that overexpression of DDR1 drives renal inflammation and fibrosis in models of chronic kidney disease. The aim of our study was to investigate whether the pharmacogenetic inhibition of DDR1 preserves kidney in 2 models of acute tubular injury, unilateral ureteral obstruction (UUO) and ischemia/reperfusion (I/R).

Methods: Severe tubular injury was induced by performing either UUO or I/R in male mice, and the expression of DDR1 was inhibited by administering oligodeoxynucleotide antisense (AS), or scrambled sequences (SCR), either from day 2 after ligation or prior to the ischemia. Mice were sacrificed 24 h (I/R) or 7 days (UUO) after surgery.

Results: DDR1 protein expression was strongly induced in tubular cells after UUO or I/R and this expression was inhibited by the AS administration. Increased uremia (BUN) showed impaired renal function in SCR+I/R animals, whereas AS treatment blunted BUN levels. Tubular damage and fibrogenesis were significantly lowered in AS+UUO and AS+I/R mice. Kim-1, NGAL, vimentin and VCAM-1 mRNA expressions were increased in SCR+I/R compared to controls and significantly decreased in mice treated with AS. Moreover, the AS-induced inhibition of DDR1 was accompanied by decreased pro-inflammatory cells infiltration and cytokines mRNA expressions in both models.

Conclusion: DDR1 inhibition protects mice from the UUO- and I/R-induced histological damage, inflammation and loss of renal function. DDR1 overexpression plays a deleterious role in these models of kidney injury, thus reinforcing the interest to develop agents capable of specifically blocking the function of this receptor.

YPO.018

Could red blood cell phenotype and function predict renal anion exchanger 1 function in distal renal tubular acidosis patients?

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Introduction: Anion Exchanger 1 (AE1, *SLC4A1*) mediates Cl^-/HCO_3^- exchange and is expressed at the basolateral membrane of α-intercalated cells in kidney (kAE1) and the red blood cell (RBC) membrane (eAE1). Mutations in *SLC4A1* lead to distinct disorders: an acidification defect manifesting as distal renal tubular acidosis (dRTA) and abnormal RBC morphology including South-East Asian ovalocytosis (SAO). Recent studies have described RBC morphological abnormalities in some dRTA patients.

Methods: From previously characterised dRTA patients with kAE1 (*SLC4A1*) mutations, RBCs were phenotyped: HCO_3^- exchange (k) through resealed ghost membranes (stopped-flow spectrofluorometry), RBC and reticulocyte indices, RBC morphology (blood smear), and RBC deformability (ektacytometry).

Results: Eight unselected families were phenotyped: the sex ratio was 3:1 (F:M) and median age 30 [11–68] years. Five exhibited a 589 substitution, one a deletion at position 906: all had a HCO_3^- transport ($k = 4.3 \pm 0.5$) of similar amplitude to healthy controls ($k = 4.1 \pm 0.6$, $P > 0.99$). Two harboured the S613F and G701D substitutions and had an intermediate ($k = 3.2 \pm 0.2$) and a low ($k = 2.0 \pm 0.0$) transport, respectively. k was closely associated ($r^2 = 0.65$, $P = 0.003$) to AE1 expression. RBC deformability was normal in all patients, except in G701D patient, who exhibited the classical pattern of SAO (ovalocytosis).

Discussion: These preliminary data show patients with classical kAE1 mutations have a normal RBC phenotype whereas those with eAE1 mutations

(G701D) have both RBC and renal phenotypes. AE1 function is closely related to its membrane expression. Further on-going analyses (Cl^- transport, new families) will help to confirm these results.

YPO.019

Role of claudin-16 in basal and PTH-stimulated ion transport in the thick ascending limb of Henle's loop (TALH)

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Introduction: Claudin-16 is specifically expressed at the tight junction of TALH. Inactivating mutations of the gene encoding claudin-16 causes Familial Hypercalciuria with Hypomagnesemia and NephroCalcinosis (FHHNC), a rare genetic disorder responsible for hypomagnesemia, hypercalciuria, nephrocalcinosis and early renal insufficiency[1]. However, the role of claudin-16 in basal and parathyroid hormone (PTH)-stimulated ion transport in the TALH remains unclear.

Materials and methods: We used ex vivo microperfusion of TALH from *Cldn16*$^{-/-}$ and *Cldn16*$^{+/+}$ mice to measure paracellular ion permeabilities and to assess the effect of basolateral PTH (10^{-10} M) on transepithelial calcium absorption.

Results: Paracellular permeabilities to calcium (P_{Ca}) and magnesium (P_{Mg}) were significantly decreased in *Cldn16*$^{-/-}$ mice (P_{Ca} = 0.18 versus 0.61×10^{-4} cm/s in *Cldn16*$^{+/+}$ mice; P_{Mg} = 0.88 versus 4.52×10^{-5} cm/s. in *Cldn16*$^{+/+}$ mice). Calcium and magnesium absorption was decreased by ~50% in TALH from *Cldn16*$^{-/-}$ mice, compared to *Cldn16*$^{+/+}$ mice. Permeabilities to sodium, chloride and potassium were unaffected in *Cldn16*$^{-/-}$ mice. PTH significantly increased calcium reabsorption in TALH from both *Cldn16*$^{+/+}$ and *Cldn16*$^{-/-}$ mice.

Discussion: Claudin-16 is required for normal paracellular permeability to calcium and magnesium in the TALH, under basal condition. However, the lack of claudin-16 does not prevent the PTH-elicited increase in calcium absorption.

YPO.020

Influence of cyclosporine dose on the main mycophenolate mofetil pharmacokinetic parameters

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Introduction: Cyclosporine (CsA) and mycophenolate mofetil (MMF) are characterized by large interindividual variability in pharmacokinetic and pharmacodynamic response, which may result in drug toxicity or lack of efficacy, and are subject to drug-drug interaction.

Objective to assess the effect of a CsA dose on the main MMF pharmacokinetic parameters.

Materials and methods: The main pharmacokinetic parameters ($AUC_{(0-12)}$ and C_{max}) of CsA and mycophenolic acid (MPA), an active metabolite of MMF, were evaluated in 83 patients after kidney transplantation using Bayesian estimator and a 3-point limited sampling strategy.

