Tuesday September 27, 2016
Olympic Museum
Quai d'Ouchy 1, 1006 Lausanne

……

LIMNA Symposium: "Muscle stem cells, Metabolism and Ageing"

……

Organizing Committee: Prof. J. Auwerx, Prof. B. Desvergne, Prof. L. Fajas, Prof. F. Pralong, Prof. B. Thorens, Prof. Kei Sakamoto and Dr. L. Descamps.

Invited Speakers

- Francesca Amati, Department of Physiology and Institute of Sports Sciences, University of Lausanne, Switzerland
- Jérôme Feige, Nestlé Institute of Health Sciences, Lausanne, Switzerland
- Pascal Maire, Institut Cochin, INSERM U1016, CNRS UMR 8104, Université Paris Descartes, France
- Rémi Mounier, Institut NeuroMyogène, CNRS UMR 5310, INSERM U1217, Université de Lyon, France
- Pura Muñoz-Cànoves, ICREA and Pompeu Fabra University (UPF, Barcelona) and Cardiovascular Research Center (CNIC, Madrid), Spain
- Mario Pende, Inserm U1151, Institut Necker Enfants Malades, Faculté de Médecine Paris Descartes, Paris, France
- Michael Rudnicki, Ottawa Hospital Research Institute, Canada

Short talks

- Tanja Sonntag, Aging of Skeletal Muscle, NIHS
- Laurent Mouchiroud, Laboratory of Integrative Systems Physiology, EPFL
- Catherine Yzydorczyk, Department of Pediatrics & DOHaD Laboratory, CHUV University Hospital and UNIL
- Emmanuel Somm, Service of Endocrinology, Diabetology and Metabolism, CHUV

Agenda

8h30-9h00 Welcome and distribution of badges

Opening

9h00 *Welcome*
Johan Auwerx

Morning session
Chairman: Johan Auwerx

9h10 **Michael Rudnicki**
"Molecular regulation of muscle stem cell asymmetric division"

9h45 **Jérôme Feige**
"Targeting the stem cell niche to restore muscle regeneration in aging"

10h20 Coffee Break

Chairman: Kei Sakamoto

10h40 **Rémi Mounier**
"Cell- and non-cell-autonomous metabolic regulations of muscle stem cell fate and skeletal muscle homeostasis"

11h15 **Pascal Maire**
"Six homeoproteins in skeletal muscle development and repair"

11h50 **Tanja Sonntag**
"*Nicotinamide riboside protects aged skeletal muscle by partially overcoming a state of NAD+ resistance in rats"*

12h05 Lunch

Afternoon session

13h00 Poster session

Chairman: Jérôme Feige

14h00 **Pura Muñoz-Cànoves**
"Loss of proteostasis in aging muscle stem cells"

14h35 **Laurent Mouchiroud**
"Dietary supplementation with Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents"

15h00 **Catherine Yzydorczyk**
"Stress-induced premature liver senescence in adulthood after transient postnatal overfeeding in mice"

15h15 **Emmanuel Somm**
"*β-Klotho deficiency protects against obesity: a crosstalk between liver, microbiota and brown adipose tissue"*

15h30 Coffee Break

Chairman: Lluis Fajas

15h50 **Mario Pende**
"mTOR signaling in muscle metabolism and ageing"

16h25 **Francesca Amati**
"Skeletal muscle mitochondrial adaptations to exercise in the elderly"

17h00 Concluding remarks and poster prize distribution

Talks abstracts

Molecular regulation of muscle stem cell asymmetric division
Michael A. Rudnicki
Ottawa Hospital Research Institute, 451 Smyth Road, Ottawa, Ontario, Canada K1H 8L6.
mrudnicki@ohri.ca.

We discovered that a subset of satellite cells in skeletal muscle are self-renewing stem cells that give rise to myogenic progenitors through asymmetric apical-basal cell divisions. Our identification of *satellite stem cells* has facilitated important insights into satellite cell biology. For example, we discovered Wnt7a/Fzd7 signaling as important intrinsic control mechanism that plays a central role in regulating the pool size of the satellite stem cell compartment by stimulating symmetric stem cell expansion. Direct injection of recombinant Wnt7a protein into muscle significantly augments regeneration. Wnt7a treated muscles were larger, contained higher numbers of satellite cells, larger caliber myofibers, and were able to generate more force upon stimulation. Thus, the regulation of asymmetric stem cell division is a key control point that impacts the efficacy of the entire regenerative program. Stem cell polarity is established by the PAR complex, comprised of PAR3/PAR6/aPKC, to regulate self-renewal and expansion. We have discovered that full-length dystrophin is expressed in satellite stem cells in skeletal muscle. We have made the seminal discovery that dystrophin regulates the establishment of PAR-mediated polarity in satellite cells. In the absence of dystrophin, the polarity effector Par1b is dysregulated, leading to the failure of Par3 to become localized to the cortex associated with the basal lamina. Importantly, this results in an abnormal increase in centrosome number, a 10-fold reduction in the numbers of satellite stem cells undergoing asymmetric divisions, and a marked decrease in the generation of myogenin-expressing progenitors. Accordingly, our data suggests that the failure of regenerative myogenesis to keep pace with disease progression in DMD is not due to muscle stem cell exhaustion, but rather is due to a cell-autonomous deficiency in asymmetric division.

