Monday April 25, 2016
Olympic Museum
Quai d'Ouchy 1, 1006 Lausanne

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Fifth LIMNA Symposium

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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

Bart Deplancke, PhD, Associate Professor, School of Life Sciences, Interschool Institute of Bioengineering, EPFL, Lausanne, Switzerland

Frédéric Gachon, PhD, Nestlé Institute of Health Sciences, Lausanne, Switzerland

Ping-Chih Ho, PhD, Assistant Professor, Department of Fundamental Oncology, University of Lausanne, Switzerland

Pere Puigserver, PhD, Professor of Cell Biology, Harvard Medical School, Dana-Farber Cancer Institute, Boston, US

Kristina Schoonjans, PhD, Associate Professor, School of Life Sciences, Interschool Institute of Bioengineering, EPFL, Lausanne, Switzerland

Agenda

8h30-9h00 Welcome and distribution of badges

Opening

9h00 **Lluis Fajas**
Welcome

Morning session

Chairman: Kei Sakamoto

9h10 **Kristina Schoonjans**
"LRH-1 as an anabolic integrator of hepatic cancer cell metabolism"

9h45 **Ping-Chih Ho**
"Metabolic communication and competition: That is why T cells fight against or cooperate with cancer cells"

10h20 Coffee Break

Chairman: Johan Auwerx

10h40 **Frédéric Gachon**
"Regulation of mouse physiology by circadian and feeding rhythms-coordinated post-transcriptional modifications"

11h15 **Marco Cassano**, Post Doc, Laboratory of Virology and Genetics, EPFL, Lab. Didier Trono
 "Polyphenic trait determines cancer susceptibility in a model of epigenetic instability"

11h30 **Joanna Ratajczak**, PhD student, Nestlé Institute of Health Sciences, Lab. Carles Canto
"The role of Nicotinamide Riboside Kinase 1 in mammalian metabolism"

11h45 **Hongbo Zhang**, PhD student, Laboratory of Integrative and Systems Physiology (LISP), EPFL, Lab. Johan Auwerx
"Improving mitochondrial function by NAD+ repletion rejuvenates adult stem cells and enhances lifespan »

12h00 Lunch

Afternoon session

13h00 **Poster session**

14h00 **Flash talks**

Chairman: Lluis Fajas

Alexandre Picard, Post Doc, Center for Integrative Genomics, Lab. Bernard Thorens, *A genetic screen identifies hypothalamic Fgf15 as a regulator of glucagon secretion*

Saifeldin Shehata, PhD student, Nestlé Institute of Health Sciences, Lab. Kei Sakamoto, *The regulation of the CDK-related PCTAIRE-1 protein kinase and its role in the brain*

Kathryn Burton, PhD student, EDM Department, CHUV, Lab. François Pralong, *Everything you always wanted to know about probiotics (but didn't dare to ask)*

Elena Katsyuba, PhD student, Laboratory of Integrative and Systems Physiology (LISP), EPFL, Lab. Johan Auwerx, *ACMSD as novel therapeutic target to boost NAD+ levels*

Sarah Sonnay, PhD student, Laboratory for Functional and Metabolic Imaging, EPFL, Lab. Rolf Gruetter, *Visual stimulation-induced functional and metabolic modifications in the cortex of the Tupaia belangeri*

Ngoc-Hien Du, Post Doc, Center for Integrative Genomics, Lab. David Gatfield, *Altered adaptation to food restriction upon total miRNA loss in mouse liver*

Laia Morató, Post Doc, Laboratory of Behavioral Genetics, Brain Mind Institute, EPFL, Lab. Carmen Sandi, *Effect of early life stress on behavioral and metabolic programming*

Anita Nasrallah, PhD student, Department of Physiology, Unil, Lab. Lluis Fajas, *The Role of CDK10 in Metabolism*

Sylviane Lagarrigue, Post Doc, Department of Physiology, Unil, Lab. Francesca Amati, *A missing link in skeletal muscle metabolism*

Svenja Groeneveld, PhD student, ISREC Institute, Lab. Etienne Meylan, *The hexosamine biosynthesis pathway in epithelial-to-mesenchymal transition in non-small cell lung cancer*

Rachana Pradhan, PhD student, Laboratory of Systems Biology and Genetics, EPFL, Lab. Bart Deplancke, *Integrative transcriptomic & epigenomic analysis of brown fat cell differentiation reveals novel transcriptional regulators*

Jasmin Hafner, PhD student, Laboratory of Computational Systems Biotechnology, EPFL, Lab. Vassily Hatzimanikatis, *"ATLAS of Biochemistry", a repository of all possible biochemical reactions for synthetic biology, metabolic engineering and metabolomics*

14h45 **Keynote talk - Pere Puigserver**
"Transcriptional control of glucose and mitochondrial bioenergetics"

15h30 **Albert Giralt Coll**, Post Doc, Department of Physiology, Unil, Lab. Lluis Fajas
"E2F1 participates in the regulation of hepatic gluconeogenesis"

15h45 Coffee break

Chairman: François Pralong

16h00 **Elise Vinckenbosch,** PhD student, CIBM, Laboratory for Functional and
Metabolic Imaging, EPFL, Lab. Rolf Gruetter
"Effects of glial TCA cycle inhibition in rodent cerebral metabolism"

16h15 Sophie Croizier, Post Doc, Center for Integrative Genomics, Lab. Bernard
Thorens
"Leptin Controls Parasympathetic Wiring of the Pancreas During Embryonic Life"

16h30 **Federica Gilardi**, Scientist, Center for Integrative Genomics, Unil, Lab. Béatrice
Desvergne
*"Epigenetic signature of WAT early aging highlights new immune players in WAT
progression to senescence"*

16h45 **Bart Deplancke**
"Decoding white and brown adipogenesis, one cell and transcription factor at a time"

17h20 Conclusions and award distribution

ABSTRACTS

Sponsored by

Kristina Schoonjans
School of Life Sciences, Interschool Institute of Bioengineering, EPFL, Lausanne, Switzerland

LRH-1 as an anabolic integrator of hepatic cancer cell metabolism

Metabolic reprogramming is a universal feature of tumor cells and is characterized by increased glycolysis and diminished oxidative phosphorylation, a phenomenon known as the Warburg effect. More recently, it has become evident that tumor cells heavily rely on glutamine metabolism to compensate for the Warburg effect and to replenish the tricarboxylic acid (TCA) cycle. However, the molecular mechanisms by which glutamine supports tumor cell metabolism remain largely unexplored. Here we will present data that underscore a pivotal role for the nuclear receptor LRH-1 as a key regulator of hepatic cancer cell metabolism. Hepatocyte-specific Lrh-1 knockout mice are protected against diethylnitrosamide-induced hepatocarcinogenesis. Acute and chronic deletion of hepatic LRH-1 interferes with glutamine-dependent anaplerosis and is attributed to the impaired flux through glutaminase 2 (GLS2), a mitochondrial enzyme involved in the deamination of glutamine. Diminished glutaminolysis in LRH-1 null livers is associated with reduced steady-state levels of the TCA intermediate, α-ketoglutarate, which in turn inhibits the mTORC1-signaling pathway to eventually block cell growth. In addition to GLS2, several other enzymes of the non-canonical glutamine processing pathway are affected in Lrh-1 knockout livers, ultimately depleting NADPH levels, which are required as cofactors for reductive biosynthetic pathways. Our studies provide novel insights into the regulatory mechanisms of glutamine fueled cancer metabolism and identify LRH-1 as a potential target for new anticancer therapeutics.

Ping-Chih Ho, Ph.D.
Department of Foundamental Oncology, University of Lausanne
Ludwig Center for Cancer Research at UNIL (LICR)

Metabolic communication and competition: That is why T cells fight against or cooperate with cancer cells

Metabolic transformation is a cardinal hallmark of most cancer cells that increases their proliferative and anti-apoptotic capacity by boosting anabolic metabolism, mainly through aerobic glycolysis and glutaminolysis. Elevated aerobic glycolysis and glucose uptake ability in cancer cells fuels their unregulated proliferation by accumulating glycolytic intermediates for generating biosynthetic macromoecules; this property of cancer cells is well known as the "Warburg effect". This metabolic property of cancer cells underscores the importance of glucose metabolism in cancer progression and it is also the foundation of the fludeoxyglucose positron emission tomography (FDG-PET), the primary method of identifying and monitoring the location and growth of primary and metastatic malignancies. Despite this knowledge, it remains unclear whether the metabolic state(s) of cancer cells modulates the functions and metabolic states of infiltrating immune and stromal cells. Our recent works focus on how metabolic communication and competition between cancer and tumor-infiltrating T cells determine anti-tumor immunity and the immunological phenotypes of infiltrating T cells. Moreover, our results suggest the intensive crosstalk between cancer and T cells shapes metabolic preference of cancer cells through an unidentified mechanism.

Frédéric Gachon
Nestlé Institute of Health Sciences, Lausanne, Switzerland

Regulation of mouse physiology by circadian and feeding rhythms-coordinated post-transcriptional modifications

Circadian clocks have been conserved throughout the evolution, allowing bacteria, animals, and plants to adapt their physiological needs to the time of day in an anticipatory way. In mammals, these pacemakers regulate many physiological processes such as sleep-wake cycles, body temperature, heartbeat, and many other aspects of the physiology. If mechanisms allowing these controls by the molecular oscillator and feeding rhythms are not completely understood yet, it is accepted that they involve rhythmic transcription of genes coding for enzymes implicated in different aspects of animal physiology. However, the potential role of post-transcriptional regulations in this process has been largely neglected for the moment. Based on our recent results using high throughput RNA sequencing and in vivo SILAC quantitative proteomic, we have now a better characterization of the rhythmic activation of signaling pathways, the mechanisms involved in their regulation by the circadian clock and feeding rhythms, and their consequences for animal physiology and metabolism. Considering the fact that perturbation of the circadian clock leads to numerous pathologies including obesity, type 2 diabetes and cancer, our results could contribute significantly to the understanding of this phenomenon.

Pere Puigserver
Harvard Medical School, Dana-Farber Cancer Institute, Boston, US

Transcriptional Control of Glucose and Mitochondrial Bioenergetics

1- **Glucose Metabolism-** Mammalian cells sense nutrient signals to reprogram energetic metabolism and trigger biological responses within the context of tissue function and whole animal physiology. This is exemplified by the nutrient fluctuations occurring during fed/fasting or diabetic conditions and the specific functions of the liver, skeletal muscle or adipose tissues. In the last years, we have extensively used the metabolic transcriptional coactivator PGC1α, a key protein in remodeling cellular metabolic programs including mitochondrial oxidative phosphorylation, as a "scaffold" bait to identify the mammalian nutrient sensing components. We have identified a new regulatory nutrient/metabolite pathway that impinges on the hyperacetylation status of PGC1α. Central sensing components within this pathway include metabolite sensitive enzymes such as the acetyl transferase GCN5 (responds to Acetyl-CoA levels), the deacetylase SIRT1 (responds to NAD+ levels), components of the canonical cAMP pathway, and cell cycle components.

