Tuesday October 29, 2019
Olympic Museum - Quai d'Ouchy 1, 1006 Lausanne

# *LIMNA Symposium:*

# *Emerging topics and technologies in metabolism*

**Organizing Committee:** Prof. Francesca Amati, Prof. Carles Cantó Alvares, Prof. Roberto Coppari, Prof. Bart Deplancke, Prof. Lluis Fajas, Prof. Nelly Pitteloud, Prof. Bernard Thorens, Prof. Kei Sakamoto, Prof. Kristina Schoonjans and Dr Marta Bellone



## Invited Speakers

- Karine Clément, INSERM/Sorbonne Université, NutriOmics, Paris, France
- Jorge Ferrer, Center for Genomic Regulation (CRG), Barcelona, Spain
- Arvand Haschemi, Medical University of Vienna, Austria
- Zoltan Kutalik, University of Lausanne, Switzerland
- Susanne Mandrup, University of Southern Denmark, Odense, Denmark
- Samuel Nobs, Weizmann Institute of Science, Rehovot, Israel
- Ganna Panasyuk, Necker Enfants Malades Institute (INEM), Paris, France

## Program

### 8h30-9h00 Welcome and distribution of badges

### Opening

9h00 Bart Deplanche

*Welcome*

### Session I

Chair: Bart Deplanche

9h10 Susanne Mandrup

*Transcriptional networks and epigenomic mechanisms driving lineage-determination of human mesenchymal stem cells*

9h45 Samuel Nobs

*On the microbiome and precision dieting*

10h20 Reyhan Sonmez Flitman

*Integrating expression data into metabolome-wide GWAS identifies genes involved in the modulation of the metabolite concentration*

### 10h35 Coffee Break

### Session II

Chair: Kristina Schoonjans

10h55 Arvand Haschemi

*Exploring Metabolic Configurations of Single Cells in Situ*

11h30 Jorge Ferrer

*Genetic mechanisms underlying diabetes: insights from the noncoding genome*

12h05 Flash poster presentations

*45" per poster*

### 12h35 Lunch





### 13h20 Poster session

### Session III

Chair: Lluis Fajas

14h20 Ganna Panasyuk

*The novel mechanisms of metabolic control by class 3 PI3K*

14h55 Zoltan Kutalik

*Disentangling the causes and consequences of metabolic diseases using large scale genetic studies*

15h30 Dassine Berdous

*Unbiased genetic screening in BXD recombinant mice unraveled Crat as a new regulator of insulin content*

### 15h45 Coffee Break

### Session IV

Chair: Francesca Amati

16h05 Ana Rodriguez Sanchez-Archidona

*A systems biology analysis of the crosstalk between liver and pancreatic β-cell function through plasma lipids*

16h20 Nele Gheldof

*From GWAS to function: identification of ALK as a candidate thinness gene*

16h35 Karine Clément

*Where the study of adipose tissue transcriptome in obesity has brought us?*

### 17h10 Concluding remarks and prizes distribution

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# TALK ABSTRACTS *IN ORDER OF PRESENTATION*

1. **Transcriptional networks and epigenomic mechanisms driving lineage-determination of human mesenchymal stem cells**

**Susanne Mandrup**
*Center for Functional Genomics and Tissue Plasticity, Functional Genomics & Metabolism Research Unit, Dept. Biochemistry & Molecular Biology, University of Southern Denmark*

Mesenchymal (stromal) stem cells (MSCs) constitute populations of mesodermal multipotent cells involved in tissue regeneration and homeostasis in many different organs. Defective differentiation and functions of MSCs contribute to age-related changes and diseases, including obesity, sarcopenia, and osteopenia. Furthermore, the plasticity and pleiotropic functions of these cells, make them relevant targets for stem cell-based regenerative therapies. We have characterized the transcriptional and epigenomic changes associated with osteoblast and adipocyte differentiation of human bone marrow-derived MSCs. Our results demonstrate that adipogenesis is driven by considerable remodeling of the chromatin landscape and de novo activation of enhancers, while osteogenesis involves activation of pre-established enhancers. Using machine-learning algorithms for in silico modeling of transcriptional regulation we identified a large and diverse transcriptional network of pro-osteogenic and anti-adipogenic transcription factors. Intriguingly, binding motifs of these factors overlap with single-nucleotide polymorphisms (SNPs) related to bone and fat formation in humans, and knockdown of single members of this network is sufficient to modulate differentiation in both directions, indicating that lineage-determination is a delicate balance between activities of many different transcription factors. To determine how adipogenic enhancers act in 3D space to drive the dramatic remodeling of chromatin during adipocyte differentiation, we applied capture Hi-C and integrated data with genome-wide mapping of transcription factors and enhancer activity. We show that highly connected enhancers drive and coordinate cell type-specific gene programs through the formation of insulated chromatin regions, inside which, enhancers converge on adipogenic gene promoters.





2. **On the microbiome and precision dieting**

**Samuel Nobs**
*Weizmann Institute of Science, Rehovot, Israel*

The composition, timing and patterning of diet has a profound impact on human health. Host microbiome interactions are a key determinant of our response to diet, thereby profoundly affecting host metabolism and immunity.

3. **Integrating expression data into metabolome-wide GWAS identifies genes involved in the modulation of the metabolite concentration**

**Reyhan Sonmez Flitman**, Rico Rueedi, Sven Bergmann
*University of Lausanne, Computational Biology Department*

Metabolome-wide GWAS (mGWAS) search for associations between metabolites and common genetic variants within large collections of samples. NMR spectral intensities reflect metabolite concentrations, which in targeted NMR are combined to quantify a limited set of metabolites. In order to avoid the often imperfect identification and quantification of metabolites, we designed a different type of mGWAS called untargeted mGWAS, that directly tests metabolome features for association with genetic variants, not discarding any data that may have eluded identification. The effect of a genetic variant on the concentration of a metabolite tends to translate, in an untargeted mGWAS, to associations with all or some of the features corresponding to the pure NMR spectrum of the metabolite. The association profile can therefore allow for identification of the underlying metabolite using a method we call metabomatching (Rueedi et al., PLoS Genetics 2014). Here, we apply metabomatching to associations with gene expression rather than genotypic variation. To this end we used RNAseq profiles of lymphoblastoid cell lines derived from 555 CoLaus subjects for which we had also urine NMR data. Specifically, we investigated the genes whose expression levels showed strong association with metabolome features. We found that ALMS1 gene expression is strongly associated to the concentration of N-Acetyl L-Aspartate (NAA) making ALMS1 the most likely candidate gene for modulating this metabolite. In summary our study provides evidence that the integration of metabolomics with gene expression data can support mQTL analysis, helping to identify the most likely gene involved in the modulation of the metabolite concentration.