Targeting the Stem Cell Niche to Restore Muscle Regeneration in Aging
Jérôme N. Feige
Aging Group, Nestlé Institute of Health Sciences, EPFL Innovation Park, Lausanne, Switzerland.

The remarkable ability of skeletal muscle to regenerate upon injury is conferred by tissue-resident stem cells called satellite cells. With age, the regenerative capacity of muscle stem cells (MuSCs) dramatically declines. Developing strategies to enhance muscle repair in elderly people is therefore required, in particular to accelerate their recovery from injuries following falls or from surgical interventions affecting muscle tissues. As the causes of MuSC dysfunction with age are multi-systemic, we have investigated age-related changes at different levels of the MuSC niche, in order to uncover synergistic ways to restore regenerative capacity in aged skeletal muscle. Our results demonstrate that interventions targeting the extracellular matrix, cell-cell interactions in the stem cell niche and circulating peptides can rescue skeletal muscle regenerative failure during aging by restoring youthful muscle stem cell function.

Cell- and non-cell-autonomous metabolic regulations of muscle stem cell fate and skeletal muscle homeostasis
Rémi Mounier, Institut NeuroMyogène, CNRS UMR 5310, INSERM U1217, Université de Lyon, France

Skeletal muscle possesses a remarkable plasticity and responds to environmental and physiological challenges by changing its phenotype in terms of size, composition and metabolic properties. Muscle fibers rapidly adapt to drastic changes in energy demands during exercise through fine-tuning of the balance between catabolic and anabolic processes. Recent advances have shed new light on the relevance of AMPK both as a multi-task gatekeeper and energy regulator in skeletal muscle. We will discuss recent findings in the AMPK functions in skeletal muscle adaptations to contraction as well as highlight its role in the control of skeletal muscle regeneration post-injury. Indeed, we demonstrated that macrophage AMPKα1 is crucial for the resolution of inflammation during skeletal muscle regeneration. Our work also establishes a new and important role of AMPKα1 in muscle stem fate choice by switching their metabolism at the time of differentiation/self-renewal during skeletal regeneration, linking for the first time self-renewal and metabolism in this context.

Six homeoproteins in skeletal muscle development and repair
Pascal Maire
Institut Cochin. INSERM U1016. CNRS UMR8104. Université Paris Descartes. Sorbonne Paris Cité. 24 rue du Fg St Jacques. Paris, France.

Six homeoproteins are expressed all along muscle development, in the embryonic dermomyotome where they control sequentially hypaxial Pax3 expression, Myf5 and Myod1 gene expression and myogenic fate engagement, and later on Myogenin and muscle sarcomeric genes expression. Six1 remains expressed at high levels in adult muscle fibers and in associated satellite cells. I will present a summary of our recent work concerning the role of Six1 and Six4 in mouse embryonic fibroblast reprogramming by MyoD and in the specific properties of Six1 in the control of the adult muscle fast phenotype.

Loss of proteostasis in aging muscle stem cells
Pura Muñoz-Cánoves
Pompeu Fabra University, ICREA and CNIC, Spain

Skeletal muscle has a remarkable capacity to regenerate by virtue of its resident Pax7-expressing stem cells (satellite cells), which are normally quiescent in the adult. Upon injury, quiescent satellite cells activate and proliferate, to subsequently differentiate and form new myofibers or self-renew to restore the quiescent satellite cell pool. Through a combination of global gene expression/bioinformatics and molecular/cellular assays, we found that resting satellite cells have basal autophagy activity, which is required to maintain the quiescent state. We will discuss our recent findings on how loss of proteostasis is causally implicated in muscle stem cell aging, and strategies to target this pathway to improve muscle regeneration.

mTOR signaling in muscle metabolism and ageing.
Mario Pende
Inserm, Paris Descartes University, France.

The mammalian Target Of Rapamycin is a master regulator of growth. mTOR is a serine/threonine protein kinase that exists in two distinct complexes in the cell (mTORC1 and mTORC2) and transduces virtually all anabolic signals from the environement: nutrients, such as glucose and amino acids, growth factor peptides, such as insulin and insulin like growth factors, oxygen, mitochondrial metabolites, energy status. mTOR is required to sustain cell responses to nutrient availability including cell growth, proliferation, macromolecule biosynthesis, and suppress autophagy. During the past ten years we have generated and characterized a wide panel of mouse mutants in the mTOR pathway. We were involved in revealing specific and interesting phenotypes that increased our knowledge of mTOR roles in pathophysiology: mutants with small cells, mutants resistant to tumorigenesis in specific tissues and after specific oncogenic, mutants with muscle dystrophy, mutants mimicking caloric restriction and promoting longevity, mutants with altered insulin action.
I will present our progress on the molecular mechanisms of cell size control and organismal longevity. I will show how nutrient sensing pathways impact on cell senescence through the activation of mTORC1-S6 kinases and the phosphorylation of ZRF1. I will also detail our efforts to understand rare human genetic diseases that arise from pathological changes in the activity of the mTOR pathway, including Tuberous Sclerosis Complex and lipin1 deficiency.