2- **Mitochondrial Bioenergetics**- Biogenesis of functional and competent mitochondria requires the import and assembly of more than 1,000 proteins synthesized mostly in the cytoplasm and few in the mitochondria. The dynamics of mitochondrial formation and assembly is a complex cellular process that ultimately shapes the bioenergetic capacity. Mitochondrial biogenesis is heavily dependent upon timely and coordinated control of genes encoding for mitochondrial proteins. This regulatory network provides a therapeutic window to treat a broad spectrum of diseases associated with mitochondrial dysregulation, including metabolic diseases, myopathies and neurodegenerative diseases. We have applied bioinformatic and genetic/proteomic tools to identify transcription factors that are pivotal to mitochondrial biology including the zinc finger YY1 and the co-activator PGC-1α.

Bart Deplancke

School of Life Sciences, Interschool Institute of Bioengineering, EPFL, Lausanne, Switzerland

Decoding white and brown adipogenesis, one cell and transcription factor at a time

Achieving a comprehensive understanding of the cellular origin of white, brown and brite (brown-in-white) fat cells and of how they arise, are maintained, and function in vivo would be of great value for tackling global health burdens such as obesity and the metabolic syndrome. In this talk, I will discuss how we exploited the analytical power of single cell transcriptomics to molecularly dissect the adipose stem cell-enriched mouse subcutaneous stromal vascular fraction. Specifically, I will describe a detailed single cell RNA-seq-based characterization of precursor populations resident in mouse subcutaneous fat, arguing for the existence of several cell subpopulations with specific transcriptomic signatures and remarkably distinct differentiation potential. In addition, I will present how we use systems genomics approaches to dissect the regulatory mechanisms underlying brown adipogenesis, enabling us to identify novel regulators of this metabolically important differentiation process. Together, the presented findings will provide several, new insights into distinct aspects of white and brown adipose biology.

SHORT TALKS ABSTRACTS

Cassano Marco

Post Doc, Laboratory of Virology and Genetics, EPFL, Lab. Didier Trono

Polyphenic trait determines cancer susceptibility in a model of epigenetic instability

Marco Cassano, Sandra Offner, Alessandra Piersigilli, Evarist Planet, Suk Min Jang, Julien Duc, Hugues Henry, Kathleen McCoy, Andrew McPherson, and Didier Trono

Hepatocellular carcinoma (HCC) represents the fifth most common form of cancer worldwide and carries a high mortality rate due to lack of effective treatment (Fattovich et al, 2004). Males are eight times more likely to develop HCC that females, an effect largely driven by sex hormones, albeit through still poorly understood mechanisms (Bosch et al. 2004). We previously identified TRIM28, a scaffold protein capable of recruiting a number of chromatin modifiers, as a crucial mediator of sexual dimorphism in the liver, with Trim28hep-/- leading to sex-specific transcriptional deregulation of a wide range of bile and steroid metabolism genes in the mouse, and male-limited development of liver adenomas (Bojkowska et al. 2012). We now demonstrated that obesity precipitates alterations of Trim28-dependent transcriptional dynamics, leading to a metabolic infection state responsible for a high penetrance of male-restricted hepatic carcinogenesis. Raising Trim28hep-/- mice in axenic conditions reverts their tumor-prone phenotype, establishing a link between gut microbiota and hepatic carcinogenesis. Moreover, androgen deprivation markedly attenuates the frequency and severity of tumors in these animals, consolidating the apparent link between epigenetic control of sex hormones metabolism and oncogenic degeneration. This work underpins how discrete polyphenic traits can contribute to a cancer-prone state in epigenetic unstable settings, and more broadly provides new evidence linking hormonal imbalances, metabolic disturbances and cancer. Furthermore, our results establish the Trim28hep-/- mouse as a relevant model to study the pathogenesis of human HCC and test new therapeutic approaches for its control.

Ratajczak Joanna
PhD student, Nestlé Institute of Health Sciences, Lab. Carles Canto

The role of Nicotinamide Riboside Kinase 1 in mammalian metabolism

Joanna Ratajczak, Magali Joffraud, Samuel A.J. Trammell, Rosa Ras, Núria Canela, Marie Boutant, Sameer S. Kulkarni, Marcelo Rodrigues, Marie Migaud, Johan Auwerx, Oscar Yanes, Charles Brenner and Carles Canto

NAD+ has emerged as a central metabolic node implicated in lifespan and healthspan regulation by being a consumed substrate required for the activity of various enzyme families, including sirtuins and poly(ADP-ribose) polymerases. Supplementation with NAD+ precursors, such as nicotinamide mononucleotide (NMN) or nicotinamide riboside (NR), protects against metabolic disease, neurodegenerative disorders and age-related physiological decline in mammals. Nicotinamide riboside kinase 1 (NRK1) is an enzyme highly expressed in the liver, responsible for the first step of NR metabolism. Using genetic gain- and loss-of-function models, we demonstrate that in mammalian cells NRK1 is rate-limiting and essential for NR- and NMN-driven NAD+ synthesis, whereas dispensable for other NAD+ precursors. Using isotopically labeled compounds, we show that NMN is extracellularly metabolized to NR for intracellular incorporation and conversion into NAD+. Moreover, we demonstrate that animals lacking NRK1 have defects in NR utilization in vivo. To gain further insight into liver function of NRK1, we generated NRK1 liver-specific KO mouse model (NRK1 LKO). Here, we show that NRK1 LKO mice are able to maintain their basal liver NAD+ levels when fed with chow diet. However, when challenged with high-fat diet, these animals display significantly lowered NAD+ values in the liver, develop glucose intolerance and insulin resistance, have impaired mitochondrial function and liver damage. Our study has unveiled a critical role of NRK1 for the metabolism of diverse forms of VitaminB3 and highlights how circulating NR metabolism is key for metabolic health.

Zhang Hongbo

PhD student, Laboratory of Integrative and Systems Physiology (LISP), EPFL, Lab. Johan Auwerx.

Improving mitochondrial function by NAD+ repletion rejuvenates adult stem cells and enhances lifespan

Hongbo Zhang, Dongryeol Ryu, Yibo Wu, Karim Gariani, Peiling Luan, Davide D'Amico, Xu Wang, Eduardo R. Ropelle, Matthias P. Lutolf, Ruedi Aebersold, Kristina Schoonjans, Keir J. Menzies and Johan Auwerx

Adult stem cells (SCs) are essential for tissue maintenance and regeneration yet are susceptible to SC senescence during aging. Here we demonstrate the importance of cellular NAD+ level and its impact on mitochondrial activity as a pivotal switch to modulate muscle stem cell (MuSC) senescence. Importantly, the induction of the mitochondrial unfolded protein response (UPRmt) and of prohibitin proteins, subsequent to increasing cellular NAD+ with the precursor nicotinamide riboside (NR), rejuvenates MuSCs in aged mice. NR also prevents MuSCs senescence in Mdx mice, a mouse model of muscular dystrophy. Extending these observations to other SC pools and on the organism as a whole, we demonstrate that NR delays neural stem cell (NSC) and melanocyte stem cell (McSC) senescence, while also increasing mouse lifespan. Strategies that conserve cellular NAD+ could therefore be utilized to reprogram dysfunctional SCs in aging and disease to improve lifespan in mammals.

Giralt Coll Albert

Post Doc, Department of Physiology, Unil, Lab. Lluis Fajas

E2F1 participates in the regulation of hepatic gluconeogenesis

Albert Giralt Coll, Pierre-Damien Denechaud, Isabel C. Lopez-Mejia, Emilie Blanchet, Caroline Bonner, Francois Pattou, Jean-Sébastien Annicotte and Lluis Fajas

The liver plays a unique role in the maintenance of lipid and glucose homeostasis. Deregulated liver metabolism contributes to the development of several pathologies such as obesity, insulin resistance and type 2 diabetes. The elucidation of the molecular mechanisms that regulate liver metabolism in normal and pathological conditions could lead to the identification of new therapeutic targets to treat metabolic disorders. We have recently demonstrated that the cdk4-Rb-E2F1 pathway participates in the regulation of glycolysis and lipogenesis in the liver. Here, we report that E2F1 also participates in the regulation of hepatic gluconeogenesis, a pathway that is abnormally upregulated during insulin resistance and that contributes to the observed hyperglycemia in this state. E2F1 -/- mice show decreased gluconeogenesis in vivo and reduced expression of liver key gluconeogenic enzymes. Accordingly, lack of E2F1 in primary hepatocytes showed reduced glucose production and gluconeogenic gene expression. Cdk4 typically increases E2F1 transcriptional activity by releasing it from the repression by pRb. Cdk4 -/- primary hepatocytes showed decreased gluconeogenesis while, on the contrary, a hyperactive form of Cdk4 (R24C) lead to an E2F1-dependent increased gluconeogenesis. On the contrary, adenoviral E2F1 overexpression increased gluconeogenic gene expression and glucose production in primary hepatocytes. Luciferase reporter assays showed that PEPCK1 promoter activity is increased in response to E2F1. Liver biopsies from obese subjects showed a correlation between PEPCK1 and E2F1 mRNA expressions, suggesting that E2F1 could contribute to the increased gluconeogenesis observed during insulin resistance. In agreement with this, E2F chemical inhibition decreased glucose production and PEPCK1 expression in a mice model of insulin resistance. Taken together, our results show that E2F1 participates in the regulation of both glucose and lipid metabolism in the liver, suggesting that E2F1 inhibition could be used as a therapeutic approach for the treatment of type 2 diabetes.

Vinckenbosch Elise
PhD student, CIBM, Laboratory for functional and metabolic imaging, EPFL, Lab. Rolf Gruetter

Effects of glial TCA cycle inhibition in rodent cerebral metabolism

Elise Vinckenbosch, Mor Mishkovsky, Blanca Lizarbe, João Das Neves Duarte, Arnaud Comment and Rolf Gruetter

High energy requirement of the brain for neuronal activity is supported by glial cells insuring regulation of energy production, delivery and utilization.[1] In this study, we examined, in vivo consequences of glial TCA cycle inhibition on cerebral metabolism by 1H MRS, long infusion 13C MRS and hyperpolarized 13C MRS.[2,3,4] Anesthetized rats were treated with fluoroaceate, inhibitor of aconitase enzyme, and scanned at 14.1 and 9.4 Tesla.1H spectra were acquired and 21 metabolites concentrations were measured overtime. [1,6-13C]glucose was used as a precursor in long infusion 13C MRS for calculating metabolic fluxes.[3] Finally [1-13C] acetate was hyperpolarized and injected to highlight glial TCA cycle intermediates.[4,5] After treatment, we observed a decrease of glutamate, aspartate, and an increase of glutamine, glucose, lactate and alanine. In [1,6-13C]glucose infusion experiments, the C3/C4 enrichment ratio was lower in glutamine than glutamate. In contrast, untreated animals exhibited practically equal ratios. During hyperpolarized acetate experiments, 2-oxoglutarate intermediate was observed and its production rate was estimated. With inhibition treatment, a reduction in the production rate of a quarter was observed. Our results are in agreement with the literature.[6] In 13C MRS experiments, C3/C4 ratios suggested that glutamate pool is generated by faster TCA cycle than glutamine after fluoroacetate exposure, consistent with the inhibition of the glial TCA cycle, the compartment where most glutamine resides. Hyperpolarized experiments confirmed a reduction in glial TCA cycle activity.