Results: A significant positive correlation between the main MPA pharmacokinetic parameters was noticed: higher CsA dose displayed higher MPA AUC exposure and higher C_{max}. Linear regression analysis showed that AUC of MMF was CsA dose dependent and accounted for 14.1% of the cases; and AUC of MMF was CsA AUC dependent and accounted for 8.6% of cases. Spearman correlation coefficient confirmed strong relationship between a CsA dose and an increase in MMF AUC exposure, and between CsA AUC exposure and MMF AUC exposure, while no relationship between MMF AUC exposure and C_0 of CsA was found.

Conclusions: The use of CsA has an impact on the main MPA pharmacokinetic parameters in a CsA dose-related manner. The usage of a low CsA dose reduces MPA AUC exposure under the therapeutic window and may lead to ineffective therapy, while the use of a high CsA dose is related to greater than 10 mg/L MPA C_{max} and increases the likelihood of adverse events.

YPO.021

Evaluation of no homeostasis actors and vegfc in intradialytic hypertension

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Introduction: 15% of hemodialysis patients show a constant increase in their systolic blood pressure (SBP) during dialysis session, known as intradialytic hypertension (IH). The mechanisms undergoing IH remain unclear and are often associated with NO and RAAS imbalance. The aim of our study is to investigate the endothelial microenvironment in order to characterize new actors involved in IH pathogenesis.
Methods: Sixty-two adult patients undergoing regular hemodialysis were enrolled in the study. Patients were divided into two groups: Control ($n = 53$) with stable SBP during the session, and IH group ($n = 9$) with an increased in SBP ≥ 10 mmHg post-dialysis from baseline measurements. Blood samples were collected from all patients for ELISA plasma concentration measurements.
Results: Plasmatic VEGFC remained unchanged in IH group while it increased after dialysis in control group ($P < 0.05$). Predialysis TNFα was lower in IH group while it tends to increase after dialysis ($P > 0.05$ versus control). Collectrine and ADMA concentrations were more important in IH group compared to control before and after dialysis ($P < 0.05$), as for as the concentrations of endothelin-1 ($P < 0.05$). Moreover, L-Citrulline was significantly lower before and after dialysis in IH group compared to control. Finally, Predialysis, ACE2 was higher in IH group compared to control and it decreased in post-dialysis ($P < 0.05$).
Discussion: Taken together, NO homeostasis actors and the endothelial inflammatory microenvironment seems to be impaired in patients with IH. In addition, these findings may pinpoint toward interesting new therapeutic targets in the treatment of IH.

YPO.022

Crystalluria for management of cystinuric patients

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Introduction: Few studies have evaluated crystalluria in cystinuria, the most common monogenic nephrolithiasis disorder.
Material and methods: We retrospectively studied pH, specific gravity, presence of cystine crystalluria and calcium phosphate crystalluria in 722 morning urine specimens from 89 patients with cystinuria followed at Necker Hospital (Paris). Well-homogenized urine samples set in a Malassez cell were examined by light microscopy within 2 h of collection.
Results: The median number [min–max] of crystalluria examinations per patient was 4.0 [1–65]. Considering the period from first to last crystalluria study [4.5 years (0–25.4)], the median number of samples studied for crystalluria per patient per year was 1.4 [0.2–7.8]. 37.0% of samples were positive for cystine crystalluria. Increasing urinary pH and decreasing urinary specific gravity significantly reduced the risk for a patient to have a cystine crystalluria whereas cysteine-binding thiols did not reduce this risk. The estimated probability of cystine crystalluria was 45, 38, 31 and 25% for a urinary pH of 6.5, 7.0, 7.5 and 8 respectively and was 47, 27 and 14% for a urinary specific gravity of 1015, 1010 and 1005 respectively. The estimated probability of calcium phosphate crystalluria was 12, 19, 28 and 40% for a urinary pH of 6.5, 7.0, 7.5 and 8 respectively.
Discussion: Crystalluria studies led us to confirm the targets of medical preventive treatment and challenged the efficacy of cysteine-binding thiols in cystinuria. Urinary specific gravity below 1005 and urinary pH above 7.5 but below 8 to avoid calcium-phosphate precipitation should be the goals of medical therapy.

YPO.023

Investigation on the role of the Kir4.2 K^+ channel in renal and pancreatic functions

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The potassium Inwardly Rectifying (Kir) channels are widely expressed and involved in many physiological and pathological processes. In the kidney, Kir4.1 plays a central role in ion transport by the distal nephron and its dysfunction is responsible for the renal traits of the inherited SeSAME syndrom. Several studies indicated a strong expression of Kir4.2, a closely related Kir4.1 homolog, in the whole kidney, but its intrarenal distribution and its role in the renal function remain unknown.

In mice, Kir4.2 mRNA was detected only in cells of the proximal convoluted tubule suggesting that Kir4.2 may play a key role in proximal tubule function. We therefore established the renal phenotype of *Kcnj15* deleted mice (Kir4.2$^{-/-}$). Kir4.2$^{-/-}$ mice showed mild, but not significant, hyperproteinuria and hyperphosphaturia and a lower urinary pH (5.8 versus 6.3; $P = 0.02$), and plasma content analysis revealed no sign of perturbation in their acid-base status.

KCNJ15 has been identified as a susceptibility gene for diabetes mellitus type 2, Kir4.2 appearing to be involved in the regulation of insulin secretion by pancreatic beta-cells. But we found no sign of hyperglycemia, polyuria and polydipsia in Kir4.2$^{-/-}$ mice, and our results from oral glucose tolerance test indicated no major pancreatic dysfunction.

More detailed functional and molecular analyses of Kir4.2$^{-/-}$ mice will complete these preliminary results which, at that stage, indicate that Kir4.2 does not support alone a critical function in kidney and pancreas, and determine whether Kir4.2 absence may be compensated by the increased expression of other K^+ channels in both tissues.