Skeletal muscle mitochondrial adaptations to exercise in the elderly

Francesca Amati
Department of Physiology and Institute of Sports Sciences, University of Lausanne, Switzerland.

Increased life expectancy is associated with alterations in skeletal muscle, encompassing metabolic (e.g. oxidative dysfunction, insulin resistance) and functional consequences (e.g. muscle wasting). Increasing evidence places the decline in mitochondria, content and function, among key skeletal muscle aging processes. Many aspects of unhealthy aging can be improved, or at least mitigated, by physical activity and exercise. Indeed, lifelong inactive subjects are able, even late in life, to recover muscle mass, as well as mitochondrial function with targeted interventions. Mitochondrial function and efficiency, is related to a dynamic capacity to modify architecture in response to external stimuli. Mitochondrial dynamic processes include the possibility to be newly generated, fused, divided (called fission) or to be discarded by mitochondria targeted autophagy (called mitophagy). Fusion, fission and mitophagy are strongly interconnected and share key actors such as Mfn2 and DRP1. These ensure the role of switch tender to determine the fate of mitochondria. The physiological and molecular mechanisms regulating this equilibrium and the adaption of these processes in aging muscle remain unknown. To decipher the molecular mechanisms involved in the "fountain of youth" effect of exercise, we investigated the subtle balance of the tripart system fusion/fission/mitophagy in skeletal muscle of older volunteers before and after an exercise intervention.

Shorts talks

Sonntag Tanja
Nicotinamide riboside protects aged skeletal muscle by partially overcoming a state of NAD+ resistance in rats

T. Sonntag, E. Migliavacca, A. Pannérec, P. Cichosz, C. Cantó and J.N. Feige

The co-factor nicotinamide adenine dinucleotide (NAD+) is a major regulator of cellular metabolism. Age-related mitochondrial dysfunction has been connected to a decline in NAD+ levels and repletion of NAD+ through NAD+ precursors like nicotinamide riboside (NR) has been shown to revert this process. In this study, we aimed to investigate whether targeting mitochondrial function through NR can be beneficial in the context of sarcopenia, the age-related loss of muscle mass and function. We tested the acute transcriptional signaling in young adult and aged sarcopenic rats following injection with NR. Using transcriptional profiling of gastrocnemius muscle, we identify molecular pathways regulated by NR supplementation and demonstrate that aging leads to a state of partial NAD+ resistance as only a subset of the pathways regulated by NR in young skeletal muscle is conserved in the aged. Altogether our results uncover novel transcriptional networks regulated by NR and establish the subset of these regulations which are conserved during aging and can reverse the molecular perturbations of sarcopenia.

Mouchiroud Laurent
Laboratory of Integrative Systems Physiology, EPFL

Dietary supplementation with Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents

Laurent Mouchiroud, Dongryeol Ryu, Pénélope Andreux, Elena Katsyuba, Norman Moullan, Amandine Nicolet-Dit-Félix, Evan Williams, Pooja Jha, Giuseppe Lo Sasso, Damien Huzard, Patrick Aebischer, Carmen Sandi, Chris Rinsch, Johan Auwerx

The biological effects of urolithins remain poorly characterized, despite wide-spread human exposure via the dietary consumption of their metabolic precursors, the ellagitannins, which are found in the pomegranate fruit, as well as in nuts and berries. We identified urolithin A (UA) as a first-in-class natural compound that induces mitophagy both in vitro and in vivo following oral consumption. In C. elegans, UA prevented the accumulation of dysfunctional mitochondria with age and extended lifespan. Likewise, UA prolonged normal activity during aging in C. elegans, including mobility and pharyngeal pumping, while maintaining mitochondrial respiratory capacity. These effects translated to rodents, where UA improved exercise capacity in two different mouse models of age-related decline of muscle function, as well as in young rats. Our findings highlight the health benefits of urolithin A and its potential application in strategies to improve mitochondrial and muscle function.