(1) Bélanger, 2011, Cell Metabolism
(2) Muir, 1986, Brain Research
(3) Gruetter, 2001, AJPEM
(4) Mishkovsky, 2012, JSCBFM
(5) Comment, 1969, Biochemistry
(6) Hassel, 1997, JCBF

Croizier Sophie
Post Doc, Center for Integrative Genomics, Lab. Bernard Thorens

Leptin Controls Parasympathetic Wiring of the Pancreas During Embryonic Life

Sophie Croizier, Vincent Prevot and Sebastien G Bouret

The autonomic nervous system plays a critical role in glucose metabolism through both its sympathetic and parasympathetic branches, but the mechanisms that underlie the development of the autonomic innervation of the pancreas remain poorly understood. Here, we report that cholinergic innervation of pancreatic islets develops during midgestation under the influence of leptin. Leptin-deficient mice display a greater cholinergic innervation of pancreatic islets beginning in embryonic life and this increase persists into adulthood. Remarkably, a single intracerebroventricular injection of leptin in embryos caused a permanent reduction in parasympathetic innervation of pancreatic β cells as well as long-term impairments in glucose homeostasis. These developmental effects of leptin likely involve a direct inhibitory effect on the outgrowth of neurites from preganglionic neurons located in the hindbrain. These studies reveal an unanticipated regulatory role of leptin on the parasympathetic nervous system during embryonic development and may have important implications for our understanding of the early mechanisms that contribute to diabetes.

Sponsored by

Gilardi Federica
Scientist, Center for Integrative Genomics, Unil, Lab. Béatrice Desvergne

Epigenetic signature of WAT early aging highlights new immune players in WAT progression to senescence.

G. Giordano Attianese, A. Naldi, K. Trang, B. Toffoli, C. Winkler, M. Baruchet, B. Desvergne and F. Gilardi

Epigenetic modifications that convey flexibility to the genome in response to environmental and nutritional inputs, participate to the regulation of the senescence process in many mammalian tissues. Here we investigate how chromatin remodeling events contribute to very early phase of white adipose tissue (WAT) aging, when systemic body decline is still negligible. WAT from 3 and 12 months old mice was collected as representative of young- and middle-adulthood, respectively. Functional enrichment analyses on differentially expressed genes showed alteration in carbohydrates/amino acids metabolism and in extracellular matrix related pathways, as expected. The genome-wide profiling of histone marks and RNA Pol II recruitment revealed a general decrease of H3K9Ac in the WAT of 12Mo mice, accompanied by a deep remodeling of Pol II recruitment on both TSS and gene body. Most importantly, the ChIP-seq analysis highlighted a significant epigenetic remodeling of a number of genes belonging to immunity-related pathways, highly enriched for ETS binding motifs and whose expression was not yet altered. However, no classical signs of WAT inflammation were detected, except for an increased number of both NK and T reg cells and lymphatic vessel hyperplasia. Unexpectedly, we also found high levels of membrane associated IgG deposition in the 12Mo WAT, likely due to their retention by the FcγRIV isotype (the human orthologue of FcγRIIIa) that we found to be specifically expressed at the membrane levels of adipocytes. The activation of FcγRIV-IgG complexes could contribute to the recruitment of NK and T reg cells, recently described as crucial players in the WAT aging. This perturbation of the inflammatory/anti-inflammatory balance could represent one of the first steps of WAT senescence.

FLASH TALKS ABSTRACTS

Picard Alexandre
Post Doc, Center for Integrative Genomics, Lab. Bernard Thorens

A genetic screen identifies hypothalamic Fgf15 as a regulator of glucagon secretion

Alexandre Picard, Josselin Soyer, Xavier Berney, David Tarussio, Maxime Jan, Frédéric Burdet , Mark Ibberson and Bernard Thorens

The counterregulatory response to hypoglycemia, which restores normal blood glucose levels to ensure sufficient provision of glucose to the brain, is critical for survival. To discover underlying brain regulatory systems, we performed a genetic screen in recombinant inbred mice to identify quantitative trait loci (QTL) controlling glucagon secretion in response to neuroglucopenia. We identified a QTL on the distal part of chromosome 7 and combined this genetic information with tranacriptomic analysis of hypothalamic. This revealed Fgf15 as the strongest candidate to control the glucagon response. Fgf15 was found to be expressed by neurons of the dorsomedial hypothalamus and the perifornical area. Intracerebroventricular injection of FGF19, the human ortholog of Fgf15, reduced activation by neuroglucopenia of dorsal vagal complex neurons and of the parasympathetic nerve, leading to a lower glucagon secretion. These data show that Fgf15 in hypothalamic neurons is a new regulator of vagal nerve activity in response to neuroglucopenia.

Shehata Saifeldin
PhD student, Nestlé Institute of Health Sciences, Lab. Kei Sakamoto

The regulation of the CDK-related PCTAIRE-1 protein kinase and its role in the brain.

Cell signalling, mediated to a large extent by protein kinase phosphorylation, plays a vital role in regulation of cellular function. PCTAIRE-1 (CDK16), is a kinase that has been implicated in many physiological processes, including insulin secretion, neurite outgrowth, and vesicle trafficking. Most recently, it has been proposed as a novel X-linked intellectual disability (XLID) gene. The precise physiological mechanisms that regulate PCTAIRE-1 remained largely obscure. We first showed that PCTAIRE-1 preferentially phosphorylated peptide motifs that differed from the classical CDK family substrate preference, suggesting a more distinct role for the kinase. We further showed that cyclin Y, a novel cyclin, robustly binds and activates PCTAIRE-1 > 100-fold. Moreover, we identified two phosphorylation sites on cyclin Y that are essential for binding the well-known adaptor protein 14-3-3, which we propose stabilises cyclin Y in a favourable PCTAIRE-1-binding conformation. Furthermore, we have cloned human PCTAIRE-1 mutants identified in XLID patients, and confirmed their failure to bind the cyclin Y-14-3-3 activating complex. In order to understand the physiological relevance of PCTAIRE-1 activity, we have utilised a chemical genetics approach that exploits the ability of an engineered PCTAIRE-1 mutant to selectively modify its substrates. We have identified PCTAIRE-1 substrates in brain that are involved in fundamental processes that regulate neuronal synaptic transmission, and are in the process of validating them. With this, we hope to lay the foundations for future studies of the role of PCTAIRE-1 in disease, and to increase our understanding of the cellular functions in which it is involved.

Burton Kathryn
PhD student, EDM Department, CHUV, Lab. François Pralong

Everything you always wanted to know about probiotics (but didn't dare to ask)

Have you ever wondered where probiotics fit in the realm of -biotic research: antibiotics, eubiotics, xenobiotics, prebiotics…? Far from a new phenomenon, probiotics date back to the early 19th century but their use in health and disease is on the rise with new technologies providing a better understanding between probiotics, the human microbiota and health. This talk aims to give an overview of probiotics, their relevance for health and the current limitations in the field.

Katsyuba Elena
PhD student, Laboratory of Integrative and Systems Physiology (LISP), EPFL, Lab. Johan Auwerx

ACMSD as novel therapeutic target to boost NAD+ levels

This is for a flash talk! Activity of the sirtuins, a set of enzymes that play an important role in metabolic homeostasis and have potential therapeutic relevance, depends on the balance between the oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD). We present here an innovative way to increase NAD+ levels by stimulating its de novo synthesis.

Sonnay Sarah

PhD student, Laboratory for Functional and Metabolic Imaging, EPFL, Lab. Rolf Gruetter

Visual stimulation-induced functional and metabolic modifications in the cortex of the Tupaia belangeri

Sarah Sonnay, Jordan Poirot, Nathalie Just, Anne-Catherine Clerc, Rolf Gruetter, Gregor Rainer and João M.N. Duarte

Neurons and astrocytes efficiently cooperate to match the metabolic demands of brain activity. However it is still unclear how astrocytic metabolism responds to increased neuronal activity. Here we aimed at investigating metabolic modification to increased neuronal activity in the primary visual cortex (V1) of the Tupaia belangeri. We developed a device composed of 2 matrices of 64 LEDs each that allows delivering continuous visual stimuli. This device was used for blood-oxygenation-dependent (BOLD) functional magnetic resonance imaging and magnetic resonance spectroscopy (MRS) experiments at 14.1 T. Reproducible and robust BOLD responses were detected in V1, where the volume of interest was placed for MRS. 1H MRS and 13C MRS during [1,6-13C]glucose infusion were performed in V1 under light isoflurane anesthesia at rest or during visual stimulation. Preliminary results from 1H MRS (n=4) indicate that, relative to baseline, stimulation induces a 35% (-0.3 μmol/g) reduction in glucose concentration, a 3% (+0.05 μmol/g) increase in lactate, a 4% (-0.1 μmol/g) decrease in aspartate and a 1% (+0.08 μmol/g) increase in glutamate. Preliminary metabolic flux analysis (n=2 stimulation, n=1 rest) with a two-compartment model of energy metabolism suggests increased glial (+12%) and neuronal (+11%) tricarboxylic acid cycle rate associated with an increase in the neurotransmission rate (+13%). Altogether, the cerebral metabolic rate of glucose oxidation increased by 5% during stimulation. These results suggest that visual stimulation-induced cortical activity under light isoflurane anesthesia is associated with increased glycolysis, and increased glial and neuronal oxidative metabolism.