YPO.024

Syndrome of inappropriate antidiuresis induces volume-dependent hypercalciuria

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Chronic hyponatremia related to the Syndrome of Inappropriate Anti-diuresis (SIAD) has been associated with osteoporosis. Mechanism of bone demineralization remains elusive. Assuming that SIAD is a euvolemic condition that does not alter renal calcium handling, the direct roles of hyponatremia or AVP have been proposed. We evaluated renal calcium excretion in patients with chronic hyponatremia, either related to SIAD or to hypovolemia.

We retrospectively included all patients referred to our Department between May 2006 and May 2014 for hyponatremia (plasma sodium <135 mM), resulting from SIAD or hypovolemic hyponatremia. None had edema, cardiac or renal insufficiency, or cirrhosis. Exploration included estimation of volemia, extracellular fluid volume (ECFV) measurement with inulin, and parameters of calcium homeostasis.

Twenty-three patients were included in SIAD group and seven in hypovolemic group. SIAD group exhibited signs of increased volemia: higher glomerular filtration rate, higher fractional excretion of uric acid, lower plasma renin. In SIAD group, ECFV was higher than in hypovolemic group and above usual values. There was no difference between the two groups regarding plasma ionized calcium, PTH, vitamin D and phosphorus. However, SIAD group exhibited calciuria above hypovolemic group, reaching levels of hypercalciuria. Furthermore, there was a strong positive correlation between calciuria and ECFV.

Our results show that: (i) SIAD is not a euvolemic but a slightly hypervolemic state; (ii) Despite hyponatremia in both groups, SIAD group only exhibited hypercalciuria; (iii) hypercalciuria was proportional to ECVF expansion. Renal loss of calcium induced by hypervolemia, may therefore contribute to bone loss in SIAD patients.

YPO.025

Study of metabolic acidosis in sickle cell disease patients

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Introduction: Metabolic acidosis is encountered in 42% of patients with sickle cell disease (SCD)(Maurel S, CJASN, 2014). This study aims at determining its mechanism.

Materials and methods: We conducted a multicentric observational study including SCD patients with [HCO_3^-] <22 mM and glomerular filtration rate estimated by CKD-EPI (eGFR) >60 mL/min/1.73 m^2. The urinary acidification test was achieved by oral administration of furosemide and fludrocortisone, increasing distal tubular Na^+ delivery, Na^+ reabsorption via principal cells and H^+ secretion via α-intercalated cells. An abnormal test was identified by a failure to lower urinary pH <5.3 and/or to increase NH_4^+ excretion rate ≥33 μEq/min at least once within 6 h.

Results: We evaluated 9 SCD patients [3 males, 40.7 years (24–49), eGFR = 113 mL/min/1.73 m^2 (74–132)]. During the test, urinary pH remained ≥5.3 in four patients and urinary $NH4^+$ excretion remained <33 in seven patients. Only one patient had a normal test.

In comparison with our historical cohort of 630 SCD patients, this nine patients had lower hemoglobin [7.0 (6.6–8.4) versus 8.7 (8–9.6) g/dL, $P = 0.0052$], higher lactate dehydrogenase [566 (507–770) versus 352 (285–450) IU/L, $P = 0.0002$] and a higher rate of high density red blood cell (>1.11) [21 (13–31) versus 12 (7–18), $P = 0.024$].

Discussion: SCD patients with metabolic acidosis have a haematological phenotype of hyperhemolysis. The impairment of urinary $NH4^+$ increase during the urinary acidification test in 80% of our patients shows that this metabolic acidosis is likely due to an impaired $NH4^+$availability, probably secondary to the medullary ischemia seen in SCD.

YPO.026

Looking for AMPK regulation of the type 2 H, K-ATPase after functional expression

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Introduction: AMP-activated protein kinase (AMPK) regulates cell energy and metabolism. It is also involved in the regulation of the activity/expression of ionic transporters. In the kidney, the 'colonic' H/K-ATPase (HKA2) is located in the apical membrane of intercalated cells of the collecting duct. It is involved in potassium homeostasis, being up-regulated during potassium depletion. We investigated whether AMPK affects HKA2 activity after expression in *Xenopus laevis* oocytes.

Methods: RNAs encoding HKA2 (HKα2 and Na/Kβ1) were injected in oocytes to express HKA2 alone or co-injected with RNAs encoding a mutated AMPK: a constitutively activated $AMPK^{\gamma R70Q}$ ($\alpha 1$, $\beta 1$, $\gamma 1^{R70Q}$) or an inactive $AMPK^{\alpha K45R}$ ($\alpha 1^{K45R}$, $\beta 1$, $\gamma 1$). The endogenous activity of AMPK was modulated by incubating HKA2-expressing oocytes with AICAR or Compound C (activator and inhibitor of AMPK, respectively). Using pH-sensitive microelectrodes, HKA2 function was evaluated by monitoring the basal intracellular pH (pH_i), and pH_i recovery after a cell acid load.

Results: HKA2-expressing oocytes were significantly more alkaline than control (water-injected) oocytes. The pharmacological activation or inhibition of the endogenous AMPK did not change this value. Compared to oocytes expressing HKA2 alone, pH_i was slighly more acidic in oocytes co-expressing HKA2 and $AMPK^{\gamma R70Q}$ or $AMPK^{\alpha K45R}$. The pH_i recovery from cell acid load was unchanged by AICAR or Compound C incubation or by the co-expression of $AMPK^{\gamma R70Q}$ or $AMPK^{\alpha K45R}$.

Discussion: Taken together, these results do not support an AMPK-mediated regulation of HKA2. This will be further discussed.

Cardiovascular Physiology

YPO.027

Nuclear PKA activity regulation by B1- and B2-adrenoceptors in adult cardiac myocytes

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Differences between β_1-adrenoceptors (β_1-ARs) and β_2-ARs in the regulation of gene expression and the mechanisms involved are not well known. Thus, we studied the mechanisms regulating cytoplasmic and nuclear PKA activation by β_1/β_2-ARs.

PKA activity was measured using targeted FRET-based reporters. Specific β_1/β_2-AR stimulation was achieved by combining isoprenaline (Iso) with a β_2/β_1-AR antagonist.