Yzydorczyk Catherine
Department of Pediatrics & DOHaD Laboratory, CHUV University Hospital and UNIL, Lausanne, Switzerland

Stress-induced premature liver senescence in adulthood after transient postnatal overfeeding in mice

Catherine Yzydorczyk, Na Li, Christine Sempoux, Dolores Mosig, Michael Bidho, Basile Keshavjee, Jean Baptiste Armengaud, Hassib Chehade, Katya Nardou, Benazir Siddeek, Mohamed Benahmed, Catherine Vergely, Umberto Simeoni

Background Epidemiological and animal studies have shown that an altered nutritional environment during critical periods of development can lead to metabolic disorders later in life. Oxidative stress (OS) could be associated with the development of these chronic diseases. The liver is involved in lipid and glucose homeostasis and is vulnerable to nutritional programming during the perinatal period. OS has been associated with stress-induced premature senescence (SIPS), and hepatocyte senescence has notably been involved in liver structure and function alterations. However, the role of SIPS in adult liver dysfunctions after a transient postnatal overfeeding (OF) is still unknown. Material and Methods Litters of C57BL/6 male mice were maintained, during lactation period, at 9 pups (normal litter, NL) or reduced to 3 in order to induce transient postnatal OF. The following parameters were studied at adulthood (7 months): i) markers of OS (reactive oxygen species, antioxidant defenses); ii) markers of SIPS (factors involved in cell cycle arrest); iii) liver structure and function (histological analysis, insulin signaling pathways and glucose transporters). Results At adulthood liver from OF vs. NL mice presented: i) higher levels of superoxide anion, decreased catalase, superoxide dismutase expressions, $p<0.01$; ii) increased p21, p53, Acp53, p16, decreased pRb/Rb expressions, $p<0.01$; iii) increased glycogen content and hepatic fibrosis, decreased IRS-1/2, pIRS-1, PI3K and pAkt/Akt expressions, $p<0.01$; decreased GLUT-2 and increased GLUT-4 levels, $p<0.05$. Conclusion A transient postnatal overfeeding led at adulthood to SIPS associated with OS and altered hepatic structure and function. This could contribute to later development of hepatic chronic disease.

Somm Emmanuel
EDM (CHUV)

β-Klotho deficiency protects against obesity: a crosstalk between liver, microbiota and brown adipose tissue

Emmanuel Somm, Henry Hugues, Stephen Bruce, Sébastien Aeby, Marta Rosikiewicz, Gerasimos P Sykiotis, Andrew Dwyer, James S Acierno Jr, Kristina Schoonjans, Luis Fajas and Nelly Pitteloud

Administration of rFGF21 or analogs is a promising metabolic treatment. β-Klotho is the obligate co-receptor for the FGF21/FGFR1c and the FGF15-19/FGFR4 signaling. We presently show that Klb-/- mice are resistant to diet-induced obesity (DIO) due to a robust elevation of energy expenditure. Beyond a derepressed bile acid (BA) synthesis, Klb-/- mice exhibit a drastic change in circulating BA composition featured by overrepresentation of cholic acid (CA) and its microbiota-derivative deoxycholic acid (DCA). A genetic approach (concomitant Tgr5 deletion) and a pharmacologic approach (antibiotic treatment abolishing bacterial conversion of CA into DCA) both reverse the DIO resistance, identifying a liver-microbiota-BAT crosstalk in Klb-/- mice. Our work demonstrates that 1) Endogenous FGF21 signaling is dispensable to resist to DIO and 2) Secondary BA (DCA) specifically activates thermogenesis, conferring to microbiota a new regulatory role in host metabolism. Pharmacological approaches targeting the FGF15-19/FGFR4/KLB pathway to selectively modulate BA pool composition could be valuable to treat metabolic disorders.

Posters

1. Dammone Gabriele

Cross-talk between regeneration and ectopic adipogenesis in muscle aging

Laura Lukjanenko1, Sonia Karaz1, Gabriele Dammone2, Alessio Palini1, Carine Winkler2, Federica Gilardi2, Beatrice Desvergne2 and Jérôme N. Feige1

1- Nestlé Institute of Health Sciences
2- Centre Intégratif de Génomique

Adipose tissue infiltration in human skeletal muscle is associated with loss of muscle function in diseases such as age-related sarcopenia or myopathies, where the regenerative capacity of muscle is altered. The Fibro-Adipogenic Progenitor (FAP) is a newly characterized cell type in skeletal muscle which supports satellite cell myogenic functions and can give rise to ectopic adipocytes. While impaired regeneration capacity during aging has been associated with satellite cell dysfunction, how FAPs contribute to the homeostasis of the muscle stem cell niche during aging has not been explored. We have previously demonstrated that muscle regeneration triggers a transient adipogenic response with the presence of ectopic adipocytes between muscle fibers and the expression of an adipogenic molecular signature. We have now analyzed how this adipogenic response influences muscle regeneration by studying regeneration in PPARγ KO mice, which fail to activate adipogenic programs. In order to characterize how age impairs the muscle stem cell niche, we combined ex-vivo assays of muscle progenitors from young and old mice to transcriptomic and proteomic profiling in the muscle stem cell niche of young and old muscle. Altogether, our results provide an integrated view of the cross-talk between myogenic and adipogenic signals in satellite cells and FAPs during muscle aging.