Du Ngoc-Hien
Post Doc, Center for Integrative Genomics, Lab. David Gatfield

Altered adaptation to food restriction upon total miRNA loss in mouse liver

Ngoc-Hien Du, Marieke Hoekstra, Bulak Arpat, Mara De Matos, Paul Franken, and David Gatfield

Recently, we have reported a comprehensive profiling of the rhythmic transcriptome in hepatocyte-specific Dicer knockout and proposed roles for miRNAs in modulating daily gene expression. In brief, we found that miRNA-mediated regulation affected the phase and amplitude of as much as 30% of the rhythmic transcriptome. In contrast, the core clock was surprisingly resilient to miRNA loss with the exception of mild posttranscriptional upregulation in Per2, Per1 and Cry2 mRNAs. However, free-running PER2:LUC rhythms measured in liver explants showed long periods in the absence of miRNAs. We concluded that upon miRNA loss, the liver clock is only functional in the entrained animal, but not when it is released from the control of the master clock. This prompted us to explore the role of miRNAs in the hepatic core clock under conditions when the relationship between the master clock in the brain and the liver clocks is brought out of equilibrium such as when food is restricted. Mice were fed ad libitum before switching to 6-h food only in the light phase, and PER2:LUC rhythms in freely moving Dicer knockout and control animals were recorded. Our experiments suggest advanced kinetics of phase-shifting in Dicer knockouts compared to controls. At the behavioural level, Dicer knockout mice lost food-anticipatory-like activities that were observed before meal in control mice. At present, we are increasing the number of analysed animals in order to precisely quantify the effect and designing experiments to pinpoint the responsible miRNA(s). Altogether, our data point to an involvement of miRNAs in regulating flexibility of the clock towards changes in entrainment conditions.

Morató Laia

Post Doc, Laboratory of Behavioral Genetics, Brain Mind Institute, EPFL, Lab. Carmen Sandi

Effect of early life stress on behavioral and metabolic programming

Laia Morató, Marie-Isabelle Guillot de Suduiraut, Jocelyn Grosse, Olivia Zanoletti, Orbicia Riccio, Céline Fournier and Carmen Sandi

Stress-related psychopathologies and obesity are pervasive phenomena in modern societies. The high prevalence and comorbidity of both pathologies supports the rationale for shared underlying biological substrate. We aim to investigate the neuronal mechanisms whereby early life stress can simultaneously induce dysregulation of behavioral coping and, particularly, peripheral metabolic responses. C57BL/6 mice were subjected to an unpredictable stress regime between P28 to P42. At adulthood, mice were tested for anxiety behavior (e.g. elevated plus maze, open field), sociability (e.g. three-chamber social-interaction test) and aggression (e.g. resident intruder test). Additionally, they were assessed for peripheral metabolic regulation (e.g. percentage of lean and fat mass, intraperitoneal glucose and insulin tolerance test). Finally, we evaluated whether differences observed at behavioral and metabolic levels were associated with changes of gene expression in the brain of stressed mice. We found that mice subjected to stress during the peripubertal period exhibited not only increased anxiety and reduced social behavior compared to their control littermates, but also a higher percentage of fat mass. These changes were concomitants with an altered expression of key metabolic genes in the hypothalamic arcuate, paraventricual nucleus and nucleus accumbens. The conclusion of our work is twofold: First, we highlight our peripubertal stress protocol as a good model to induce simultaneous alterations in behavior and peripheral metabolism. Second, we identified metabolic genes in specific brain areas that might represent the shared underlying neurobiological mechanisms between obesity and stress-induced psychopathologies.

Nasrallah Anita
PhD student, Department of Physiology, Unil, Lab. Lluis Fajas

The Role of CDK10 in Metabolism

Anita Nasrallah, Isabel Lopez-Mejia, Albert Giralt Coll and Lluis Fajas

CDK10 has been known to have several isoforms, each involved in different processes and functions. One isoform has been shown to be implicated in cell cycle progression, proliferation, migration, colony formation, and anchorage-independent growth of several cancers. Another isoform has been proven to bind to CyclinM and has a different role that has not been clearly identified yet. Based on our recent screening of several mouse tissues, our data indicate the high expression of CDK10 in metabolic tissues, i.e. liver, muscle, heart, adipose tissues. As compared among adipose tissues, it was shown that BAT had the highest expression as compared to other white adipose tissues. For this reason, we hypothesize that CDK10 has an important role in general in metabolic tissues, and in particular in brown adipose tissue. Thus, we generated adipose tissue-specific CDK10 knockouts and are studying the effect of this protein on metabolism. We also plan to find new functions as well as new targets and binding partners of CDK10.

Lagarrigue Sylviane
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A missing link in skeletal muscle metabolism

Sylviane Lagarrigue, Luc Pellerin and Francesca Amati

Monocarboxylate transporters (MCTs) belong to the solute carrier family SLC16A. They are highly conserved bidirectional proton-linked carriers that allow energy substrates like lactate to be either released or taken up by different cells. In muscle, two low affinity isoforms have been classically describe; while MCT1 (SLC16A1) is present in all fiber types and is thought to be mainly a lactate influx carrier, MCT4 (SLC16A3) expression is limited to glycolytic fibers and is thought to be the lactate efflux carrier. The higher affinity isoform MCT2 (SLC16A7) is known to be present in a few cell types exhibiting high oxidative metabolism (e.g. neurons). However, its presence in muscle cells has not been thoroughly investigated. Here we show that MCT2 is strongly expressed by a large subset of oxidative muscle fibers in humans. Further, its expression is localized not only in the sarcolemma and in the intramyofibrillar region. This is of high interest as MCT2 allows not only lactate but also ketone bodies (KB) to enter cells even at relatively low concentrations. While it has been demonstrated that KB are a major oxidative fuel in fasting skeletal muscle at rest or during low intensity exercise, the transporter and energy substrate model was yet to be described. In 90 seconds, we will convince you that MCT2 is in human skeletal muscle biopsies and that cultured primary cells use KB. We anticipate this finding to be the starting point of renewed interest for KB metabolism in humans.

Groeneveld Svenja
PhD student, ISREC Institute, Lab. Etienne Meylan

The hexosamine biosynthesis pathway in epithelial-to-mesenchymal transition in non-small cell lung cancer

Svenja Groeneveld, Bernard Moret, Simona Rossi and Etienne Meylan

Glutamine-Fructose-6-Phosphate Transaminase 2 (GFPT2) catalyzes the rate limiting step of the hexosamine biosynthesis pathway (HBP), a metabolic branch of glycolysis that produces UDP-GlcNAc, a substrate for protein modifications. We discovered that in tumors from non-small cell lung cancer (NSCLC) patients, the expression of GFPT2 correlates with GLUT3, a glucose transporter we have recently reported to be involved in epithelial-to-mesenchymal transition (EMT) in this malignancy. To examine the connection between the HBP and EMT, in particular if and how GFPT2 is regulated by the EMT-inducing transcription factor Snail, we have combined overexpression and silencing techniques in vitro and in vivo. We found that mesenchymal NSCLC cell lines display higher levels of GFPT2 expression and protein O-GlcNAc modification compared to epithelial cell lines. Induction of an EMT in epithelial cells by TGF-β or Snail led to an up regulation of GFPT2 and an increase in O-GlcNAc levels. Accordingly, these changes were also observed upon overexpression of Snail in a mouse model of human NSCLC. Overexpression of GFPT2 by itself did not induce an EMT and siRNA-mediated inhibition of GFPT2 expression did not inhibit Snail-mediated EMT in epithelial cells. However, shRNA- or siRNA-mediated suppression of GFPT2 in mesenchymal cells increased expression of the epithelial protein E-cadherin and diminished that of the mesenchymal transcription factor ZEB1, respectively. We conclude that enhanced HBP activity goes along with an EMT. The expression of GFPT2 appears to be positively regulated by Snail. Surprisingly, GFPT2 seems to be neither necessary nor sufficient for an EMT to occur. Our data indicate, however, that GFPT2 might be involved in the maintenance of a mesenchymal phenotype.

Pradhan Rachana

PhD student, Laboratory of Systems Biology and Genetics, EPFL, Lab. Bart Deplancke

Integrative transcriptomic & epigenomic analysis of brown fat cell differentiation reveals novel transcriptional regulators

Rachana N. Pradhan, Johannes J. Bues, Petra C. Schwalie, Wanze Chen, Julie Russeil, Monica Albarca, Sunil Raghav and Bart Deplancke

Brown adipocytes are specialized to regulate energy expenditure via mitochondrial uncoupling, making them attractive therapeutic targets to tackle obesity & the associated metabolic syndrome. However, little is known about the transcriptional regulatory basis of brown fat cell (BFC) differentiation. To address this, we investigated changes in gene expression & chromatin state using RNA-seq & H3K27ac ChIP-seq, respectively, across differentiation of a murine-derived brown pre-adipocyte cell line. We detected ~1,000 genes to be differentially expressed during differentiation. A majority of these differentially expressed genes (DEGs) also showed significant changes in H3K27ac, suggesting active remodeling of the chromatin state during BFC differentiation. Among DEGs, we detected up-regulation of known positive regulators of adipogenesis such as PPARg/CEBPA & the BFC marker UCP-1, as expected. We identified novel transcriptional activators & repressors of BFC differentiation by delineating differentially expressed transcription factor (TF)-coding genes that showed co-expression with known regulators of BFC differentiation as well as differences in expression in brown versus white fat cells. Additionally, we looked for over-represented transcription factor binding sites (TFBS) in differentially acetylated regions, likely implicated in the transcriptional control of BFC differentiation. To assess the phenotypic effect of ~30 putative regulators highlighted by our integrative analysis, we set up a lentivirus mediated knockdown screen, revealing Elf3, Sox18 as positive regulators & Crem, Sox13 as negative regulators. In sum, our integrative approach fills important gaps in our understanding of the adipogenic & thermogenic transcriptional circuit during BFC differentiation.

Hafner Jasmin
PhD student, Laboratory of Computational Systems Biotechnology, EPFL, Lab. Vassily Hatzimanikatis

"ATLAS of Biochemistry", a repository of all possible biochemical reactions for synthetic biology, metabolic engineering and metabolomics

Jasmin Hafner, Noushin Hadadi, Katerina Zisaki and Vassily Hatzimanikatis

Cellular metabolism is very complex and we are still far away from a complete understanding, as shown by knowledge gaps in metabolic networks and the continuous growth of biochemical databases. Furthermore, recent progress in the field of metabolomics produced vast amounts of newly identified metabolites which need to be integrated into existing metabolic networks. To face this challenge, we created a database of all the biochemically possible reactions between known metabolites, using the computational framework BNICE.ch. The database "ATLAS of Biochemistry" was generated by applying known enzymatic reaction mechanisms on all metabolites present in the Kyoto Encyclopedia of Genes and Genomes (KEGG), thus reconstructing known KEGG reactions as well as novel, hypothetical reactions between known KEGG compounds. This extrapolation of the known metabolism resulted in a network of more than 130'000 known and novel reactions, each connecting two or more KEGG compounds. Each reaction is annotated with an estimated value for the Gibbs free energy of reaction and an EC-number up to the third level. Also, to each novel reaction, we assigned its structurally most similar KEGG reaction. We showed that several reactions recently added to KEGG already exist in ATLAS as novel reactions, thus demonstrating its predictive power. The databased is available online, including tools to search the data and to reconstruct possible metabolic routes within the ATLAS network. Results from a pathway search can propose previously unidentified enzymatic activities, bridge gaps in metabolic models and provide potential targets for protein and metabolic engineering. Hence, our work may be of great interest for genome annotation, metabolic engineering, synthetic biology and metabolomics research.