4. **Exploring Metabolic Configurations of Single Cells in Situ**

**Arvand Haschemi**
*Department of Laboratory Medicine, Medical University of Vienna, Austria*

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Cellular metabolism is a highly dynamic process and able to quickly adapt in response to environmental factors. Up to now, metabolic configurations of specific cell types and their adaptations were primarily studied in vitro, neglecting the impact of the respective tissue microenvironments. Recently, we have developed and published a method to assess the metabolic configuration of single cells within their native tissue microenvironment by visualization and quantification of multiple enzymatic activities in combination with antibody-based cell type identification. Determination of enzymatic activities and kinetics of enzymes located within different branches of primary carbohydrate metabolism provides valuable information on the actual configuration and adaptation of glycolysis, the pentose phosphate pathway and the TCA cycle. I will present various applications of this novel tool to profile single cell metabolism and assess cellular metabolic reprogramming in immunology, tumor biology and endocrinology research. These examples will demonstrate the importance of in situ studies for investigating the complex interplay of cellular metabolism and functions in health and disease.

5. **Genetic mechanisms underlying diabetes: insights from the noncoding genome**

**Jorge Ferrer**
*Regulatory genomics and diabetes lab. Genome Regulation Center-CRG and CIBERDEM. Carrer Aiguader 88, Barcelona, Spain 08003.*

Most genetic mechanisms that are currently known to underlie developmental processes and human diseases have been discovered by analyzing a very small fraction of the genome that encodes for protein-coding sequences. Recent studies have revealed that large portions of the noncoding genome contain functional regulatory elements and noncoding transcripts. It is thus reasonable to presume that understanding the function of such elements can shed new light into developmental and cellular mechanisms, and uncover new causes of human disease.
The molecular mechanisms that lead to Type 2 diabetes are largely unknown. Recent work has shown that common DNA variants in human pancreatic islet enhancers contribute to the risk of developing common polygenic forms of type 2 diabetes. Many of these diabetes risk variants are located in islet enhancer hubs that control one or more cell-specific target genes. The current challenge lies in using this information to dissect the mechanisms through which these regulatory variants impact pancreatic islets and polygenic diabetes. This effort could be assisted by the analysis of rare highly penetrant natural or artificial mutations, which can be used to provide insights into how noncoding DNA variants contribute to disease mechanisms.
Rare recessive mutations in an enhancer near PTF1A have been shown to cause neonatal diabetes associated with severe pancreas hypoplasia. I will discuss how modeling mutations in this enhancer provide unsuspected insights into the developmental and molecular mechanisms that underpin this regulatory defect.
Defects in long noncoding RNAs (lncRNAs) provide another potential mechanism of disease. Inhibition of several lncRNAs can impact gene expression and insulin secretion in cellular assays, but their physiological and disease relevance remains largely unexplored. I will discuss a genetic





model that shows how disruption of a lncRNA can lead to severe pancreatic beta cell dysfunction and diabetes.

6. **The novel mechanisms of metabolic control by class 3 PI3K**

**Ganna Panasyuk**
*Institut Necker-Enfants Malades (INEM), Paris, France*

The capacity of cells to engage in energy utilization is ochestrated by nutrient sensing signal transduction pathways together with nutrient sensing transcription factors such nuclear receptor superfamily. In the team, we aim to understand how cellular metabolic activities are coordinated by these nutrient sensing pathways in response to nutrient fluctuations. We focus on class 3 phosphatidylinositol 3-kinase (PI3K) signalling that functions in every eukaryotic cell from yeast to mammals. Its lipid kinase activity is essential for vesicular trafficking and functional lysosomal degradation by autophagy. Recently, we discovered a novel role of class 3 PI3K in metabolic adaptation to fasting. We have demonstrated that, in liver, selective autophagy driven by class 3 PI3K is essential for transcriptional control of lipid catabolism and mitochondrial activity. Using the model of hepatocyte specific inactivation of an essential regulatory subunit of class 3 PI3K, Vps15, we discovered that class 3 PI3K couples transcriptional induction of mitochondrial biogenesis and lipid catabolism by activating nuclear receptor PPARa, a master regulator of fasting catabolism. We have shown that transcriptional repressors of PPARa, NCoR1 and Hdac3 proteins, are degraded by selective autophagy and in this way PPARa activity is activated in fasting. Now, in the team, we extend these findings further to other metabolic states to discover how class 3 PI3K governs metabolic homeostasis.

7. **Disentangling the causes and consequences of metabolic diseases using large scale genetic studies**

**Zoltan Kutalik**
*Statistical Genetics Group, University of Lausanne*

Observational studies can mostly estimate correlations between potential risk factors and diseases, which has limited utility for public health interventions. Mendelian randomisation methods leverage information from genetic correlates of risk factors and complex diseases in order to identify causal relationships and its extent. I will present several extensions of this principle. These extensions led to identification of different obesity subtypes with distinct cardio-metabolic consequences. Furthermore, another new method (distinguishing genetic confounding and causation) revealed that - as opposed to most analyses - obesity does not lead to lower educational attainment. Finally, such methods also enable the detection of key driving forces in mate-choice.