2. Sonntag Tanja

Nicotinamide riboside protects aged skeletal muscle by partially overcoming a state of NAD+ resistance in rats

T. Sonntag, E. Migliavacca, A. Pannérec, P. Cichosz, C. Cantó and J.N. Feige

The co-factor nicotinamide adenine dinucleotide (NAD+) is a major regulator of cellular metabolism. Age-related mitochondrial dysfunction has been connected to a decline in NAD+ levels and repletion of NAD+ through NAD+ precursors like nicotinamide riboside (NR) has been shown to revert this process. In this study, we aimed to investigate whether targeting mitochondrial function through NR can be beneficial in the context of sarcopenia, the age-related loss of muscle mass and function. We tested the acute transcriptional signaling in young adult and aged sarcopenic rats following injection with NR. Using transcriptional profiling of gastrocnemius muscle, we identify molecular pathways regulated by NR supplementation and demonstrate that aging leads to a state of partial NAD+ resistance as only a subset of the pathways regulated by NR in young skeletal muscle is conserved in the aged. Altogether our results uncover novel transcriptional networks regulated by NR and establish the subset of these regulations which are conserved during aging and can reverse the molecular perturbations of sarcopenia.

3. Pannérec Alice

Maintenance of a robust neuromuscular system as a novel strategy to protect skeletal muscle from sarcopenia

Alice Pannérec, Margherita Springer and Jérôme N. Feige

Declining muscle mass and function is one of the main drivers of loss of independence in the elderly. Sarcopenia, the age-related loss of skeletal muscle mass and function, is associated with numerous cellular and endocrine perturbations, and it remains challenging to identify those changes that play a causal role and could serve as targets for therapeutic intervention. Using a rat model of natural aging, we have found a differential susceptibility of certain muscles to age-related decline as aging rats specifically lose muscle mass and function in the hindlimbs, but not in the forelimbs. By performing a comprehensive comparative analysis of these muscles, we demonstrate that regional susceptibility to sarcopenia is dependent on neuromuscular junction fragmentation, loss of motoneuron innervation, and reduced excitability. Remarkably, muscle loss in elderly humans also differs in vastus lateralis and tibialis anterior muscles in direct relation to neuromuscular dysfunction, suggesting that maintenance of the neuromuscular system is key for healthy muscle aging. We further found that circulating levels of one neurotrophic factor are decreasing with age in rats. In vitro analysis using a nerve/muscle co-culture assay revealed that this neurotrophic factor protects the neuromuscular system from damage, suggesting that maintaining proper circulating levels with advancing age would have beneficial effect on muscle. Indeed, when rats were treated with this specific factor at the onset of sarcopenia we observe a protective effect on muscle mass. Taken together, our results demonstrate that maintenance of the neuromuscular system is key to protect from sarcopenia and we provide evidence for the role of one neurotrophic factor in this process.

4. Mouchiroud Laurent

Dietary supplementation with Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents

Laurent Mouchiroud, Dongryeol Ryu, Pénélope Andreux, Elena Katsyuba, Norman Moullan, Amandine Nicolet-Dit-Félix, Evan Williams, Pooja Jha, Giuseppe Lo Sasso, Damien Huzard, Patrick Aebischer, Carmen Sandi, Chris Rinsch, Johan Auwerx

The biological effects of urolithins remain poorly characterized, despite wide-spread human exposure via the dietary consumption of their metabolic precursors, the ellagitannins, which are found in the pomegranate fruit, as well as in nuts and berries. We identified urolithin A (UA) as a first-in-class natural compound that induces mitophagy both in vitro and in vivo following oral consumption. In C. elegans, UA prevented the accumulation of dysfunctional mitochondria with age and extended lifespan. Likewise, UA prolonged normal activity during aging in C. elegans, including mobility and pharyngeal pumping, while maintaining mitochondrial respiratory capacity. These effects translated to rodents, where UA improved exercise capacity in two different mouse models of age-related decline of muscle function, as well as in young rats. Our findings highlight the health benefits of urolithin A and its potential application in strategies to improve mitochondrial and muscle function.

5. Shaqiri Albulena

How to counteract perceptual and cognitive decline in healthy aging by nutrients?

Albulena Shaqiri, Karin Pilz, Aaron Clarke, Daniela Herzig, Marina Kunchulia, Michael H. Herzog

Almost nothing is known about how nutrition affects perception and cognition. Several studies have shown that certain ingredients of food can improve performance in certain cognitive or perceptual tests (and can thus counteract decline in healthy aging). However, surprisingly, performance levels in cognitive and perceptual tests correlate very little with each other and, thus, it remains unknown whether nutrition can improve general abilities. We will study the effects of nutrition by using a battery of well-chosen perceptual and cognitive tests. In addition, we will record the EEG, in order to understand the underlying neural causes of nutrition on aging. Our long term goal is to create a platform for human perceptual and cognitive testing within the Integrative Food and Nutrition Center, in cooperation with laboratories in the Lemanic region working on animal and molecular models of nutrition and aging.