POSTER ABSTRACTS

Poster 1

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Affiliation: Laboratory of Computational Systems Biotechnology (LCSB), Swiss Federal Institute of Technology (EPFL)

Title of presentation: "ATLAS of Biochemistry", a repository of all possible biochemical reactions for synthetic biology, metabolic engineering and metabolomics

Authors list: Jasmin Hafner Noushin Hadadi Katerina Zisaki Vassily Hatzimanikatis

Abstract: Cellular metabolism is very complex and we are still far away from a complete understanding, as shown by knowledge gaps in metabolic networks and the continuous growth of biochemical databases. Furthermore, recent progress in the field of metabolomics produced vast amounts of newly identified metabolites which need to be integrated into existing metabolic networks. To face this challenge, we created a database of all the biochemically possible reactions between known metabolites, using the computational framework BNICE.ch. The database "ATLAS of Biochemistry" was generated by applying known enzymatic reaction mechanisms on all metabolites present in the Kyoto Encyclopedia of Genes and Genomes (KEGG), thus reconstructing known KEGG reactions as well as novel, hypothetical reactions between known KEGG compounds. This extrapolation of the known metabolism resulted in a network of more than 130'000 known and novel reactions, each connecting two or more KEGG compounds. Each reaction is annotated with an estimated value for the Gibbs free energy of reaction and an EC-number up to the third level. Also, to each novel reaction, we assigned its structurally most similar KEGG reaction. We showed that several reactions recently added to KEGG already exist in ATLAS as novel reactions, thus demonstrating its predictive power. The databased is available online, including tools to search the data and to reconstruct possible metabolic routes within the ATLAS network. Results from a pathway search can propose previously unidentified enzymatic activities, bridge gaps in metabolic models and provide potential targets for protein and metabolic engineering. Hence, our work may be of great interest for genome annotation, metabolic engineering, synthetic biology and metabolomics research.

Poster 2

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Affiliation: Laboratory for Functional and Metabolic Imaging (LIFMET), EPFL

Title of presentation: Hyperpolarized MRS as a tool to study cerebral metabolism in real-time

Authors list: Mor Mishkovsky, Elise Vinckenbosch, Arnaud Comment, Rolf Gruetter

Abstract: The advent of dissolution dynamic nuclear polarization (DNP) led to the emergence of a new kind of magnetic resonance (MR) measurements providing the opportunity to probe metabolism in vivo in real time. It has been shown that, following the injection of hyperpolarized substrates prepared using dissolution DNP, specific metabolic bioprobes that can be used to differentiate between healthy and pathological tissue in preclinical and clinical studies can be readily detected by MR thanks to the tremendous signal enhancement. The present study relates to hyperpolarized molecules that can be used to monitor brain function and cerebral metabolism. We focus on molecules that have been commonly used for thermally-polarized MR spectroscopic measurements, notably glucose, lactate and acetate but this time in their hyperpolarized form. This enabled us to access earlier stages of their metabolic pathways, and to measure in real time their turn-over in vivo. The challenges, constraints and future opportunities that this technology could offer for brain study will be discussed.

Poster 3

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Title of presentation: Altered glycogen metabolism in the brain of insulin-resistant Goto-Kakizaki rats measured in vivo by 13C magnetic resonance spectroscopy

Authors list: João M.N. Duarte, Sakura S. Nussbaum, Rolf Gruetter, Ana F. Soares

Abstract: The role of glycogen in the diabetic brain remains to be elucidated. We investigated insulin resistance-induced alterations of brain glycogen metabolism in the living brain by means of magnetic resonance spectroscopy (MRS) in vivo at 14.1 T. [1-13C]glucose was infused into adult Wistar and insulin-resistant Goto-Kakizaki (GK) rats under isoflurane anaesthesia. Localised 13C MRS was performed in a volume of 600 µL within the brain with a modified SIRENE pulse sequence. The 13C MRS experiment measured brain glucose and glycogen signals over at least 8 hours. Then, rats were sacrificed with a focused microwave fixation device, and the brain was stored for extraction of glycogen and water-soluble metabolites. Fractional enrichment (FE) and content of glucose and glycogen were determined by MRS in vitro. Time courses of glycogen 13C labelling measured in vivo were modelled together with FE and concentration determined in brain extracts to estimate glycogen turnover. Brain glycogen concentrations were similar in both experimental groups (6.7±0.9 µmol/g in controls and 6.7±0.6 µmol/g in diabetic rats). FE of brain glycogen was lower in GK rats than in Wistar rats, suggesting that insulin resistance reduces 13C incorporation. Indeed, with a mathematical model of glycogen labelling from [1-13C]glucose, we estimated a glycogen turnover of 0.72±0.33 µmol/g/h in controls and 0.21±0.08 µmol/g/h in GK rats. These data demonstrate that insulin resistance slows down brain glycogen metabolism despite normal brain glycogen content, which may have implications for the adequate support of neuronal function, especially during increased brain activity or during reduced energy availability such as hypoglycaemia. Supported by CIBM and FNS.

Poster 4

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Title of presentation: The regulation of the CDK-related PCTAIRE-1 protein kinase and its role in the brain.

Abstract: For both poster and flashtalk: Cell signalling, mediated to a large extent by protein kinase phosphorylation, plays a vital role in regulation of cellular function. PCTAIRE-1 (CDK16), is a kinase that has been implicated in many physiological processes, including insulin secretion, neurite outgrowth, and vesicle trafficking. Most recently, it has been proposed as a novel X-linked intellectual disability (XLID) gene. The precise physiological mechanisms that regulate PCTAIRE-1 remained largely obscure. We first showed that PCTAIRE-1 preferentially phosphorylated peptide motifs that differed from the classical CDK family substrate preference, suggesting a more distinct role for the kinase. We further showed that cyclin Y, a novel cyclin, robustly binds and activates PCTAIRE-1 > 100-fold. Moreover, we identified two phosphorylation sites on cyclin Y that are essential for binding the well-known adaptor protein 14-3-3, which we propose stabilises cyclin Y in a favourable PCTAIRE-1-binding conformation. Furthermore, we have cloned human PCTAIRE-1 mutants identified in XLID patients, and confirmed their failure to bind the cyclin Y-14-3-3 activating complex. In order to understand the physiological relevance of PCTAIRE-1 activity, we have utilised a chemical genetics approach that exploits the ability of an engineered PCTAIRE-1 mutant to selectively modify its substrates. We have identified PCTAIRE-1 substrates in brain that are involved in fundamental processes that regulate neuronal synaptic transmission, and are in the process of validating them. With this, we hope to lay the foundations for future studies of the role of PCTAIRE-1 in disease, and to increase our understanding of the cellular functions in which it is involved.

Poster 5

Last Name: Sonnay

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Title of presentation: Visual stimulation-induced functional and metabolic modifications in the cortex of the Tupaia belangeri

Authors list: Sarah Sonnay, Jordan Poirot, Nathalie Just, Anne-Catherine Clerc, Rolf Gruetter, Gregor Rainer, João M.N. Duarte

Abstract: Neurons and astrocytes efficiently cooperate to match the metabolic demands of brain activity. However it is still unclear how astrocytic metabolism responds to increased neuronal activity. Here we aimed at investigating metabolic modification to increased neuronal activity in the primary visual cortex (V1) of the Tupaia belangeri. We developed a device composed of 2 matrices of 64 LEDs each that allows delivering continuous visual stimuli. This device was used for blood-oxygenation-dependent (BOLD) functional magnetic resonance imaging and magnetic resonance spectroscopy (MRS) experiments at 14.1 T. Reproducible and robust BOLD responses were detected in V1, where the volume of interest was placed for MRS. 1H MRS and 13C MRS during [1,6-13C]glucose infusion were performed in V1 under light isoflurane anesthesia at rest or during visual stimulation. Preliminary results from 1H MRS (n=4) indicate that, relative to baseline, stimulation induces a 35% (-0.3 µmol/g) reduction in glucose concentration, a 3% (+0.05 µmol/g) increase in lactate, a 4% (-0.1 µmol/g) decrease in aspartate and a 1% (+0.08 µmol/g) increase in glutamate. Preliminary metabolic flux analysis (n=2 stimulation, n=1 rest) with a two-compartment model of energy metabolism suggests increased glial (+12%) and neuronal (+11%) tricarboxylic acid cycle rate associated with an increase in the neurotransmission rate (+13%). Altogether, the cerebral metabolic rate of glucose oxidation increased by 5% during stimulation. These results suggest that visual stimulation-induced cortical activity under light isoflurane anesthesia is associated with increased glycolysis, and increased glial and neuronal oxidative metabolism.

Poster 6

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Affiliation: CIBM LIFMET

Title of presentation: Effects of glial TCA cycle inhibition in rodent cerebral metabolism

Authors list: Elise Vinckenbosch, Mor Mishkovsky, Blanca Lizarbe, João Das Neves Duarte, Arnaud Comment and Rolf Gruetter

Abstract: High energy requirement of the brain for neuronal activity is supported by glial cells insuring regulation of energy production, delivery and utilization.[1] In this study, we examined, in vivo consequences of glial TCA cycle inhibition on cerebral metabolism by 1H MRS, long infusion 13C MRS and hyperpolarized 13C MRS.[2,3,4] Anesthetized rats were treated with fluoroacetate, inhibitor of aconitase enzyme, and scanned at 14.1 and 9.4 Tesla.1H spectra were acquired and 21 metabolites concentrations were measured overtime. [1,6-13C]glucose was used as a precursor in long infusion 13C MRS for calculating metabolic fluxes.[3] Finally [1-13C] acetate was hyperpolarized and injected to highlight glial TCA cycle intermediates.[4,5] After treatment, we observed a decrease of glutamate, aspartate, and an increase of glutamine, glucose, lactate and alanine. In [1,6-13C]glucose infusion experiments, the C3/C4 enrichment ratio was lower in glutamine than glutamate. In contrast, untreated animals exhibited practically equal ratios. During hyperpolarized acetate experiments, 2-oxoglutarate intermediate was observed and its production rate was estimated. With inhibition treatment, a reduction in the production rate of a quarter was observed. Our results are in agreement with the literature.[6] In 13C MRS experiments, C3/C4 ratios suggested that glutamate pool is generated by faster TCA cycle than glutamine after fluoroacetate exposure, consistent with the inhibition of the glial TCA cycle, the compartment where most glutamine resides. Hyperpolarized experiments confirmed a reduction in glial TCA cycle activity.