8. **Unbiased genetic screening in BXD recombinant mice unraveled Crat as a new regulator of insulin content.**

**Dassine Berdous**, Xavier Berney, Ana Rodriguez Sanchez-Archidona, Clara Roujeau, Maxime Jan, and Bernard Thorens
*CIG, University of Lausanne*

Background: Pancreatic ß cells secrete insulin in response to a rise in extracellular glucose concentration to maintain normoglycemia. This secretory response is potentiated by exendin-4 (GLP1 receptor agonist). Impaired insulin secretion is central in the onset of type 2 diabetes, a disease with a multifactorial and polygenic origin. Aim: Here, we aimed at identifying novel regulators of glucose-stimulated insulin secretion (GSIS) and exendin-4 potentiation using a genetic screen in recombinant BXD inbred mice. Results: We identified a locus that was associated to insulin secretion in response to high glucose with exendin-4 and a positive correlation between insulin secretion and content. Combining QTL mapping and islet gene expression allowed us to identify carnitine acetyltransferase (Crat) as a likely regulator of insulin secretion and content. Crat is a mitochondrial enzyme catalyzing the interconversion of acetyl-CoA to acetyl-carnitine. The role of this enzyme in ß-pancreatic cells has not been investigated yet. By overexpression and knockdown studies in ß-cell lines we established that Crat plays a role in insulin content regulation. Using mice with ß-cell-specific knockout of Crat we showed that Crat inactivation was associated with reduced insulin content, when islets were exposed to high concentration of glucose and palmitate. We also showed that ß Crat KO mice were glucose intolerant compared to their control littermates, when fed with a high fat diet. Perspectives: We propose that Crat represents a target in β-cells of type 2 diabetic animal model and patients that deserves further investigation as it may constitute a pharmacological target to improve insulin content and insulin secretion.

9. **A systems biology analysis of the crosstalk between liver and pancreatic β-cell function through plasma lipids**

**Ana Rodriguez Sanchez-Archidona**, Celine Cruciani-Guglielmacci, Jessica Denom, Leonore Wigger, Florence Mehl, Christian Klose, Kai Simmons, Philippe Delerive, Celine Cruciani-Guglielmacci, Christophe Magnan, Mark Ibberson and Bernard Thorens
*CIG, University of Lausanne*

Background and Aim Quantitative measurement of circulating metabolites may help to identify tissue specific metabolic pathways that are modulated by or causal for a disease condition. The aim of the study was to explore associations between plasma lipid concentrations and gene expression in pancreatic β-cells and insulin target tissues in a mouse model of pre-diabetes to uncover molecular mechanisms and biomarkers of diabetes susceptibility. Material and Methods Three genetically different mouse strains (C57Bl/6, DBA/2J and BALB/cJ) were fed with a high fat





diet (HFD) or a regular chow (RC) for different periods of time: 5, 13 and 13 days. Liver and pancreatic islets tissue samples were taken at the end of these three periods of time and subjected to RNA-Seq analysis. Plasma was also sampled at the end of each time point in order to evaluate 560 lipids, basal plasma glucose and insulin levels. We performed weighted gene co-expression network analysis (WGCNA) on RNA-Seq data from the isolated tissues and searched for correlation between tissue-specific gene expression modules and plasma lipids, glucose and insulin. Results We identified liver and beta-cell gene modules strongly correlated with the plasma concentration of a group of triglycerides (TGs) and sphingomyelins (SMs). In liver, a gene module highly anti-correlated with plasma TGs and correlated with SMs was enriched in genes controlling plasma membrane and mitochondrial carnitine transporters and β-oxidation. In pancreatic islets, TGs were strongly correlated with a module containing a large number of genes controlling insulin secretion. This β-cell module was also strongly correlated with liver gene modules enriched in “ER stress” and “gluconeogenesis” genes and both these liver and β-cell modules showed similar correlation with basal insulinemia. Furthermore, plasma TGs and insulin measurements were strongly correlated. Conclusions We have established two coordinated axes of communication between the liver and the pancreatic islets transcriptomes: one through plasma TGs and another through insulin. Our study also found evidence of association of plasma TGs concentrations with lipid metabolism in liver and insulin secretion, which suggests that the two axes are indeed part of a regulatory network that operates in a coordinated manner to control insulin secretion. It also points out to specific genes that may be targeted to revert the pre-diabetic state. Grant Acknowledgement EU-IMI IMIDIA, EU-IMI2 RHAPSODY, JDRF, ANR and SNF BetaDiaMark

10. **From GWAS to function: identification of ALK as a candidate thinness gene**

Michael Orthofer, Armand Valsesia, Reedik Magi, Jorg Hager, Josef M. Penninger, **Nele Gheldof**
*Nestlé Institute of Health Sciences, Lausanne*

Background and aims: Despite living in our modern world´s obesogenic environment that promotes weight gain, some individuals have the unique capacity to maintain a very low stable body weight, a state called constitutional thinness (CT). CT individuals show a persistent extreme low BMI (generally lower than 18 kg/m2) but have normal metabolic, psychological, feeding and exercise profiles and often a strong desire to gain weight. It is currently not clear why CT individuals have resistance to weight gain, but it has been suggested that genetics play a major role in the predisposition of such people to remain thin. Materials and methods: Using data from the population-wide Estonian biobank, we performed a genome-wide association study (GWAS) on thin versus control individuals. We followed up by evaluating the effect of knockdown of the gene in which one of the top variants was located on metabolism and adiposity in fly and several mouse models. Results: From the thinness GWAS study, we found that one of the top intragenic hits was located within ALK (Anaplastic Lymphoma Receptor Tyrosine Kinase), a gene belonging to the insulin receptor superfamily. Using fly adiposity screens and several mouse models, we found that knock-out of Alk resulted in thin fly and mice markedly resistant to diet-and leptin-

mutation-induced obesity with many striking similarities to the human thin phenotype. We genetically mapped this effect of ALK to hypothalamic neurons in the central nervous system. Mechanistically, Alk deficient mice showed normal food intake but increased energy expenditure and sympathetic outflow-mediated white adipose tissue lipolysis. This resulted in reduced feeding efficiency, i.e. reduced weight gain per calories consumed. Conclusion: In conclusion, our human cohort data and functional validation in animal models have identified ALK as one of the first thinness gene involved in the resistance to weight gain.