For a similar activation of cytoplasmic PKA, nuclear PKA activity was 3-fold higher with β_1-AR than with β_2-AR stimulation. Inhibition of G_i proteins, caveolae disruption, and inhibition of GRK2-mediated desensitization potentiated β_2-AR-induced cytoplasmic but not nuclear PKA activity. PDE4 inhibition strongly potentiated cytoplasmic and nuclear PKA responses to both β_1/β_2-AR stimulation. In contrast, PDE3 inhibition had no significant effect on β_1-AR induced PKA activation in both compartments, while it increased cytoplasmic but not nuclear PKA activity upon β_2-AR stimulation. Downregulation of mAKAP, a nuclear envelope associated scaffold protein, decreased β_1-AR stimulation of nuclear PKA activity. Consistently, β_1-ARs but not β_2-ARs were able to induce the expression of the PKA-regulated pro-apoptotic gene, ICER. Results show that i) β_1/β_2-ARs differentially regulate cytoplasmic versus nuclear PKA activities, ii) nuclear PKA activation can be dissociated from bulk cytoplasmic PKA activity upon β_2-AR stimulation; and iii) PDE4 and mAKAP are critical components of nuclear PKA signalling.

YPO.028

Endothelium-dependent relaxation and T-type voltage gated calcium channels in mice pulmonary artery

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T-type voltage-gated calcium channels (T-VGCC) are involved in the regulation of pulmonary arterial tone. In addition to their roles in smooth muscle cell contraction, we investigate if T-VGCC could control vascular relaxation via endothelial calcium signaling regulation in mouse pulmonary arteries. Immunofluorescence labelings indicates that endothelial cells (as well as smooth muscle cells) express T-VGCC proteins (Cav3.1 and Cav3.2 isotypes). We show that nitric oxide (NO)-dependent relaxation induced by acetylcholine is reduced in the presence of T-VGCC antagonists (mibefradil, NNC 55-0396) and in Cav3.1 knock-out mice. Acetylcholine induces an endothelial intracellular calcium increase which is reduced by the same type of inhibitors. By contrast, endothelium dependent relaxation induced by β2 adrenergic stimulation (with procaterol), or TRPV4 agonist (GSK1016790A), is not inhibited by mibefradil or in Cav3.1 knock-out mice. Furthermore, in pathological condition (pulmonary hypertension induced by a chronic hypoxia), acetylcholine-mediated relaxation is reduced and even more in Cav3.1 knock-out mice. In summary, in mice pulmonary arteries, acetylcholine mediated relaxation is calcium-dependent and require T-VGCC. The present findings suggest that calcium influx through T-VGCC mediate a dual effect in pulmonary artery: a contraction when it occurred in smooth muscle cells and a dilatation when it occurred in endothelial cells.

YPO.029

Slug: a common target of invasive carcinoma and atherosclerosis?

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Atherosclerosis, a chronic vascular inflammatory disease, is the leading cause of mortality worldwide. Phenotypic transition of vascular smooth muscle cells (VSMC) plays a pivotal role in this disease. This phenomenom allows these cells to migrate from the media to the intima of arteries where they secrete numerous pro-inflammatory mediators. Migration and inflammation are also features of cancer cells. Molecules that regulate these properties, especially in carcinoma cells, have been identified. For example, the transcription factor Slug, by allowing epithelial to mesenchymal transition of pre-invasive carcinoma cells participates in their transformation into invasive ones.

Here we investigated the role of Slug on VSMC transdifferentiation by using small interfering RNA strategy in primary cultures of rat aortic smooth muscle cells. Our results show that Slug extinction inhibits by about 50% IL-1ß induction of pro-migratory and pro-inflammatory genes such as MMP 9, inducible nitric oxide synthase and CCL3 and CXL2 chemokines. These results demonstrate that Slug is involved in IL-1β-induced VSMC phenotypic transition. We show that IL-1β up-regulates Slug protein level in

VSMC independently from a genic regulation. In carcinoma cells, Slug induction by carcinogenic molecules usually involves glycogen synthase kinase-3β inhibition. However we demonstrate that IL-1β doesn't inhibit this enzyme in CMLV which excludes its involvement in IL-1β-induced Slug expression in VSMC which molecular mechanisms remain to be determined.

All together, our results highlight the potential role of Slug in atherogenesis, a role which would come to be added to the well established one that it plays in tumour metastasis.

YPO.030

Extracellular calpain/calpastatin balance is involved in the progression of pulmonary hypertension

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Introduction: Excessive growth of pulmonary-arterial (PA) smooth muscle cells (SMCs) is a major component of pulmonary arterial hypertension (PAH). The calcium-activated neutral cysteine proteases calpains-1 and -2, expressed by PA-SMCs, contribute to PH but are tightly controlled by a single specific inhibitor, calpastatin. To investigate calpastatin during pulmonary hypertension (PH) progression and its potential role as an intracellular and/or extracellular effector.

Methods and results: We assessed calpains and calpastatin in patients with idiopathic PAH and mice with hypoxic or spontaneous (SM22-5HTT$^+$ strain) PH. To assess intracellular and extracellular roles for calpastatin, we studied effects of the calpain inhibitor PD150606 on hypoxic PH in mice with calpastatin overexpression driven by the cytomegalovirus promoter (CMV-Cast) or C-reactive protein (CRP) promoter (CRP-Cast), inducing increased calpastatin production ubiquitously and in the liver, respectively. Chronically hypoxic and SM22-5HTT$^+$ mice exhibited increased lung calpastatin and calpain-1 and -2 protein levels and activity, both intracellularly and extracellularly. Prominent calpastatin and calpain immunostaining was found in PA-SMCs of remodeled vessels in mice and PAH patients, who also exhibited increased plasma calpastatin levels. CMV-Cast and CRP-Cast mice showed similarly decreased PH severity compared to wild-type mice, with no additional effect of PD150606 treatment. In cultured PA-SMCs from wild-type and CMV-Cast mice, exogenous calpastatin decreased cell proliferation and migration with similar potency as did PD150606 and suppressed fibronectin-induced potentiation.

Conclusion: These results indicate that calpastatin limits PH severity via extracellular mechanisms. They suggest a new approach to the development of treatments for PH.