6. Diessler Shanaz

Systems genetics approach to identify the molecular pathways controlling sleep, focus on metabolic data

Shanaz Diessler, Maxime Jan, Yann Emmenegger, Debra Skene, Ioannis Xenarios, Paul Franken.

Disrupted sleep is prevalent in our 24/7 society and can have far-reaching adverse, clinical effects such as increased risk for metabolic disorders. Although sleep and the response to sleep loss are known to have strong genetic determinants, genetic heterogeneity and complex gene-by-environment interactions will ultimately determine vulnerability to develop sleep-associated diseases. We took a systems genetics approach using a mouse genetic reference population to map the molecular pathways involved in regulating sleep by combining multi-level information from genotype, to brain and liver transcriptomes (RNAseq), to plasma metabolome (targeted LC/MS, FIA), and to the sleep-wake phenome (EEG/EMG, locomotor activity) with sleep deprivation as an environmental challenge. We studied 33 BXD lines both under baseline and sleep deprivation conditions, constructed genotype maps using 11k SNPs and quantified 325 sleep/EEG/activity phenotypes, >12k transcripts in cortex and liver, and 124 plasma metabolites. Quantitative Trait Locus (QTL) mapping identified 61 genome wide-significant phenotype or phQTLs, 21 significant metabolic or mQTLs, and several thousand expression or eQTLs in cortex and liver. Most QTLs were broad containing many candidate genes. To prioritize candidate genes within the QTL region, phenotype-gene expression correlations, eQTLs, and SNPs impact prediction on protein function were determined. Apart from this genomic analysis of sleep traits, our data show that sleep deprivation profoundly impacts both central and peripheral gene expression levels and the blood metabolome. Network analyses integrating transcriptome, metabolome, and sleep phenome data allowed us to identify critical nodes and gene module.

7. Martinez Carreres Laia

CDK4 is a lysosomal regulator via mTORC1

Martínez-Carreres, L., Orpinell, M., Fajas, L.

Cyclin Dependent Kinase 4 (CDK4) is a serine/threonine kinase belonging to the CDK family, which exerts its function when associated to a Cyclin partner. When CDK4 associates with D-type cyclins in the G1 phase of the cell cycle, it phosphorylates target proteins such as the Retinoblastoma (Rb) protein. The phosphorylated Rb releases the transcription factor E2F1, which in turn will drive the transition from the G1 phase to S phase of the cell cycle by activating its target genes. In addition to the well established role of CDK4 in cell cycle progression, work from our laboratoy has provided extensive proof that CDK4 plays alternative but crucial roles in the Insulin Signaling Pathway as well. In the present study, we show that the function of CDK4 contributes to the regulation of lysosomal biology in cancer cells. CDK4 inhibition or depletion leads to increased lysosomal size and number, which may correlate with induction of autophagy and lysosomal biogenesis, respectively. Consistent with its role as a regulator of lysosomal biology, the function of mTORC1 is impaired upon CDK4 inhibition or depletion, as evidenced by a decrease in the phosphorylation status of its downstream targets. Moreover, CDK4 inhibition or depletion blunts mTORC1 recruitment to the lysosomal membrane, even in the presence of amino acids, thus mimicking starvation conditions. Taken together, our results show that CDK4 is a novel regulator of mTORC1 activity and lysosomal biology.

8. Lemos Vera

A SUMO-dependent LRH-1/OSBP pathway promoting nonalcoholic fatty liver disease

Sokrates Stein*, Vera Lemos*, Pan Xu, Xu Wang, Dongryeol Ryu, Hadrien Demagny, Thomas F. Lüscher, Maaike H. Oosterveer and Kristina Schoonjans

The nuclear receptor liver receptor homolog-1 (LRH-1) is an important regulator of intermediary metabolism in the liver. Here, we assessed the contribution of LRH-1 SUMOylation to the development of nonalcoholic fatty liver disease (NAFLD). SUMOylation-defective Lrh-1 K289R mice developed NAFLD and early signs of nonalcoholic steatohepatitis (NASH) when challenged with a lipogenic high-fat high-sucrose diet. Moreover, we show that the Lrh-1 K289R mutation induces the expression of the oxysterol binding protein-like 3 (OSBPL3), enhances SREBP-1 processing and promotes de novo lipogenesis. Mechanistically, we demonstrate that ectopic expression of OSBPL3 facilitates SREBP-1 processing in wildtype mice, while silencing of Osbpl3 in the livers of Lrh-1 K289R mice reverses the lipogenic phenotype. These findings suggest that compromised LRH-1 SUMOylation predisposes to the development of NAFLD under lipogenic conditions.