11. **Where the study of adipose tissue transcriptome in obesity has brought us?**

**Karine Clément**
*Sorbonne Université, INSERM, NutriOmics research Unit, Pitié-Salpêtrière hospital Paris, F-75006 France; Karine.clement@inserm.fr*

The moderate increase in circulating inflammatory factors found in obese subjects and the discovery of inflammatory cell accumulation in adipose tissue (WAT) has opened fascinating researches in obesity and diabetes. Substantial knowledge has been brought initially by the study of tissue pangenomic gene expression in rodents and in human metabolic disorders. In early 2000, we started to explore the gene profiling in WAT. Large scale gene expression (mostly microarray) showing an enrichment of inflammatory and extracellular matrix genes in human obese WAT prompted us to pursue the exploration of WAT structure and function. These features are deeply altered associated with adipose cell hypertrophy and immune cells accumulation which include macrophages, lymphocytes and mast cells. Together with inflammatory cell accumulation, the evaluation of transcriptomic interaction characterizing human WAT demonstrated the strong relationship linking inflammatory processes to extra cellular matrix (ECM) remodelling. Our group then showed that interstitial fibrosis accumulates in obese WAT as in many organs affected by low-grade inflammation in chronic diseases (i.e. liver, lung, kidney). We also provided insights into the composition of WAT fibrosis showing a different pattern and distinct pathophysiological significance in subcutaneous and omental WAT. An important finding was also that high level of scWAT fibrosis associated with diminished bariatric surgery-induced weight loss in severe obesity. Finally, combining mouse and human experiments, we detected progenitor cells which might contribute to fibrosis development.
Grant acknowledgments for your research. National Agency of Research (CAPTOR) and European Community Frameworks (ADAPT, FLIP, HEPADIP and DIOGENES), French foundation for medical research and EFSD





# POSTER ABSTRACTS

Presenter Poster number

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## 1. Dissecting the mechanisms of molecular and cellular variation in human adipogenesis

Daniel Alpern, Vincent Gardeux, Magda Zachara, Gerard Llimos, Julie Russeil, Pernille Rainer, Olga Pushkareva, Marco Tello, Audrey Bourdilloud, Christian Caprara, Gianni Soldati, Bart Deplancke
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Human adipose stromal/stem cell (hASC) can be extracted in abundance from subcutaneous lipoaspirate samples and are broadly used for the research and cell-based therapies. Capable of differentiation under defined culturing conditions into mature adipocytes, these cells provide a robust model for studying the molecular cues of obesity in humans. ASC also serves as a valuable resource for investigating the impact of the genetic variation on shaping molecular and cellular phenotypes and arguably complex traits at the organismal level. To study the functional genome organization in the variable genetic background we have gathered 45 hASC culture samples from non-related individuals. We have differentiated each sample into mature adipocytes and collected cellular phenotyping data and a large body of genomic, performing molecular characterization of each sample at both differentiation time points. By integrating the transcriptomics and chromatin states data with the intracellular lipid content information we have revealed striking and reproducible donor-specific variation in the adipogenic differentiation capacity of each hASC sample. Further analyses, including single cell transcriptomics, will be required to determine to which extent this cellular variation is driven by genetic variation or by differences in stromal cell population structure or both. We anticipate that this study might help to better characterize the molecular mechanisms of determining the adipogenic capacity of individual hASC samples and contribute to the interpretability of obesity-related non-coding GWAS variants, which remain largely uncharacterized.





## 2. Role of ECRG4 in hypercalciuric mouse models

Daniela Cabuzu1, Suresh K. Ramakrishnan1 and Olivier Bonny1,2
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Introduction: Kidney stone is one of the most frequent urinary tract diseases, affecting 10% of the population and displaying a high recurrence rate. The stone formation process is complex, resulting from an impaired balance between urine promoters (calcium and uric acid) and inhibitors of crystallization. Therapeutic possibilities remain underexplored. Esophageal cancer-related gene-4 (ECRG4) was identified by differential microarray analysis as a gene upregulated in the DCT/CNT segment of the hypercalciuric kidney specific NCX1-KO mouse model. ECRG4 encodes a 17kDa protein of unknown function that has been described as a potential tumour suppressor and antiapoptotic gene. Under physiological conditions it is expressed at very low level in several tissues, such as heart and brain. We hypothesized that ECRG4 might be upregulated by calcium and might protect against deleterious effect of hypercalciuria and stone formation. Materials and Methods: We used several established mouse models of hypercalciuria and looked at renal mRNA ECRG4 expression, compared to controls. We used ECRG4 KO and control littermate mice to characterize the basal mineral metabolism in metabolic cages. We exposed the mice to two different diets known to induce (i) hypercalciuria: a diet enriched in dihydrotachysterol (DHTS, an analog of vitamin D) (1.5%) for 7 days or (ii) hypercalciuria and crystal deposits in the kidney: a diet enriched in calcium (1.5%) and hydroxyproline (2%, a precursor of oxalate) for 8 days. Also we used another established mouse model of hypercalciuria: Claudin 2 KO (CLD2) mice. Results: We could show a stronger expression of the ECRG4 transcript in the hypercalciuric mouse models compared to controls: CLD2 KO mice (vs. control littermates) and DHTS fed mice (vs. regular diet). Next, we compared the mineral metabolism parameters of the ECRG4 KO vs. control mice, but could not identify differences. When challenging the ECRG4 KO and control mice by feeding them calcium oxalate rich diet (vs regular diet), our preliminary results show an increase in urine calcium/creatinine ratio for the ECRG4 KO mice at the end of the treatment, compared to the controls. The intrarenal deposits were evaluated in the kidney tissue sections by Pizzalato staining and the quantification showed a significantly increased number of deposits in the ECRG4 KO mice vs. control mice. Moreover, the ECRG4 KO kidneys developed more fibrosis compared to the control mice, indicated by the Masson trichrome staining.