YPO.031

Development of tissular injuries in chronic exercice-induced ischemia: validation of a human sartorius muscle model, in peripheral arterial disease

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Assessment of physiopathological mechanisms in peripheral arterial disease (PAD), i.e. inflammatory process, endothelial or mitochondrial dysfunctions, has mainly been realized in experimental models with acute limb ischemia. However, it is not reflective of adaptations observed during chronic exercice-induced ischemia in Human. Our aim was to compare in a same muscle, structural and functional alterations between a portion subjected to exercice-induced ischemia and a portion without any ischemia.

In 24 patients, distal and proximal biopsies of sartorius muscle were performed during a vascular surgery (femoral bypass or veinous sampling for coronary bypass). Twelve patients presented a PAD, 12 patients had coronary syndrome without any PAD. Before surgery, each patient performed a treadmill test with measurement of exercise transcutaneous oxygen pressure on proximal and distal sartorius regions, to evaluate exercise-induced ischemia in the distal portion. From biopsies several analyses were performed: (i) immunohistochemistry to quantify inflammatory cell infiltration, capillary density and arterial remodelling, and (ii) mitochondrial respiratory chain complex activities measurement.

During exercise, only patients with PAD showed a significant distal sartorius ischemia; this portion seemed to show increases of capillary density (20%)

and inflammatory cell infiltration (30%), compared to proximal portion. Moreover, mitochondrial respiratory was impaired in muscle portion subjected to exercise-induced ischemia: complexe I activity related to citrate synthase was decreased from 40%.

This model allowed us to characterize the exercise muscular ischemia in Human. It will be promising to better understand physiopathological mechanisms of chronic ischemia, irrespective of inter-individual variability, patient being its own control subject.

YPO.032

Aldosterone-independent regulation of EnNaC surface expression and endothelial nanomechanics – 'feedforward' activation by high Na^+

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In contrast to ENaC in the kidney, endothelial ENaC (EnNaC) seems to be activated by Na^+ in a 'feedforward' manner: In the absence of aldosterone, high extracellular Na^+ (>145 mM) increases EnNaC membrane expression *in vitro*, leading to a stiff endothelial cortex and decreased nitric oxide (NO) release. Interestingly, EnNaC transcription is not affected by high Na^+.

Here, we aimed to elucidate the underlying mechanisms of Na^+-dependent changes of the endothelium in an aldosterone synthase (Cyp11B2) deficient ($AS^{-/-}$) mouse model.

Therefore, we employed ECs of *ex vivo* aorta preparations from wild-type (WT) and AS(−/−) to detect αEnNaC surface expression and cortical stiffness by Atomic Force Microscopy (AFM). EnNaC surface expression (−16%) and cortical stiffness (−22%) were reduced in AS(−/−), compared to WT, whereas NO secretion was exclusively detectable in AS(−/−). EnNaC inhibition with benzamil decreased stiffness in WT (−56%) and AS−/− (−22%), while mineralocorticoid receptor antagonism diminished the cortical stiffness only in the WT (−19%). In the absence of aldosterone, high Na^+ increased EnNaC surface expression *ex vivo* (+19%) and cortical stiffness *in vivo* (+44%) and *ex vivo* (+41%). Exposure to aldosterone adjusted the stiffness of AS(−/−) to the WT level.

We conclude that high Na^+ *per se* leads to retention of EnNaC in the endothelial membrane which stiffens the EC cortex. Aldosterone in turn regulates the gene expression and subsequent membrane insertion of EnNaC. Thus, high Na^+ *per se* might be sufficient to cause endothelial dysfunction by increasing EC cortical stiffness, a downregulator of NO production.

Exercise Physiology

YPO.033

Label-free quantitative protein profiling of the human muscle during ultra-endurance exercise

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Introduction: Sports events lasting more than 6 h are defined as ultra-endurance exercise. Ultra-endurance is associated with stresses that mechanically damage muscle cells and lead to injured proteins and organelles. Previous investigations provided evidence that ultra-endurance exercise is associated with a coordinated up-regulation of the ubiquitin-proteasome and autophagy-lysosomal proteolytic pathways (Jamart et al., 2012). The purpose of the present investigation was to assess the resulting modifications in the muscle proteome.

Materials and methods: Ten men, experienced ultra-endurance athletes ran for 24 h on a treadmill. Muscle biopsy samples were taken from the vastus lateralis muscle 2 h before starting and immediately after finishing exercise. Athletes ran 150 ± 16 km with an effective running time of 18 h:42 min (±41 min). Label-free quantitative protein profiling ('Shot-Gun') was performed according to Théron et al. (2014) to quantify and compare proteomes before and after ultra-endurance running.

Results: Shot-Gun proteomics of the Human muscle homogenate identified 633 proteins, and among them 96 were differentially expressed after ultra-endurance running. Most of the proteins were under-represented after exercise. Functional interaction networks indicated that ultra-endurance strongly altered the mitochondrial proteome suggesting enhanced mitophagy. Our results also revealed important modifications

related to the cytoskeleton, cytodetoxification, proteostasis and membrane repair.

Discussion: This study describes the most extensive proteomic analysis of Human muscle adaptation to ultra-endurance exercise. Many potential biomarkers may represent novel starting points to elucidate the mechanisms of muscle adaptation to extreme exercise.

YPO.034

Effects of prior respiratory exercise on VO_2 and intercostal muscle oxygenation kinetics during maximal incremental exercise

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Prior peripheral heavy exercise delayed the fatigue occurrence, speeded VO_2 kinetic and improved muscle oxygenation during a subsequent high intensity exercise. Given the respiratory muscles fatigue contribution to the exercise performance limitation in trained subjects, the aim of this study was to investigate the effect of prior respiratory exercise (PRE) on respiratory muscle fatigue (RMF), inspiratory muscle oxygenation and overall VO_2 kinetics during a maximal incremental exercise (MIE).