9. Poster canceled

10. Somm Emmanuel

β-Klotho deficiency protects against obesity: a crosstalk between liver, microbiota and brown adipose tissue

Emmanuel Somm, Henry Hugues, Stephen Bruce, Sébastien Aeby, Marta Rosikiewicz, Gerasimos P Sykiotis, Andrew Dwyer, James S Acierno Jr, Kristina Schoonjans, Luis Fajas and Nelly Pitteloud

Administration of rFGF21 or analogs is a promising metabolic treatment. β-Klotho is the obligate co-receptor for the FGF21/FGFR1c and the FGF15-19/FGFR4 signaling. We presently show that Klb-/- mice are resistant to diet-induced obesity (DIO) due to a robust elevation of energy expenditure. Beyond a derepressed bile acid (BA) synthesis, Klb-/- mice exhibit a drastic change in circulating BA composition featured by overrepresentation of cholic acid (CA) and its microbiota-derivative deoxycholic acid (DCA). A genetic approach (concomitant Tgr5 deletion) and a pharmacologic approach (antibiotic treatment abolishing bacterial conversion of CA into DCA) both reverse the DIO resistance, identifying a liver-microbiota-BAT crosstalk in Klb-/- mice. Our work demonstrates that 1) Endogenous FGF21 signaling is dispensable to resist to DIO and 2) Secondary BA (DCA) specifically activates thermogenesis, conferring to microbiota a new regulatory role in host metabolism. Pharmacological approaches targeting the FGF15-19/FGFR4/KLB pathway to selectively modulate BA pool composition could be valuable to treat metabolic disorders.

11. Yzydorczyk Catherine

Stress-induced premature liver senescence in adulthood after transient postnatal overfeeding in mice

Catherine Yzydorczyk, Na Li, Christine Sempoux, Dolores Mosig, Michael Bidho, Basile Keshavjee, Jean Baptiste Armengaud, Hassib Chehade, Katya Nardou, Benazir Siddeek, Mohamed Benahmed, Catherine Vergely, Umberto Simeoni

Background Epidemiological and animal studies have shown that an altered nutritional environment during critical periods of development can lead to metabolic disorders later in life. Oxidative stress (OS) could be associated with the development of these chronic diseases. The liver is involved in lipid and glucose homeostasis and is vulnerable to nutritional programming during the perinatal period. OS has been associated with stress-induced premature senescence (SIPS), and hepatocyte senescence has notably been involved in liver structure and function alterations. However, the role of SIPS in adult liver dysfunctions after a transient postnatal overfeeding (OF) is still unknown. Material and Methods Litters of C57BL/6 male mice were maintained, during lactation period, at 9 pups (normal litter, NL) or reduced to 3 in order to induce transient postnatal OF. The following parameters were studied at adulthood (7 months): i) markers of OS (reactive oxygen species, antioxidant defenses); ii) markers of SIPS (factors involved in cell cycle arrest); iii) liver structure and function (histological analysis, insulin signaling pathways and glucose transporters). Results At adulthood liver from OF vs. NL mice presented: i) higher levels of superoxide anion, decreased catalase, superoxide dismutase expressions, $p<0.01$; ii) increased p21, p53, Acp53, p16, decreased pRb/Rb expressions, $p<0.01$; iii) increased glycogen content and hepatic fibrosis, decreased IRS-1/2, pIRS-1, PI3K and pAkt/Akt expressions, $p<0.01$; decreased GLUT-2 and increased GLUT-4 levels, $p<0.05$. Conclusion A transient postnatal overfeeding led at adulthood to SIPS associated with OS and altered hepatic structure and function. This could contribute to later development of hepatic chronic disease.

12. Burton Kathryn

Two-Week Intake of Dairy Probiotic or Prebiotic Suppresses the Postprandial Inflammatory Response Associated with a High-Fat Meal

KATHRYN J. BURTON, GRÉGORY PIMENTEL, MARTA ROSIKIEWICZ, UELI BUTIKOFER, UELI VON AH, MARIE-JEANNE VOIROL, SÉBASTIEN AEBY, GILBERT GREUB, FRANÇOIS P. PRALONG, GUY VERGÈRES, NATHALIE VIONNET.

Intestinal dysbiosis has been associated with metabolic diseases including type 2 diabetes mellitus and obesity. Intake of probiotics and prebiotics may modify the gut microbiota by respective delivery of live microorganisms to the microbiota and selective promote growth (or alter activity) of microorganisms present in the microbiota. Limited research has examined the impact of these interventions on the microbiota and their benefits on metabolic disease risk remain controversial. To explore the role of these interventions on the gut microbiota and metabolic health, fourteen healthy men (age 24.6 ± 4.7 years, BMI 21.8 ± 1.8 kg/m^2) were recruited in a 12 week randomised cross-over study. Yogurt containing Lactobacillus rhamnosus GG and acidified milk (2% δ-gluconolactone) were tested for 2 weeks (400g/day). Further to fasting assessments, a standardised high fat meal test was used to assess how the dairy products modulated the postprandial inflammatory response. The faecal microbiota was evaluated at 8 stages during the study by 16S rRNA sequencing (Illumina MiSeq). Daily intake of either probiotic yogurt or acidified milk did not affect fasting markers of metabolic health or inflammation. Conversely, for both dairy interventions, the postprandial response of interleukin 6, tumour necrosis factor-α and chemokine ligand 5 to a high fat test meal was significantly reduced (iAUC, $p<0.0001$). The microbiota showed marked change at the genera level following the dairy interventions with increased levels of Lactobacillus for both interventions and increased Bifidobacteria after acidified milk ($p<0.0001$). Short-term probiotic or prebiotic interventions appear to have benefits on postprandial inflammatory responses associated with a high fat meal, with parallel microbiota change.