3. Activation of a thermogenic transcriptional program by YY1 dephosphorylation in brown adipocytes.

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The balance between energy intake and energy expenditure is a complex physiological system that relies on cellular and endocrine mechanisms. Alteration of this equilibrium by intrinsic or extrinsic factors can lead to metabolic disorders including obesity, a worldwide epidemic. The elevation of energy expenditure through brown adipose tissue thermogenesis has revealed a promising strategy to combat metabolic disorders. Particularly since the discovery of brown adipose depots in adult humans, several signaling and transcriptional pathways leading to brown adipose tissue activation have been identified. However, it is not fully understood how different transcription factors coordinate the activation of thermogenesis or the balance between lipogenic versus catabolic pathways. Yin Yang 1 (YY1) is a ubiquitous transcription factor involved in the general activation of transcription of multiple biological processes including basal metabolism. Our previous work showed that the transcription factor YY1 activates brown adipose tissue thermogenesis. We have now identified that a key phosphorylation site at S120 plays a switching role between thermogenic activation versus YY1-dependent cell growth and anabolic functions. Particularly, we have now shown that adrenergic signaling leads to the dephosphorylation of YY1 at S120. The phosphorylation status of YY1 is controlled by the kinase CK2 and the phosphatase PP2A. Interestingly, transcriptomic RNAseq experiments showed that YY1(S120) dephosphorylation directly leads to activation of a thermogenic gene program including UCP1 expression in brown adipocytes. In addition, YY1(S120) dephosphorylation results in the upregulation of JMJD1A, a H3K9-specific demethylase, which expression is directly mediated by YY1 shown by chromatin immunoprecipitation. Furthermore, mice fed with an anabolic stimulus such as high fat diet leads to increased YY1(S120) phosphorylation in brown adipose tissue. Our results show a complementary axis of the control of Ucp1 expression and how general transcriptional factors such as YY1, control tissue specific functions by sensing physiological signals to orchestrate downstream metabolic effectors.

4. Human fibroblasts as a model to investigate genetic and epigenetic contributions to regulation of circadian rhythms across human diseases

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Disruption of circadian rhythms has been increasingly implicated in the pathology of various human diseases, ranging from neurodegenerative diseases to metabolic disorders. How the circadian clock is molecularly linked to predisposition to complex diseases remains however





largely unknown. In reverse, how pathological conditions affect circadian traits at molecular levels is still elusive. While studies using human subjects/tissues are challenging, human cells, including skin fibroblasts and induced pluripotent stem cells (iPSCs) can be obtained noninvasively, and provide unlimited resources for long-term assessments and molecular manipulations. Importantly, primary skin fibroblasts express robust circadian rhythms that reflect human circadian physiology. In addition, iPSC reprogramming not only erases epigenetic landmarks acquired throughout a subject's lifetime, but also stops the circadian clock. By comparing circadian traits between skin fibroblasts and iPSC-derived fibroblasts, we aims to distinguish the contribution of genetic vs. epigenetic variants associated with aberrant clock function linked to human pathology. In collaboration with the University of Oxford StemBANCC consortium, we first characterised rhythms in primary skin fibroblasts collected from different patients: diabetes (71 patients), Alzheimer's disease (AD, 22), Parkinson disease (PD, 147), bipolar disorder (BD, 39), neuropathy (44), and healthy matched control subjects (35). We found that while circadian amplitudes were dampened across different diseases, circadian period lengths were prolonged in a disease-specific manner, i.e. only in cells from BD and neuropathic patients. In addition, single disease-specific circadian phenotypes were also detected. In PD, longer period length was associated with less robust rhythms. In neuropathy, almost half of the lines had very low amplitude and in some cases even lost rhythms. In conclusion, our results suggest that cellular circadian oscillations are affected by disease state. Comparison of these rhythms with those from iPSC-derived fibroblasts together with high-throughput characterization of genetic and epigenetic differences correlated across cell lines from different individuals will help to identify cell-intrinsic (i.e. genetic) and acquired (i.e. epigenetic) characteristics explaining these phenotypes.

5. Brain creatine deficiency, increased grooming and structural cerebellar changes in a new KI rat model of creatine transporter deficiency

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BACKGROUND Creatine (Cr) is synthesized by a 2-step pathway (AGAT and GAMT), and transported by SLC6A8. Cerebral Cr deficiency syndromes, due to AGAT, GAMT or SLC6A8 deficiencies, are inborn errors of metabolism causing severe neurodevelopmental delays and intellectual disability, characterized by absence of brain Cr measured by magnetic resonance spectroscopy (MRS). While AGAT and GAMT deficiencies can be improved with Cr treatment, the X-linked SLC6A8 deficiency cannot. Pathological mechanisms are still largely unknown. We present further details of characterization of our new rat model of SLC6A8 deficiency. METHODS Knock-in (c.1166A>G; p.Tyr389Cys) Sprague-Dawley rats were generated using CRISPR/Cas9 technology. Brain Cr was measured in different brain regions by 1H-MRS (horizontal 9.4T MRI system). CNS, kidney and liver were analyzed by immunohistochemistry on cryosections and by western blotting. Grooming and rearing were recorded in an arena (1 m diameter) for 10 min and

analyzed using The Observer® XT11. RESULTS Our Slc6a8Y389C/y rat strain was established based on one same missense point mutation described in human and abolishing completely the Cr transporter activity. Mutant males rats showed strong decreases in brain Cr (-80%), body weight gain from weaning, and rearing unsupported, but significant more time in grooming (as compared to age-matched WT). In kidney, AGAT levels were increased (suggesting up-regulation) while SLC6A8 was still expressed but intracellularly delocalized. GAMT levels appeared unchanged in liver, as well as those from SLC6A8, AGAT and GAMT in brain. Furthermore, mutant rat males showed structural changes in cerebellum: Astrocytic fibers were disorganized, while the granular layer of cerebellar cortex appeared thinner in KI animals. DISCUSSION Our results validate this rat model as a promising tool to better understand SLC6A8 deficiency. It may help to comprehend and treat human pathology.