(1)Bélanger,2011,CellMetabolism(2)Muir,1986,BrainResearch(3)Gruetter,2001,AJPEM(4)Mishkovsky,2012,JSCBFM(5)Comment.1969,Biochemistry(6)Hassel,1997,JCBF

Poster 7

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Affiliation: EPFL, LCSB

Title of presentation: Study of non-alcoholic fatty liver disease using a novel enrichment analysis that integrates transcriptomics and metabolite concentrations data

Authors list: Vikash Pandey and Vassily Hatzimanikatis

Abstract: Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of liver damages ranging from steatosis to steatohepatitis (NASH), and rarely, progression to liver cirrhosis. Molecular interactions of NAFLD are poorly understood and its analysis requires a detailed understanding of the underlying metabolic and regulatory processes on the molecular level. Thus, to understand the molecular interactions of NAFLD, we developed a novel method we call minimal network enrichment analysis (MiNEA), and we performed MiNEA using constraints inferred from metabolite concentrations and transcriptomics data from a NASH mouse model system. Furthermore, we compare whether the molecular interactions of NAFLD differ between mouse and human, we also performed the MiNEA analysis using transcriptomics data from humans with NASH. In both human and mouse oxidative stress pathways are found to be upregulated through superoxide anion synthesis in NASH phenotype. Additionally, in mouse, synthesis of ceramide is identified as upregulated in steatosis but downregulated in NASH. Glutamine synthesis is upregulated in mouse steatosis, while it is downregulated in human NASH.

Poster 8

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Title of presentation: The role of Nicotinamide Riboside Kinase 1 in mammalian metabolism

Authors list: Joanna Ratajczak, Magali Joffraud, Samuel A.J. Trammell, Rosa Ras, Núria Canela, Marie Boutant, Sameer S. Kulkarni, Marcelo Rodrigues, Marie Migaud, Johan Auwerx, Oscar Yanes, Charles Brenner, Carles Canto

Abstract: NAD+ has emerged as a central metabolic node implicated in lifespan and healthspan regulation by being a consumed substrate required for the activity of various enzyme families, including sirtuins and poly(ADP-ribose) polymerases. Supplementation with NAD+ precursors, such as nicotinamide mononucleotide (NMN) or nicotinamide riboside (NR), protects against metabolic disease, neurodegenerative disorders and age-related physiological decline in mammals. Nicotinamide riboside kinase 1 (NRK1) is an enzyme highly expressed in the liver, responsible for the first step of NR metabolism. Using genetic gain- and loss-of-function models, we demonstrate that in mammalian cells NRK1 is rate-limiting and essential for NR- and NMN-driven NAD+ synthesis, whereas dispensable for other NAD+ precursors. Using isotopically labeled compounds, we show that NMN is extracellularly metabolized to NR for intracellular incorporation and conversion into NAD+. Moreover, we demonstrate that animals lacking NRK1 have defects in NR utilization in vivo. To gain further insight into liver function of NRK1, we generated NRK1 liver-specific KO mouse model (NRK1 LKO). Here, we show that NRK1 LKO mice are able to maintain their basal liver NAD+ levels when fed with chow diet. However, when challenged with high-fat diet, these animals display significantly lowered NAD+ values in the liver, develop glucose intolerance and insulin resistance, have impaired mitochondrial function and liver damage. Our study has unveiled a critical role of NRK1 for the metabolism of diverse forms of VitaminB3 and highlights how circulating NR metabolism is key for metabolic health.

Poster 9

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Title of presentation: Improving mitochondrial function by NAD+ repletion rejuvenates adult stem cells and enhances lifespan

Authors list: Hongbo Zhang, Dongryeol Ryu, Yibo Wu, Karim Gariani, Peiling Luan, Davide D'Amico, Xu Wang, Eduardo R. Ropelle, Matthias P. Lutolf, Ruedi Aebersold, Kristina Schoonjans, Keir J. Menzies*, Johan Auwerx*

Abstract: Adult stem cells (SCs) are essential for tissue maintenance and regeneration yet are susceptible to SC senescence during aging. Here we demonstrate the importance of cellular NAD+ level and its impact on mitochondrial activity as a pivotal switch to modulate muscle stem cell (MuSC) senescence. Importantly, the induction of the mitochondrial unfolded protein response (UPRmt) and of prohibitin proteins, subsequent to increasing cellular NAD+ with the precursor nicotinamide riboside (NR), rejuvenates MuSCs in aged mice. NR also prevents MuSCs senescence in Mdx mice, a mouse model of muscular dystrophy. Extending these observations to other SC pools and on the organism as a whole, we demonstrate that NR delays neural stem cell (NSC) and melanocyte stem cell (McSC) senescence, while also increasing mouse lifespan. Strategies that conserve cellular NAD+ could therefore be utilized to reprogram dysfunctional SCs in aging and disease to improve lifespan in mammals.

Poster 10

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Title of presentation: A conserved class of histone demethylases regulate longevity in response to mitochondrial stress

Authors list: Carsten Merkwirth, Virginija Jovaisaite, Jenni Durieux, Olli Matilainen, Sabine D. Jordan, Pedro M. Quiros, Kristan K. Steffen, Evan G. Williams, Laurent Mouchiroud, Sarah N. Uhlein, Suzanne C. Wolff, Reuben J. Shaw, Johan Auwerx, Andrew Dillin

Abstract: Across eukaryotic species, mild mitochondrial stress can have beneficial effects on the lifespan of organisms. Mitochondrial dysfunction activates an unfolded protein response (UPRmt), a stress signaling mechanism designed to ensure mitochondrial homeostasis. Perturbation of mitochondria during larval development in C. elegans not only delays aging but also maintains UPRmt signaling, suggesting an epigenetic mechanism that modulates both longevity and mitochondrial proteostasis throughout life. Here we identify the conserved histone lysine demethylases jmjd-1.2/PHF8 and jmjd-3.1/JMJD3 as positive regulators of lifespan in response to mitochondrial dysfunction across species. Reduction-of-function of the demethylases potently suppresses longevity and UPRmt induction, while gain-of-function is sufficient to extend lifespan in an UPRmt- dependent manner. A systems genetics approach in the BXD mouse reference population further indicated conserved roles of the mammalian orthologs in longevity and UPRmt signaling. These findings illustrate a novel and evolutionary conserved epigenetic mechanism that determines the rate of aging downstream of mitochondrial perturbations.

Poster 11

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Title of presentation: Epigenetic signature of WAT early aging highlights new immune players in WAT progression to senescence.

Authors list: G. Giordano Attianese, A. Naldi, K. Trang, B. Toffoli, C. Winkler, M. Baruchet, B. Desvergne and F. Gilardi

Abstract: Epigenetic modifications that convey flexibility to the genome in response to environmental and nutritional inputs, participate to the regulation of the senescence process in many mammalian tissues. Here we investigate how chromatin remodeling events contribute to very early phase of white adipose tissue (WAT) aging, when systemic body decline is still negligible. WAT from 3 and 12 months old mice was collected as representative of young- and middle-adulthood, respectively. Functional enrichment analyses on differentially expressed genes showed alteration in carbohydrates/amino acids metabolism and in extracellular matrix related pathways, as expected. The genome-wide profiling of histone marks and RNA Pol II recruitment revealed a general decrease of H3K9Ac in the WAT of 12Mo mice, accompanied by a deep remodeling of Pol II recruitment on both TSS and gene body. Most importantly, the ChIP-seq analysis highlighted a significant epigenetic remodeling of a number of genes belonging to immunity-related pathways, highly enriched for ETS binding motifs and whose expression was not yet altered. However, no classical signs of WAT inflammation were detected, except for an increased number of both NK and T reg cells and lymphatic vessel hyperplasia. Unexpectedly, we also found high levels of membrane associated IgG deposition in the 12Mo WAT, likely due to their retention by the FcγRIV isotype (the human orthologue of FcγRIIIa) that we found to be specifically expressed at the membrane levels of adipocytes. The activation of FcγRIV-IgG complexes could contribute to the recruitment of NK and T reg cells, recently described as crucial players in the WAT aging. This perturbation of the inflammatory/anti-inflammatory balance could represent one of the first steps of WAT senescence.

Poster 12

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Title of presentation: Congenital portosystemic shunts in C57BL/6J mice are associated with confounding metabolic alterations.

Authors list: Ana Francisca Soares, Hongxia Lei, Rolf Gruetter

Abstract: (For a flash talk) C57BL/6J mice, widely used in preclinical research, exhibit sporadic congenital portosystemic shunts (PSS). As a result, nutrient- and hormone-rich portomesenteric blood bypasses the liver and drains directly to the systemic circulation. Therefore, portosystemic shunting may have an important impact in liver-mediated blood detoxification and metabolic control. We used high resolution gradient echo magnetic resonance imaging (GRE MRI) to visualize PSS in male C57BL/6J mice and 1H MR spectroscopy in vivo to identify alterations in liver and brain metabolites resulting from PSS. We also characterized whole body glucose homeostasis by performing oral glucose tolerance tests. PSS were identified in ~12% of mice directly obtained from Charles Rivers Laboratories, with maximum width and length of 2.9±0.1mm and 1.1±0.2mm. These mice showed high cerebral glutamine content, twice the value of source- and age-matched controls, which indicates lack of hepatic ammonia detoxification. Mice with PSS also showed increased hepatic lipid content, lower fasting glycemia and hampered glucose clearance during an oral glucose challenge. Such metabolic abnormalities are consistent with defects in hepatic glucose metabolism secondary to the lack of exposure to glucose itself and pancreatic hormones and point to a metabolic shift in fuel homeostasis in favor of lipid utilization. In conclusion, we demonstrate that high resolution GRE MRI can be used to diagnose PSS in mice; PSS alters the cerebral neurochemical profile and intra-hepatic energy stores; and hampers the participation of the liver in the control of glucose homeostasis. The identification of these mice in the scope of preclinical research is highly desired so that metabolic confounders are avoided.