Eleven trained subjects performed in counterbalanced order 2 MIE; one was preceded by PRE. Maximum inspiratory (MIP) and expiratory (MEP) pressures were measured before and after the completion of each MIE to estimate RMF. PRE consists on the succession of 2′ bouts duration of breathing against 85% MIP followed by 1′ recovery period during 30′. During the 2 MIE, respiratory variables were assessed using gas analyzer and intercostal muscle oxygenation using near-infrared spectroscopy.

PRE reduced RMF and decreased overall VO_2 and deoxyhemoglobin kinetics (60–100% MAS) during MIE. The VO_2 measured during MIE preceded with PRE does not reach VO_{2max}. The same MAS has been found in the 2 trials.

PRE decreased fatigue and intercostal muscle deoxyhemoglobin kinetics during MIE. This result was accompanied with a decrease in overall VO_2 kinetics and could be explained by reduced intercostal muscle oxygenation. Given to the lack of reaching VO_{2max} during MIE with PRE, peripheral system limitation could be suggested.

YPO.035

Impact of cardiac rehabilitation on the obstructive sleep apnoea in the coronary artery disease

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Introduction: Obstructive sleep apnoea (OSA) syndrome is improved by physical activity in the general population. This has not been demonstrated in patients with coronary artery disease (CAD). We aimed to determine a correlation between cardiac rehabilitation and OSA syndrome in CAD patients.

Methods: Forty-five CAD patients were included in cardiac rehabilitation programme of St-Etienne University Hospital. Patients were classified according to the severity of OSA syndrome. The number of events per hour was reported as the apnoea-hypopnoea index (AHI) measured from the Holter ECG and Electrocardiogram-Derived Respiratory (EDR). An AHI less than 5 was considered normal. An AHI of 5–14 was mild, 15–29 was moderate and more than 30 events per hour characterized severe OSA. Cardiopulmonary exercise testing (CPET) and baroreflex (BRS) were performed to assess respectively VO_2 max and autonomic nervous system at the beginning and at the end of the cardiac rehabilitation.

Results: The reduction in AHI was significant in CAD patients with severe OSA syndrome (8.15 ± 12, $P = 0.019$). This correlation was even stronger than VO_2 max and BRS were improved (10.2 ± 8, $P < 0.05$ with a gain over 20% of VO_2 max and BRS) at the end of the rehabilitation.

Conclusion: Severe OSA syndrome is improved by cardiac rehabilitation among CAD patients. Autonomic Nervous System regulation by physical activity might be key for alternative therapy for OSA syndrome.

YPO.036

Impact of melatonin ingestion at different times-of-day on short-term performances and biochemical responses in soccer players

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Introduction: Reciprocal benefits between melatonin (MEL) and physical exercise have been well reviewed. Nevertheless, scientific literature showed contradictory results concerning the optimal time-of-day of MEL ingestion. This study aimed to investigate the effect of MEL ingestion at different times-of-day on short-term performances and biochemical responses in soccer players.

Materials and methods: Twelve professional soccer players (17.2 ± 1.01 years; 1.70 ± 0.05 m; 64.0 ± 7.60 kg) performed a repeated sprint (RSA) test (6 × (20 m + 20 m)/20s-passive-recovery) at different times-of-day (08:00 h, 12:00 h, 16:00 h and 20:00 h) after a double-blind randomized ingestion of MEL (5 mg) or placebo (PLA). Rating of perceived exertion (RPE), plasma lactate [La] and glucose [GLC] were measured.

Results: The results showed a significant treatment and time-of-day effects ($P < 0.05$) on RSA performances. RSA_{best} were higher at 16:00 h compared to 08:00 h only with PLA. Moreover, RSA_{best} was affected at 12.00 h, 16:00 h and 20:00 h ($P < 0.05$), whereas RSA_{mean} was not affected during MEL condition compared to PLA. Alternatively, $RSA_{decrease}$ was reduced at 16:00 h and 20:00 h ($P < 0.05$) in MEL condition compared to PLA. The values of [La] and [GLC] were significantly lower at 16:00 h with MEL compared to PLA ($P < 0.05$). However, RPE scores were not significantly different between conditions.

DISCUSSION: The results suggested that diurnal MEL (5 mg) ingestion affects essentially very short-term performances (RSA_{best}) in the afternoon and evening but not in the morning. However, RSA_{mean} performances were not affected with MEL. The decline of $RSA_{decrase}$ and [La] measures at 16:00 h with MEL could reflect the potential effect of MEL in the recovery during RSA.

YPO.037

Effects of two types of treadmill-training on cardiomyocytes remodelling in spontaneously hypertensive rats

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Hypertension is an important public-health problem because of its high frequency and its associated risk of cardiovascular disease. Exercise training offers a potential non-pharmacological therapy for cardiovascular diseases including hypertension. Studies have shown that interval exercise training may have positive effects on maximal aerobic capacity in healthy subjects and in those with cardiovascular diseases. High intensity intermittent exercise (HIIE) has been shown to induce many adaptations normally associated with traditional endurance exercise.

The aim of this study was to compare the effects of HIIE training versus moderate intensity continuous exercise (MICE) training on cardiac remodelling in spontaneously hypertensive rats (SHR).

After eight weeks of treadmill training Vmax and mean arterial pressure (MAP) were measured. Cardiomyocytes were isolated and investigated.

Vmax in trained SHR increased to 40 m/min while it was reduced (from 28 to 25 m/min) in untrained SHR. After training MAP was lower in trained SHR versus untrained SHR regardless the type of training. This was accompanied by an increase of the left ventricular weight. At the cellular level, the space organization regularity of the transverse tubular system increased in trained SHR versus untrained SHR to an intermediate level between untrained SHR and control ones (Wistar-Kyoto). In trained SHR, calcium spontaneous events analysis revealed a drastic reduction of the release site density and firing frequency which are more marked with EIHI than with ECIM training. In conclusion: training in SHR leads to mitigation of the deleterious hypertensive characteristics and our experiments suggest that HIIE could be used in humans.

YPO.038

Exercise before sleep reduces heart rate variability but does not affect cortisol awakening response in the next morning.