6. Role of splicing factor SRSF2 in normal and insulin resistant adipose tissue

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Alternative splicing is a key post-transcriptional modification which contributes to proteome diversity by allowing a single gene to code for multiple protein isoforms. SRSF2 is a member of the serine- and arginine-rich (SR) family of RNA binding proteins that was first discovered as a regulator of alternative pre-mRNA splicing. A significant amount of studies has identified the implication of SRSF2 in various human pathologies, but not in metabolic disorders such as diabetes and obesity. Thus, we set out to investigate the role SRSF2 during metabolic syndrome, particularly in insulin resistance in adipose tissue, using in vitro and in vivo models.
First, we used 3T3-L1 as a cellular model for white adipocytes, and observed that the expression of SRSF2 was increased during differentiation of 3T3-L1 preadipocytes into mature adipocytes. Moreover the expression levels and cellular localization of SRSF2 were modified upon insulin stimulation. Interestingly, the insulin-induced SRSF2 expression is maintained in insulin resistant conditions (TNF, IL-6 and Palmitate) in 3T3-L1 adipocytes. To model insulin resistance in in vivo, C57BL/6J mice were put on high-fat diet (HFD) for 20 weeks. We observed a specific increase in SRSF2 levels in perigonadal white adipose tissue (pgWAT) in HFD fed mice, but no difference in another adipose tissue depot, the subcutaneous white adipose tissue (scWAT). In order to elucidate the role of SRSF2 in WAT function in vivo, Srsf2 adipose tissue specific knockout (Srsf2 ATKO) mice were generated and placed on both control diet (CD) and HFD. Srsf2 ATKO mice showed lower fat mass, smaller scWAT, smaller pgWAT with increased frequency of smaller adipocytes and smaller brown adipose tissue (BAT) with decreased lipid area, as well as impaired insulin sensitivity under CD. This phenotype was amplified upon HFD intervention, except for the increased frequency of smaller adipocytes in the pgWAT. In addition, Srsf2 ATKO mice in CD also had a higher RER in the light phase, suggesting a reduction in lipid metabolism and an increased carbohydrate utilization.





Analysis of RNA sequencing data from WAT samples showed significant alterations in the alternative splicing of genes encoding for proteins involved in lipid metabolism, such as Pex16, Acat2, Mttp, Apoc2, and Apoc1, suggesting that adipose tissue Srsf2 participates in WAT lipid metabolism in normal and pathological conditions, at least in part by regulating WAT alternative splicing. Overall, this study contributes to the understanding of the role adipose tissue SRSF2 in the control of whole-body energy homeostasis, particularly through its role in alternative splicing.

7. FKBP10 regulates protein translation to sustain lung cancer growth

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Cancer therapy is limited by lack of specificity. Thus, identifying molecules that are selectively expressed by and relevant for cancer cells is of paramount medical importance. Recently, we have shown that imposing a hyper-caloric diet (HCD) before the onset of KRAS-driven lung tumors inhibits tumorigenesis in KrasG12D mice. To determine the molecular mechanism underlying the anti-tumor action of this HCD feeding regimen, we performed unbiased assays and identified FK506-Binding Protein 10 (FKBP10), an endoplasmic reticulum chaperone that contains four peptidyl-prolyl-cis-trans-isomerase (PPIase) domains, as a putative mediator. Here, we combined in vivo and in vitro functional approaches to establish the specificity and relevance of FKBP10 in KRAS-driven lung tumorigenesis and unravel its mechanism of action. By analyzing human tumor datasets and surgically collected healthy and tumor lung tissues, we found that FKBP10 is selectively expressed in tumor lesions, whereas it is not detected in healthy parenchyma of adults. Furthermore, FKBP10 expression negatively correlates with patient survival affected by lung adenocarcinoma. Genetically-mediated reduction of FKBP10 expression before or after tumor-onset caused a dramatic diminution of Kras-driven lung tumor burden in mice. Results from gain- and loss-of-function assays show that FKBP10 boosts growth and stemness capacity of lung cancer cells, via its PPIase activity. Moreover, we revealed that FKBP10 is associated with ribosomes and its downregulation leads to a significant reduction of protein translation elongation at the beginning of Open Reading Frames, particularly upon insertion of proline residues. Thus, our results unveil FKBP10 as a cancer-selective molecule with a key role in translational reprogramming, stem-like traits and growth of lung cancer.

8. Systemic and central nervous system metabolic alterations in Alzheimer's disease

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Metabolic alterations play an important role in Alzheimer's disease (AD) on both the systemic and central nervous system (CNS) level. To study their extent and significance in AD, quantitative metabolomics was applied to samples from clinically well-characterized AD patients. The association of the observed metabolic alterations with core pathological processes of AD was explored to understand their relation with amyloid pathology and tau-related neurodegeneration. Metabolic profiling, i.e. untargeted metabolomics and targeted quantification, was performed on paired plasma and CSF samples from clinical and biomarker-confirmed AD patients (n=40) and cognitively healthy controls without cerebral AD pathology (n=34). Targeted quantification focused on identified deregulated pathways, such as the TCA cycle and its anaplerotic pathways, as well as the neuroactive tryptophan pathway. Concentrations of several TCA cycle and beta-oxidation intermediates were higher in plasma of AD patients, whilst amino acid concentrations were significantly lower. Similar alterations in these energy metabolism intermediates were observed in CSF, which were strongly correlated with blood-brain barrier permeability. Alterations of several amino acids were associated with CSF Amyloid $\beta$ 1-42. The tryptophan catabolites kynurenic acid and quinolinic acid showed significantly higher concentrations in CSF of AD patients, which, together with other tryptophan pathway intermediates, were correlated with either CSF Amyloid $\beta$ 1-42, or tau and phosphorylated Tau-181. These results revealed AD-associated systemic dysregulation of nutrient sensing and oxidation and CNS-specific alterations in the neuroactive tryptophan pathway. The specific association of amino acids and tryptophan catabolites with AD CSF biomarkers suggests a close relationship with core AD pathology.