Poster 13

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Title of presentation: AMPK-dependent gene expression profiles in response to small molecule AMPK activators

Authors list: Caterina Collodet 1,2, Laurent Bultot 1, Sylviane Metairon 1, Frederic Raymond 1, Kei Sakamoto 1,2, Patrick Descombes 1,2

Abstract: AMP-activated protein kinase (AMPK) is an important regulator for energy homeostasis at both cellular and whole-body levels. AMPK elicits diverse metabolic effects by directly phosphorylating various targets. For example, AMPK phosphorylates and inactivates acetyl-CoA carboxylase-1 (ACC1) and HMG-CoA reductase, key enzymes of fatty acid and sterol biosynthesis, respectively. Moreover numerous studies have shown that the activation of AMPK leads to increased fatty acid oxidation through phosphorylation of ACC and insulin-independent glucose uptake in skeletal muscle involving phosphorylation of TBC1D1, whereas AMPK signaling to ACC is required for the lipid-lowering and insulin-sensitizing effects of metformin. In addition to acute regulation of cellular targets through phosphorylation, AMPK activation has been implicated to promote metabolic reprogramming in the longer term via effects on gene expression. For example, treatment of rodents with pharmacological activators of AMPK such as AICAR resulted in improved insulin sensitivity and exercise endurance, which was associated with enhanced expression of genes/proteins involved in mitochondrial functions. However, there are no comprehensive and systematic analysis on AMPK-dependent gene expression linked to metabolic adaptation/reprogramming. To gain insights into AMPK-dependent metabolic adaptations through gene reprogramming, we have compared the effect of two different AMPK activators on global gene expression profiles from wild type and AMPK $\alpha1/\alpha2$-null mouse embryonic fibroblast cells (MEF). MEFs were treated with small molecule AMPK activators, or vehicle for 4 and 12 hours and gene expression analysis was performed using microarray. We will present our initial microarray analysis and discuss AMPK-dependent gene expression signatures in response to different AMPK activators.

Poster 14

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Title of presentation: E2F1 participates in the regulation of hepatic gluconeogenesis

Authors list: Authors: Albert Giralt Coll, Pierre-Damien Denechaud, Isabel C. Lopez-Mejia, Emilie Blanchet, Caroline Bonner, Francois Pattou, Jean-Sébastien Annicotte and Lluis Fajas Département de Physiologie, Université de Lausanne, rue du Bugnon 7, 1005 Lausa

Abstract: The liver plays a unique role in the maintenance of lipid and glucose homeostasis. Deregulated liver metabolism contributes to the development of several pathologies such as obesity, insulin resistance and type 2 diabetes. The elucidation of the molecular mechanisms that regulate liver metabolism in normal and pathological conditions could lead to the identification of new therapeutic targets to treat metabolic disorders. We have recently demonstrated that the cdk4-Rb-E2F1 pathway participates in the regulation of glycolysis and lipogenesis in the liver. Here, we report that E2F1 also participates in the regulation of hepatic gluconeogenesis, a pathway that is abnormally upregulated during insulin resistance and that contributes to the observed hyperglycemia in this state. E2F1 -/- mice show decreased gluconeogenesis in vivo and reduced expression of liver key gluconeogenic enzymes. Accordingly, lack of E2F1 in primary hepatocytes showed reduced glucose production and gluconeogenic gene expression. Cdk4 typically increases E2F1 transcriptional activity by releasing it from the repression by pRb. Cdk4 -/- primary hepatocytes showed decreased gluconeogenesis while, on the contrary, a hyperactive form of Cdk4 (R24C) lead to an E2F1-dependent increased gluconeogenesis. On the contrary, adenoviral E2F1 overexpression increased gluconeogenic gene expression and glucose production in primary hepatocytes. Luciferase reporter assays showed that PEPCK1 promoter activity is increased in response to E2F1. Liver biopsies from obese subjects showed a correlation between PEPCK1 and E2F1 mRNA expressions, suggesting that E2F1 could contribute to the increased gluconeogenesis observed during insulin resistance. In agreement with this, E2F chemical inhibition decreased glucose production and PEPCK1 expression in a mice model of insulin resistance. Taken together, our results show that E2F1 participates in the regulation of both glucose and lipid metabolism in the liver, suggesting that E2F1 inhibition could be used as a therapeutic approach for the treatment of type 2 diabetes.

Poster 15

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Title of presentation: CDK4 controls fatty acid oxidation via the repression of AMPK activity

Authors list: I.C. Lopez-Mejia1, S. Lagarrigue1, P.D. Denechaud1, B. Delacuisine1, C. Chavey 2, B. Viollet3 and L. Fajas1 1Université de Lausanne, Switzerland; 2 IGMM Montpellier, France; 3 Institut Cochin, France

Abstract: Specific cellular functions, such as proliferation, survival, growth, and senescence, are triggered by specific stimuli and require a specific adaptive metabolic response. Our results point to the participation of the cell cycle regulator CDK4 in key steps of mitochondrial function and energy homeostasis. When fatty acid oxidation (FAO) and glycolysis were measured in MEFs from Cdk4+/+, Cdk4-/- and Cdk4R24C/R24C (carrying an allele encoding for a CDK4 protein that is resistant to the inhibition by the Ink4 family members) embryos, we observed that the deletion of Cdk4 led to an increase in FAO and a decrease in anaerobic glycolysis. On the other hand, the hyperactive Cdk4 mutant drove a significantly decreased FAO and an extremely high glycolytic rate. Interestingly, the increased oxidative activity in Cdk4-/- MEFs was correlated to an increased AMPK activity, suggesting that CDK4 may be a repressor of AMPK. We demonstrated that CDK4 interacts with AMPK, and phosphorylates several AMPK subunits including the AMPK α2 subunit. Interestingly, AMPK α2-/- MEFs, but not AMPK α1-/- MEFs, exhibit the same metabolic phenotype as Cdk4R24C/R24C MEFs, that is to say increased glycolysis and decreased FAO. Moreover, the AMPK activator A769662 fails to activate FAO in Cdk4-/- MEFs and in AMPK α2-/- MEFs, suggesting that in AMPKa2 is repressed by CDK4 in order to restrain FAO. We identified and validated 4 different phosphorylation sites for CDK4 in AMPKa2. When those sites were been mutated into alanine, AMPK activity was increased and trigger higher FAO and higher phosphorylation of ACC. To summarize, CDK4 participates in energy homeostasis through the modulation of the activity of the α2 containing AMPK complexes via direct phosphorylation of 4 different residues.

Poster 16

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Title of presentation: Leptin Controls Parasympathetic Wiring of the Pancreas During Embryonic Life

Authors list: Sophie Croizier, Vincent Prevot, Sebastien G Bouret

Abstract: The autonomic nervous system plays a critical role in glucose metabolism through both its sympathetic and parasympathetic branches, but the mechanisms that underlie the development of the autonomic innervation of the pancreas remain poorly understood. Here, we report that cholinergic innervation of pancreatic islets develops during midgestation under the influence of leptin. Leptin-deficient mice display a greater cholinergic innervation of pancreatic islets beginning in embryonic life and this increase persists into adulthood. Remarkably, a single intracerebroventricular injection of leptin in embryos caused a permanent reduction in parasympathetic innervation of pancreatic β cells as well as long-term impairments in glucose homeostasis. These developmental effects of leptin likely involve a direct inhibitory effect on the outgrowth of neurites from preganglionic neurons located in the hindbrain. These studies reveal an unanticipated regulatory role of leptin on the parasympathetic nervous system during embryonic development and may have important implications for our understanding of the early mechanisms that contribute to diabetes.

Poster 17

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Title of presentation: A genetic screen identifies hypothalamic Fgf15 as a regulator of glucagon secretion

Authors list: Alexandre Picard, Josselin Soyer, Xavier Berney, David Tarussio, Maxime Jan, Frédéric Burdet, Mark Ibberson and Bernard Thorens.

Abstract: The counterregulatory response to hypoglycemia, which restores normal blood glucose levels to ensure sufficient provision of glucose to the brain, is critical for survival. To discover underlying brain regulatory systems, we performed a genetic screen in recombinant inbred mice to identify quantitative trait loci (QTL) controlling glucagon secretion in response to neuroglucopenia. We identified a QTL on the distal part of chromosome 7 and combined this genetic information with tranacriptomic analysis of hypothalamic. This revealed Fgf15 as the strongest candidate to control the glucagon response. Fgf15 was found to be expressed by neurons of the dorsomedial hypothalamus and the perifornical area. Intracerebroventricular injection of FGF19, the human ortholog of Fgf15, reduced activation by neuroglucopenia of dorsal vagal complex neurons and of the parasympathetic nerve, leading to a lower glucagon secretion. These data show that Fgf15 in hypothalamic neurons is a new regulator of vagal nerve activity in response to neuroglucopenia.

Poster 18

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Presentation: Abstract submission

Title of presentation: Susceptibility to Sarcopenia Correlates with Neuro-Muscular Dysfunction during Aging of Skeletal Muscle

Authors list: Alice Pannérec, Margherita Springer, Eugenia Migliavacca, Guillaume Jacot, Sonia Karaz, Jérôme N. Feige

Abstract: Sarcopenia is the age-related loss of skeletal muscle mass and function leading to impaired mobility in the elderly population. It is well established that the progression of sarcopenia is multifactorial and influenced by lifestyle, genetics and nutrition. However, the specific mechanisms driving sarcopenia are still not well understood. Using a rat model of natural aging, we have found that hindlimb muscle mass progressively declines with age, while forelimb muscle mass remains very stable. Electromyography measurement confirmed this difference as we observed a decline in CMAP amplitude specifically in hindlimb muscles, suggesting that the regional decline in muscle mass mirrored a regional decline in neuromuscular transmission. At the molecular level, transcriptomic analyses revealed that gene expression is perturbed in an age- and sarcopenia-dependent manner. In particular, post-synaptic genes known to be regulated in muscle by neuromuscular input were specifically de-regulated in sarcopenic muscles. Finally, neuromuscular junction fragmentation and neuromuscular innervation measured by retro-tracer labeling of spinal cord motoneurons were both specifically altered with age in the muscles susceptible to sarcopenia. Altogether these findings provide a strong molecular, histological and phenotypic correlation between neuromuscular dysfunction and sarcopenia, and suggest that maintenance of the neuromuscular system is key for healthy muscle aging.

Poster 19

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Title of presentation: Loss of Fibronectin from the Aged Stem Cell Niche Affects the Regenerative Capacity of Skeletal Muscle

Authors list: Laura Lukjanenko, Jerome N. Feige, C. Florian Bentzinger.

Abstract: Muscle regenerative capacity is decreased with age and many pathways, including JAK-STAT, Erk-MAPK and p38 signaling were previously described to be deregulated with age, leading to cell-autonomous defects. However, the common upstream triggers of aging that lead to intrinsic satellite cell changes and loss of function still remain unclear. We discovered that the induction of Fibronectin (FN) upon muscle injury, is decreased in the aged stem cell niche. FN is an extracellular matrix component which regulates muscle stem cell function. We also uncovered an impairment in adhesion capacity of old satellite cells, which appears as a novel dysfunction of old satellite cells. We further showed that FN is able to rescue old satellite cells adhesion and proliferation in vitro. In vivo, intramuscular administration of FN rescued muscle regeneration in old mice. To understand the molecular mechanisms triggering impaired satellite cell function as a result of FN loss in their niche, we performed phosphoantibody and transcriptomic arrays. This revealed that lower levels of FN in the satellite cell niche leads to a deregulation of Integrin signalling through p38, ERK MAP kinase and FAK signalling; some of which were already described to be deregulated with age and causal for muscle stem cell dysfunction. Using loss of function approaches, we showed that these pathways respond to the loss of fibronectin from the extracellular matrix during aging and are required for FN to rescue satellite cell function. Altogether, we described that FN is a critical element of the muscle stem cell niche, whose loss with age not only affects satellite cell function but also leads to a profound de-regulation of several major pathways controlling cell-autonomous defects of muscle stem cells with age.