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Introduction: Exercise activates hypothalamic-pituitary-adrenal axis (HPA) and autonomic nervous system (ANS) and generally causes beneficial changes in homeostatic balance. However, the health benefits of late-night exercise programs on the activity of HPA and ANS is not known. The aim of this study was to assess effects of pre-sleep exercise on sleep quality and on cortisol awakening response (CAR) and heart rate variability (HRV) measurements in the following morning.
Materials and methods: Medical students ($n = 20$ males, 20–24 year-old) filled Karolinska Sleep Diary on the day before exercise program. In the following morning, they provided salivary samples for the assessment of CAR (samples at 0, 15, 30 and 60 min post-awakening) and had a 5-min electrocardiogram recording for the determination of HRV. In the next night, an exercise program consisting of a 90-min football match was implemented at 09:30 p.m. and all procedures were repeated. Cortisol concentrations were measured in the salivary samples and time- and frequency-domain parameters of HRV were calculated.
Results: Pre-sleep exercise did not affect ($P > 0.05$) CAR parameters (0, 15, 30, 60 min cortisol concentrations, mean concentration, area under the curve) and sleep parameters (sleep duration, disturbed sleep, awakening problems) but decreased time-domain parameters and increased frequency-domain parameters of HRV ($P < 0.05$).
Discussion: The results suggest that pre-sleep exercise is associated with changed HRV activity rather than changes in CAR and, therefore, it might be concluded that pre-sleep exercise affects ANS activity rather than HPA activity in the next morning.

Nutritional Physiology

YPO.039

Modulation of taste receptor mRNA expression, inflammation and junction permeability in the lingual papillae in diet-induced obese mice

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Introduction: It is well established that chronic high fat intake contributes to obesity, which alters taste perception and generates inflammation. The aim of this study was to evaluate the mRNA expression of the proteins that can be altered in the lingual papillae in diet-induced obese mice.
Materials and methods: We conducted our study on female C57BL/6j mice aged between 6 and 10 weeks. The animals were fed the standard diet (STD) or a high fat diet (HFD). After 15 weeks, the animals were sacrificed and the tongues were removed. RT-PCR was used to assess the expression of mRNA of taste receptors, inflammatory cytokines and tight junction proteins.
Results: HFD fed animals gained weight progressively. HFD increased mRNA expression of sweet, umami, bitter taste receptors and decreased that of CD36, a fat taste receptor. The obese animals expressed higher IL-1ß, IL-6, IL-12, TNFα and F4/80 mRNA in the tongue papillae than control animals. Results have shown a modulation in the expression of mRNA encoding tight junction proteins like ZO-1, claudins 4, 7 and 8 in HFD group as compared to STD fed group animals.
Discussion: Our results show that HFD triggers alterations in gustatory perception, associated with modulation of lingual epithelium permeability. This phenomenon along with local papillary inflammation might play a key role in diet-induced obesity.

YPO.040

Orosensory detection of dietary fat is decreased in $CB_1R-/-$ mice

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Introduction: Recent evidences have shown the existence of a sixth taste modality dedicated to the orosensory detection of dietary lipids. Interestingly, obese subjects exhibit a high spontaneous preference for fat. Endocannabinoid system, *via* its CB_1 receptor (CB_1R), is also involved in overeating of fat-rich foods. We elucidated cell signaling mechanisms in taste bud cells (TBC) from $CB_1R^{-/-}$ and wild-type mice.
Materials and methods: We employed behavioral tests (two-bottle preference and licking tests), RT-qPCR, Western Blot and calcium imaging studies on freshly isolated TBC from $CB_1R^{-/-}$ and wild-type mice.
Results: Behavioral studies showed that the absence of CB_1R gene was associated with low preference for fatty solutions. No difference in CD36 and GPR120 gene and protein expressions were observed in TBC in $CB_1R^{-/-}$ mice. Finally, measurement of Ca^{2+} signaling using rimonabant, a CB_1R antagonist, and linoleic acid shows that rimonabant and linoleic acid act synergistically *via* different pathways and triggers a rise of $[Ca^{2+}]i$.
Discussion: For the first time, we show that CB_1R is involved in fat perception and is a potential target for the treatment of obesity. Indeed, cannabinoid agonists are used in the treatment of cachexia in cancer patients. Therefore, cannabinoid antagonists could contribute to the management of obesity.

YPO.041

Long-term high fat feeding, but not neonatal immune challenge, affects cytokine levels in rats

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Intestinal permeability to bacteria is reported to increase with high-fat diets. Aim of this study was to immunize the rat pups against *Escherichia coli*, a common intestinal bacterium, and to investigate the effects of high fat (HF) feeding on biochemical parameters and cytokines.

Female ($n = 32$) and male ($n = 32$) rat pups were injected intraperitoneally either 100 μg/kg Escherichia coli cell wall constituent (lipopolysaccharide, LPS) or sterile saline solution on postnatal day 7. Following weaning, they were divided into two subgroups and were either offered standard chow or HF diet until day 150. All animals were decapitated, organs were weighed and blood samples were removed for TNF-alpha, IL-1beta, CRP, IFN-gamma, IL-4, triglyceride and cholesterol analyses.

Liver, thymus and kidney weights were higher in males and in HF groups ($P < 0.05$). HF increased total cholesterol and triglycerides levels. Cholesterol levels were higher in females than males ($P < 0.05$). Serum concentrations of TNF-alpha and IL-4 did not differ between the groups ($P > 0.05$). Serum concentrations of IL-1 beta and IFN-gamma were higher but CRP was lower in HF groups

HF successfully increased blood cholesterol and triglyceride levels. Differed cytokine levels in HF but not in LPS groups, suggests that rather than early bacterial immunization, inflammatory changes caused by HF might be more pronounced. Additionally, there were sexually dimorphic strategies to increased fat intake.