9. The thermogenic control of brown adipocyte function by nutritional status

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Adipocytes play a key role in the physiological regulation of energy balance. White or brown adipocytes play opposite energy storing or consuming/thermogenic functions respectively and both can respond to nutritional status by modulating varying metabolic processes such as lipogenesis and lipolysis. While increased thermogenesis has been observed in brown adipocytes shortly after a meal, prolonged high nutritional status seems to convert brown adipocytes towards a "whitening" phenotype with increased lipogenesis and reduced thermogenesis. Since the mechanism behind these opposing observations remains unclear, in this research, we try to understand how nutritional flux can impact brown adipocyte function and its metabolic pathways. Chronic pyruvate treatment was given to brown adipocytes in vitro to perturb their nutritional. Upon chronic pyruvate treatment, the expression of Ucp1, the surrogate marker of thermogenesis, was significantly reduced. In addition, basal and state3 (oligomycin) oxygen consumption of the chronically treated brown cells was remarkably lower than the control and showed a blunted response to norepinephrine, suggesting a decreased thermogenic capacity. In order to identify the molecular players of this pyruvate-dependent changes in brown adipocytes thermogenesis and metabolism, RNAseq and untargeted metabolomics were performed. First, Gene Set





Enrichment Analysis (GSEA) identified a compromised thermogenic gene program. The increased levels of NADPH identified by metabolomics suggest an influence of the oxidative stress in the decreased thermogenic phenotype. Furthermore, the comparison of RNAseq and metabolomics data has led to the identification of Amine Oxidase Cupper-containing 3 (Aoc3) as a potential regulator of thermogenesis. Aoc3 was a previously identified gene which is highly up-regulated during adipogenesis. Despite of the high expression, its function in adipocytes remains unknown. Our ongoing experiments will identify its specific role in the context of metabolic status sensing and thermogenesis.

10. Increased ammonium production and reduced appetite in a knock-in rat model for glutaric aciduria type I challenged with high lysine diet

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Background: A knock-in rat model for glutaric aciduria type I (GA-I) was recently created by our group in order to develop a better model for pathophysiological investigations and treatment trials. p.R402W, the rat equivalent of the most common Caucasian mutation p.R411W, was introduced into the GCDH gene of Sprague Dawley (SD) rats by CRISPR/Cas9 technology. The rat strain was named SD-Gcdhem1Dba. Methods: Weight gain, food intake and different blood parameters were evaluated in wild type SD (WT) and homozygous SD-Gcdhem1Dba (KI) rats at the age of 6 weeks under normal diet (ND) and after 21 days of high lysine diet (HLD). N=4 for each condition. Results: No significant differences in weight gain, food intake and BMI were observed between KI and WT rats under ND. When exposed to HLD, KI rats showed highly diminished food intake resulting in highly decreased weight gain and moderate reduction of BMI. In comparison to WT rats, ammonium was highly increased in blood of KI rats under ND and mildly elevated after exposure to HLD. A concomitant decrease of urea concentrations was observed in KI rats. Glutamate increased significantly in WT rats challenged with HLD, while it remained stable in KI rats. A mild decrease of glutamine was observed in KI rats under HLD. Aminoadipate increased significantly in KI and WT rats under HLD. Interestingly, under HLD the concentrations of saccharopine did not increase in WT rats and even decreased in KI rats. Discussion: In our rat model for GA-I we observed a negative effect on appetite in KI rats challenged by HLD resulting in growth retardation and lower BMI. We further confirmed an increased ammonium production probably secondary to a combined effect of enhanced glutaminase reaction and inhibition of urea cycle. The observed increase of aminoadipate without significant increase of saccharopine in WT and KI rats under HLD may suggest that the increased lysine flux uses an alternative degradation pathway.

11. The transcriptional repressor Scrt1 regulates pancreatic $\beta$-cell proliferation and maturation

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Background: Glucose-induced insulin secretion, a unique feature of fully differentiated β-cells, is acquired only after birth and is preceded by a phase of intense proliferation. These events occurring in the neonatal period are critical for the establishment of an appropriate functional β-cell mass covering the insulin needs throughout life. However, key regulators of gene expression involved in the cellular reprogramming along maturation remain to be elucidated. Methods: This project addressed this issue by taking advantage of a new methodology called ATAC-seq permitting a fine genome-wide mapping of chromatin accessibility. ATAC-seq assay was used to compare open chromatin regions in newborn versus adult rat β-cells. These regions were then correlated with the expression profiles of mRNAs to unveil the regulatory networks governing functional β-cell maturation. Results: We obtained a genome-wide picture of chromatin accessible sites (~100'000) among which 10 % were differentially accessible during maturation. Half of these sites are in the proximity of genes displaying differential expression in newborn and adult rat islets. An enrichment analysis of transcription factor binding sites revealed that 35 transcription factors could explain these changes. While the importance of some of them, including REST, FoxO1 and JunB, is already known, the role of others remains to be determined. We focused on Scrt1 a transcriptional repressor whose expression is upregulated in adult islets. Downregulation of Scrt1 did not affect insulin secretion in response to glucose, but restored an elevated proliferation rate in adult β-cells, suggesting an involvement of this repressor in post-natal maturation. To further understand the role of Scrt1 in the regulation of the β-cell transcriptome, we performed an RNAseq on FAC-sorted β-cells from adult rats. Differential expression analysis between siScrt1 and control samples revealed 168 genes were significantly impacted (111 down-regulated and 57 up-regulated), including several genes related to proliferation and/or β-cell development (Notch1, Parp16, Ppp3r1, NFATc1 and NFATc2). Next, we compared genes affected by Scrt1 silencing and the ones differentially expressed upon maturation (in postnatal P10 versus adult rat islets) and found a set of 62 genes changing in both data set. Interestingly, a significant anti-correlation (correlation test p-value = 0,013) was observed between the fold-changes from the comparison between siScrt1 versus siCtl in adult rat beta-cells and P10 versus adult islets. Conclusions: In the present study, the comparison of open chromatin sites between newborn and adult rat islets using ATAC-seq allowed us to found several known and unforeseen key transcriptional regulators acting at cis-regulatory sites during β-cell maturation. Among them, we could identify Scrt1, an important transcriptional repressors





implicated in the switch between the proliferative and the functional state of β-cells along pancreatic islet maturation.