13. Schiffrin Mariano

Pparg null mice show a sex dimorphism in Nonalcoholic Fatty Liver Disease

Mariano Schiffrin, Carine Winkler, Laure Quignodon, Chiara Sardella, Aurélien Naldi, Harald Köfeler, Federica Gilardi and Béatrice Desvergne

Abstract: Peroxisome proliferator-activated receptor gamma (Pparg is required for adipocyte differentiation. Pparg null mice develop non-alcoholic fatty liver disease (NAFLD) with massive fat storage in the liver due, at least in part, to the inability to stock the lipids in the lacking adipose tissue. Interestingly, this hepatic lipid accumulation is gender-related and is characterized by a higher content of lipid droplets, hepatic triglycerides (TGs) and neutrophil infiltration in females compared to males at 20 weeks. The aim of this project was to characterize the sexual-specific evolution of liver steatosis in this mouse model and to establish the molecular basis of this sex specificity in mouse. We found that genes involved in lipid droplet synthesis were induced in Pparg null females, whereas genes involved in microsomal w-oxidation were induced in Pparg null males. In addition, females had a higher hepatic content of short chain highly saturated TG species compared to males. Sex dimorphism of hepatic TG and of neutrophil infiltration was abolished by gonadectomy in Pparg null mice, indicating that sex hormones are involved in the liver phenotype. Hepatic gene expression is physiologically sex-biased in control mice. Importantly, we found that the sex-dependent expression of many sex-related genes was strongly altered in Pparg null mice, but also in another independent mouse model of NAFLD, the Ob/Ob mice. Our results suggest that the growth hormone/STAT5b pathway, which controls hepatic sex-biased gene expression, is dysregulated in NAFLD associated with metabolic syndrome. Our study, by highlighting the different response of males and females to lipid accumulation in the liver, will allow gaining insights into NAFLD sex dimorphism in disease development.

14. Magda Zachara

Single-cell RNA-seq-based functional characterization of adipose stem cells

Magda Zachara[1], Petra Catalina Schwalie[1], Hua Dong[2], Julie Russeil[1], Nassila Akchiche[2], Christian Wolfrum[2], Bart Deplancke[1]

[1]Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne and Swiss Institute of Bioinformatics, Lausanne, Switzerland; [2]Institute of Food Nutrition and Health, Eidgenössische Technische Hochschule Zürich, Schwerzenbach, Switzerland

Given the recent rise in the global incidence of obesity and its associated pathologies, there is a great interest in exploring mechanisms underlying disturbed energy balance and its resulting obesity and pathological effects. Fat depots consist of a heterogeneous and dynamic mix of cell types, which makes their molecular and developmental characterization highly challenging. Single-cell sequencing technologies can provide unprecedented resolution into the molecular composition of complex systems. Here, we employed single-cell RNA-seq to molecularly dissect adipogenic precursor populations that reside in a mouse subcutaneous stromal vascular fraction (msSVF). Using the SMART-seq protocol implemented on the Fluidigm C1 system, we obtained high quality transcriptome data of adipose stem cells and detected over 4,000 expressed genes per cell. We used dimensionality reduction and clustering techniques to group the cells according to their gene expression similarity, allowing us for the first time to delineate three distinct subpopulations. Specifically, we found that population 1 exhibits high expression of genes with development and differentiation-related functions as well as with secreted protein coding potential; population 2 shows high expression of pro-adipogenic genes, suggesting that these cells may already be pre-committed to the adipocyte lineage, whereas population 3 features genes that exhibit a significantly anti-correlated expression pattern with pre-adipocyte genes. To further understand functional differences between these three distinct cell groups, we used annotated surface markers to physically separate the three cell populations using flow cytometry and tested their differentiation potential. The highest adipogenic differentiation potential was observed for population 1, whereas strikingly, population 3 was completely refractory to adipogenic differentiation, consistent with their anti-adipogenic molecular character. Moreover, depleting the msSVF of population 3 cells dramatically increased overall differentiation, even though the latter cells constitute less than ~ 3% of msSVF cells. This finding has significant fundamental and biomedical implications, as it suggests that population 3 negatively controls adipocyte differentiation, making these cells attractive targets to combat fat cell accumulation and thus obesity. Together, our results emphasize the power of single-cell analysis approaches, as these allowed us to uncover several new msSVF cell types that may dramatically alter our understanding of fat cell dynamics in health and disease.