Poster 20

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Title of presentation: THE DETERMINANTS OF THE PREFERRED WALKING SPEED IN OBESE INDIVIDUALS

Authors list: 1 Davide Malatesta, 1 Aitor Fernández Menéndez, 1 Gilles Saudan, 1 Ludovic Sperisen, 2 Didier Hans, 3 Mathieu Saubade and 1 Grégoire Millet

Abstract: INTRODUCTION Obese adults have a slower preferred walking speed (PWS) compared to normal subjects. This may minimize external mechanical work and cost of walking. The minimization of perceived exertion may be another factor that regulates locomotion in normal subjects. The aim of this study was to examine the energetics, mechanics and perceived exertion that determine PWS in obese individuals. METHODS Twenty obese adults were recruited [age 33.4 ± 6.9 yr; BMI 33.6 ± 2.7 kg.m-2]. PWS was obtained after 10 min of treadmill habituation. 5-min walking trials at six speeds (PWS, 0.56, 0.83, 1.11, 1.39, 1.67 m.s-1) were performed with 5-min resting in between. Gas exchanges were measured. Gross energy cost of walking (GCw), rate perceived exertion (RPE), RPE cost of walking (RPECw) and ground reaction forces (GRFs) were assessed. External mechanical work (Wext) and inverted-pendulum recovery (R) were calculated. U-shaped relations of GCw, Wext, R & RPECw with walking speed were used to assess the optimal walking speed (OWS). RESULTS AND DISCUSSION At PWS, GCw (3.2 ± 0.4 J.kg-1.m-1) was higher than at OWS (3.1 ± 0.4 J.kg-1.m-1; $P < 0.05$). R at PWS 0.72 ± 0.04) was lower than at OWS (0.74 ± 0.04; $P < 0.05$). RPE at PWS (10.8 ± 1.7) was similar than at OWS (11 ± 1.8; $P > 0.05$). Wext at PWS (0.28 ± 0.04 J.kg-1.m-1) was higher than at OWS (0.25 ± 0.05 J.kg-1.m-1; $P < 0.05$). Multiple regression ($r = 0.63$; $P < 0.05$) showed that ~40% of the variance in PWS was accounted for by R and Wext. Hence, obese subjects may select a PWS to minimize R, GCw & RPE. R and Wext seem to be the main determinants of PWS, maybe due to a higher body mass that induce a greater work in obese subjects, highlighting the importance of body mass loss to decrease Wext and increase PWS in these individuals.

Poster 21

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Title of presentation: The hexosamine biosynthesis pathway in epithelial-to-mesenchymal transition in non-small cell lung cancer

Authors list: Svenja Groeneveld Bernard Moret Simona Rossi Etienne Meylan

Abstract: Glutamine-Fructose-6-Phosphate Transaminase 2 (GFPT2) catalyzes the rate limiting step of the hexosamine biosynthesis pathway (HBP), a metabolic branch of glycolysis that produces UDP-GlcNAc, a substrate for protein modifications. We discovered that in tumors from non-small cell lung cancer (NSCLC) patients, the expression of GFPT2 correlates with GLUT3, a glucose transporter we have recently reported to be involved in epithelial-to-mesenchymal transition (EMT) in this malignancy. To examine the connection between the HBP and EMT, in particular if and how GFPT2 is regulated by the EMT-inducing transcription factor Snail, we have combined overexpression and silencing techniques in vitro and in vivo. We found that mesenchymal NSCLC cell lines display higher levels of GFPT2 expression and protein O-GlcNAc modification compared to epithelial cell lines. Induction of an EMT in epithelial cells by TGF-β or Snail led to an up regulation of GFPT2 and an increase in O-GlcNAc levels. Accordingly, these changes were also observed upon overexpression of Snail in a mouse model of human NSCLC. Overexpression of GFPT2 by itself did not induce an EMT and siRNA-mediated inhibition of GFPT2 expression did not inhibit Snail-mediated EMT in epithelial cells. However, shRNA- or siRNA-mediated suppression of GFPT2 in mesenchymal cells increased expression of the epithelial protein E-cadherin and diminished that of the mesenchymal transcription factor ZEB1, respectively. We conclude that enhanced HBP activity goes along with an EMT. The expression of GFPT2 appears to be positively regulated by Snail. Surprisingly, GFPT2 seems to be neither necessary nor sufficient for an EMT to occur. Our data indicate, however, that GFPT2 might be involved in the maintenance of a mesenchymal phenotype.

Poster 22

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Title of presentation: Polyphenic trait determines cancer susceptibility in a model of epigenetic instability

Authors list: Marco Cassano, Sandra Offner, Alessandra Piersigilli, Evarist Planet, Suk Min Jang, Julien Duc, Hugues Henry, Kathleen McCoy, Andrew McPherson, and Didier Trono

Abstract: Hepatocellular carcinoma (HCC) represents the fifth most common form of cancer worldwide and carries a high mortality rate due to lack of effective treatment (Fattovich et al, 2004). Males are eight times more likely to develop HCC that females, an effect largely driven by sex hormones, albeit through still poorly understood mechanisms (Bosch et al. 2004). We previously identified TRIM28, a scaffold protein capable of recruiting a number of chromatin modifiers, as a crucial mediator of sexual dimorphism in the liver, with Trim28hep-/- leading to sex-specific transcriptional deregulation of a wide range of bile and steroid metabolism genes in the mouse, and male-limited development of liver adenomas (Bojkowska et al. 2012). We now demonstrated that obesity precipitates alterations of Trim28-dependent transcriptional dynamics, leading to a metabolic infection state responsible for a high penetrance of male-restricted hepatic carcinogenesis. Raising Trim28hep-/- mice in axenic conditions reverts their tumor-prone phenotype, establishing a link between gut microbiota and hepatic carcinogenesis. Moreover, androgen deprivation markedly attenuates the frequency and severity of tumors in these animals, consolidating the apparent link between epigenetic control of sex hormones metabolism and oncogenic degeneration. This work underpins how discrete polyphenic traits can contribute to a cancer-prone state in epigenetic unstable settings, and more broadly provides new evidence linking hormonal imbalances, metabolic disturbances and cancer. Furthermore, our results establish the Trim28hep-/- mouse as a relevant model to study the pathogenesis of human HCC and test new therapeutic approaches for its control.

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Poster 23

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Title of presentation: Integration of metabolomics data in a genome-scale metabolic model of the malaria parasite *Plasmodium falciparum* unveils its essential metabolic capabilities and nutritional requirements

Authors list: Anush Chiappino Pepe[a,b], Stepan Tymoshenko[a,b], Meriç Ataman[a,b], Dominique Soldati-Favre[c], Vassily Hatzimanikatis[a,b,1]

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Key words: genome-scale metabolic model, *Plasmodium falciparum*, thermodynamics-based flux analysis, essential metabolic capabilities, nutritional requirements

Abstract: The identification of novel and more efficient antimalarial therapies is a highly pressing need to fight against multi-drug-resistant parasites. Computational methods that analyze the metabolism of the pathogen can provide testable hypotheses and guide the experimental efforts for the identification of drug targets in metabolic networks. In this study, we aim at answering the following questions: (i) which of the substrates available at the blood stage are essential for growth simulation? (ii) what metabolic enzymes are essential in silico for replication? (iii) what are the intracellular metabolites that determine the directionality and function of metabolic enzymes? For this purpose, we have first developed iPfa, a newly reconstructed genome-scale metabolic model of P. falciparum, which extends the scope of the existing metabolic models of this parasite. We have then performed advanced computational analyses of iPfa, which involve the integration in iPfa of metabolomics data measured in P. falciparum at the blood stage and its thermodynamics-based flux analysis (TFA). In our computational studies, we identify the minimal nutritional requirements of P. falciparum at the blood stage and we identify the in silico minimal media, which is composed of only 23 substrates that are required for growth. We also identify the genes and enzymes that may represent drug targets for the blood-stage P. falciparum infection. With TFA and metabolomics data integrated, we predict 63 genes and 25 pairs of genes to be essential for the intraerythrocytic growth of the parasite. In total, we found supporting evidence for 35 of these predictions and no information was found for 28 genes that remain to be tested. We also identify metabolites, like glutamine or CDP-ethanolamine, whose intracellular

concentration determines the directionality and function of key reactions in the metabolic network. Our computational results provide novel insights about the metabolic capabilities and nutritional requirements of *P. falciparum* that can guide experimental efforts towards better understanding of the pathogen's physiology and, ultimately, towards the identification of novel antimalarial drug targets.

Poster 24

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Title of presentation: Altered adaptation to food restriction upon total miRNA loss in mouse liver

Authors list: Ngoc-Hien Du, Marieke Hoekstra, Bulak Arpat, Mara De Matos, Paul Franken, and David Gatfield

Abstract: Recently, we have reported a comprehensive profiling of the rhythmic transcriptome in hepatocyte-specific Dicer knockout and proposed roles for miRNAs in modulating daily gene expression. In brief, we found that miRNA-mediated regulation affected the phase and amplitude of as much as 30% of the rhythmic transcriptome. In contrast, the core clock was surprisingly resilient to miRNA loss with the exception of mild posttranscriptional upregulation in Per2, Per1 and Cry2 mRNAs. However, free-running PER2::LUC rhythms measured in liver explants showed long periods in the absence of miRNAs. We concluded that upon miRNA loss, the liver clock is only functional in the entrained animal, but not when it is released from the control of the master clock. This prompted us to explore the role of miRNAs in the hepatic core clock under conditions when the relationship between the master clock in the brain and the liver clocks is brought out of equilibrium such as when food is restricted. Mice were fed ad libitum before switching to 6-h food only in the light phase, and PER2::LUC rhythms in freely moving Dicer knockout and control animals were recorded. Our experiments suggest advanced kinetics of phase-shifting in Dicer knockouts compared to controls. At the behavioural level, Dicer knockout mice lost food-anticipatory-like activities that were observed before meal in control mice. At present, we are increasing the number of analysed animals in order to precisely quantify the effect and designing experiments to pinpoint the responsible miRNA(s). Altogether, our data point to an involvement of miRNAs in regulating flexibility of the clock towards changes in entrainment conditions.