YPO.042

Insulin treatment in type 2 diabetic patients modulates immune cell profile: implication of Th1/Th2 polarization

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Background: Low-grade inflammation in insulin resistance has been associated with type 2 diabetes mellitus. We aim to assess Th1/Th2 cytokines along with the frequencies of innate and adaptive immunity cells in insulin-treated type 2 diabetic (T2D) patients.
Methods: Forty five T2D patients and forty three age-matched healthy control subjects were selected. Serum concentrations of T-helper type 1 (Th1) and Th2 cytokines and the frequencies of innate and adaptive immunity cells were assessed.
Results: T2D patients were hyperglycemic and showed higher level of insulin, normal levels of triglycerides and total-cholesterol and without any change in HDL-cholesterol. Compared to healthy subjects, T2D patients exhibited significantly decreased frequencies of neutrophils, without any change in monocytes, eosinophils and natural killer cells. The percentages of total lymphocytes ($CD3^+$) and $CD8^+$ T-cells decreased whereas those of regulatory T-cells increased without any change in $CD4^+$ T-cells in T2D patients. Interestingly, the frequency of effector $CD4^+$ T-cells and B-cells were increased in T2D patients. Serum concentrations of IL-2, IFN-γ and IL-4

decreased while that of IL-10, an anti-inflammatory cytokine, significantly enhanced in T2D patients, suggesting a differentiation of CD4 + Th cells towards (IL-10-producing) effector Th2-cells, concomitant with increased levels of B-cells in T2D patients.
Conclusions: Insulin-treated type 2 diabetes mellitus is associated with an anti-inflammatory profile related to differentiation of $CD4^+$ Th-cells towards Th2 phenotype concomitant with increased frequency of B-cells, and this may probably be beneficial to prevent certain infections.

Integrative Biology

YPO.043

Inactivation of myostatin: a potential therapeutic tool against autosomal dominant centronuclear myopathy

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The unique mouse model for autosomal dominant centronuclear myopathy (KI-$Dnm2^{R465W/+}$), associated to mutation of the *dynamin-2* gene (*dnm2*) reproduces some of the human clinical features, notably muscle atrophy and weakness. Myostatin (MSTN), is a master negative regulator of skeletal muscle mass. We hypothesized, that inactivation of *mstn* could limit muscle atrophy and weakness reported in the KI mouse. To validate this, we intercrossed KI mice with mice inactivated for *mstn* (KO-*mstn*) to generate a double mutated lineage (KIKO).

Animals were followed during 12 months. Muscle force, and motricity were significantly altered in 1-month old KI mice. A significant loss of muscle mass and volume (microRMI) were observed in KI from 2-months of age. From 2 to 12 months, all these parameters remained below of control values. When compared to KI mice, KIKO mice presented an increase of muscle grip strength and less affected motor skills. In agreement with these data, muscle mass and volume were increased during all the study.

Molecular analyzes showed that inactivation of *mstn* allowed for an increase of several proteins involved in the IGF1/Akt/mTOR pathway, but also a down regulation of several factors involved in ubiquitin-proteasome. Overall, we demonstrated that inactivation of *mstn* improves muscle mass and function of KI mice.

These results are very promising since genetic inactivation of *mstn* showed a real benefit for KI mice. The perspective to this work is to evaluate the efficiency of an anti-mstn based pharmacological approach to restore muscle function after the establishment of the disease.

Other Allied Disciplines

YPO.044

Does the vestibular system influences circadian rhythmicity? Highlights

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Introduction: Classically considered as the organ of the equilibrium, the vestibular system is likely to influence circadian rhythms regulation (Fuller et al., 2002). To confirm this statement, our team has studied how vestibular decease affects circadian rhythms in animals and humans.
Methods: In animals, 18 rats received either a transtympanic injection of sodium arsenilate ($n = 9$) allowing selective peripheral hair cell degeneration (Vignaux et al., 2012) or of NaCl solution ($n = 9$). Circadian rhythms of temperature and locomotor activity (act) were recorded before and after injection by telemetry. In human, we recorded rest-activity cycle at home and circadian rhythms at laboratory in 9 patients suffering from idiopathic BVL.
Results: BVL in rat causes altered thermoregulation and a disappearance of circadian rhythms of temperature and locomotor activity during 1 week after BVL. This was not related to a masking effect of the disrupted activity, since act and temperature were not correlated anymore after BVL. Human patients displayed a twice higher nocturnal actigraphy than healthy participants with reduced sleep efficiency (78.8%). Patients had a marked temperature rhythm but with a significant phase advance (1 h13) and higher variability of the peak time (from 2:24PM to 9:25PM), leading to a desynchronization with the rest-activity cycle contrary to healthy participants.
Discussion: These findings support the hypothesis that vestibular inputs to the circadian clock may represent key sensory inflow regarding animal movement, including in humans. Hence vestibular inputs may represent salient input to the circadian clock that enhances the stabilization and precision of both external and internal entrainments.

Sleep Physiology

YPO.045

Effect of the chronic intermittent hypoxia on muscle function in a mouse model of obstructive sleep apnea

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Obstructive sleep apnea (OSA) is characterized by recurrent episodes of upper airway (UA) collapse leading to chronic intermittent hypoxia (ChIH), a dominant feature of OSA known to be associated to an increased reactive oxygen species production. There is evidence of structural and functional abnormalities in UA muscles but the exact origin of this dysfunction is hardly known and probably multifactorial. To better understand the contribution of ChIH and associated oxidative stress (OS) to OSA pathophysiology, C57BL/6J mice were exposed to ChIH (FIO_2 6–21%, 30 s/30 s) 8 h/day using a standardized and well-controlled device (Chodzynsky et al., 2013, PLoS One). After 35 days, ChIH mice recapitulate key features of OSA (polycythemia, cardiac and metabolic alterations). The analysis of muscle contractile properties has shown a significant increase in sternohyoïd muscle fatigability while diaphragm exhibited an improvement of fatigue resistance. An improved recovery was seen in the sternohyoïd at 35 days as compared to other time-points. No change was observed in EDL (fast) and soleus (slow) muscles as regards to mechanical properties. However, in all investigated muscles, the evaluation of myofiber size has revealed a surface distribution shift towards a greater cellular cross section indicative of a hypertrophy, without change in fibre-type distribution.

In conclusion, ChIH *per se* is sufficient to induce a UA muscle dysfunction and structural alterations of respiratory and limb muscles in a mouse model of OSA. Moreover, current analyses of protein carbonylation and lipid peroxidation indicate an OS in limb muscles.

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