12. The Role of Monocarboxylate Transporter-1 in Rescuing the Brain from Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) is a major complication of obesity. Certain observations regarding NAFLD induced neuropsychiatric and neurochemical alterations have been reported but mechanisms are unknown. In this context, monocarboxylate transporter-1 (MCT1) haploinsufficient mice, which resist high fat diet (HFD) induced hepatic steatosis represent an interesting model. Using a mouse model of NAFLD (HFD+high fructose/high glucose in water [HF/HG]) we investigated the development of cognitive deficits and state of cerebral oxygenation and cerebrovascular reactivity. Behavioural tests (open field [OF]/novel object recognition/forced swimming test [FST], morris water maze) were performed in mice fed control diet (NC; WT+NC, MCT1+/-+NC) or HFD HF/HG (WT+HFD HF/HG, MCT1+/-+HFD HF/HG) for 16 weeks. Baseline PO2 (in somatosensory cortex) and in response to systemic hypercapnia (10% CO2) was monitored under anaesthesia by a fluorescence method. Microelectrode biosensors were used for measurements of lactate release by cortical slices. EchoMRI was performed to assess lean/fat mass. Increased fat mass (not lean mass) was observed in WT and MCT1+/- mice (50% less) on HFD HF/HG compared to NC controls. Liver mass was only significantly higher in WT+HFD HF/HG mice compared to NC controls. Behavioural tests did not reveal any significant differences between groups except for FST and OF, which indicated an anxiety and depression-related behaviour in the WT+HFD HF/HG group compared to their controls. This was not observed with MCT1+/-+HFD HF/HG mice. WT+HFD HF/HG mice had a lower cerebral PO2 baseline with preserved PO2 response induced by systemic hypercapnia compared to NC controls, while the MCT1+/- groups remained unchanged. Tonic lactate release was unaltered between all groups although the MCT1+/-+HFD HF/HG group indicated a trend of decreased lactate tone. Our results suggest that NAFLD is associated with an anxiety and depression-related behaviour and decreased cerebral PO2 baseline. MCT1 haploinsufficient mice were resistant to the reported phenotypes, suggesting a link between liver metabolism and neuropathophysiological alterations in NAFLD.

13. Lipid control of anoxic death: 1-deoxydihydroceramide causes ischemia-reperfusion injury by impairing chaperonin-mediated protein folding

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Ischemic heart disease and stroke are the most common causes of death world-wide. Anoxia, the lack of oxygen, is common to both these pathologies and triggers profound metabolic and cellular changes. Sphingolipids have been implicated in anoxia injury, but the pathomechanism is unknown. Here we show that anoxia-associated injury causes accumulation of the non-canonical sphingolipid, 1-deoxydihydroceramide (DoxDHCer). Anoxia causes an imbalance between serine and alanine resulting in a switch from normal serine-derived sphinganine biosynthesis to non-canonical alanine-derived 1-deoxysphinganine. 1-deoxysphinganine is incorporated into DoxDHCer which impairs actin folding via the cytosolic chaperonin TRiC, leading to growth arrest in yeast and increased cell death upon ischemia-reperfusion injury in worms and mouse hearts. Prevention of DoxDHCer accumulation in worms and in mouse hearts resulted in decreased anoxia-induced injury. These findings unravel key metabolic changes during oxygen deprivation and point to novel strategies to avoid tissue damage and death.

14. Metabolic reprogramming of antitumor CD8 T cell immunity

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Adoptive cell transfer (ACT) therapies are successfully used in the clinic, however a large fraction of patients remains unresponsive, and the therapeutic effectiveness of the responding fraction can be improved. The limited efficacy of this therapy is mainly due to the terminally differentiated state of transferred T cells, which limits their proliferation and long-lasting antitumor response. Memory CD8+ T cells display specific phenotypic and functional characteristics endowing them with the ability to provide a more potent and long-lasting antitumor immune response than their terminally differentiated counterparts. The development and fitness of memory T cells was recently shown to be associated with specific metabolic pathways. We aimed to metabolically reprogram CD8+ T cells in order to generate fitter memory-like T cells prior to ACT. To do so, we performed a pharmacological inhibition of the metabolic enzyme isocitrate dehydrogenase 2 (IDH2) during the priming of CD8+ T cells. We have found that IDH2 inhibition during T cell activation led to an increased memory formation and to an enhanced tumor growth inhibition upon ACT into melanoma tumor-bearing mice. Interestingly, IDH2 inhibition was associated with increased histone methylation and acetylation. These histone modifications were required to induce the observed memory phenotype, since the concurrent treatment of metabolically reprogrammed cells with a histone acetyltransferase inhibitor abrogated the phenotypic changes and the





enhanced antitumoral function induced by single agent metabolic inhibition. We hypothesize the metabolic intervention resulted in altered levels of metabolic intermediates used in epigenetic modifications, such as ⊠-ketoglutarate and acetyl-CoA. These results suggest a novel strategy to promote stable memory T cell differentiation by epigenetic processes induced by metabolic reprogramming during T cell priming. These findings might be exploited to optimize ACT immunotherapy against cancer.

15. Automated analysis of large-scale NMR data generates metabolomic signatures and links them to candidate metabolites

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Identification of metabolites in large-scale 1H NMR data from human biofluids remains challenging due to the complexity of the spectra and their sensitivity to pH and ionic concentrations. In this work, we tested the capacity of three analysis tools to extract metabolite signatures from 968 NMR profiles of human urine samples. Specifically, we studied sets of co-varying features derived from Principal Component Analysis (PCA), the Iterative Signature Algorithm (ISA) and Averaged Correlation Profiles (ACP), a new method we devised inspired by the STOCSY approach. We used our previously developed metabomatching method to match the sets generated by these algorithms to NMR spectra of individual metabolites available in public databases. Based on the number and quality of the matches, we concluded that ISA and ACP can robustly identify ten and nine metabolites, respectively, half of which were shared, while PCA did not produce any signatures with robust matches.

16. Hyperpolarised L-[1-$^{13}$C] Lactate as a Neuroprotectant and Metabolic Biosensor for Ischemic Stroke in Mice

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Stroke is the second most common cause of death and the third leading cause of disability worldwide. Approximately 85% of strokes are of the ischemic subtype that can be treated by restoring blood flow through thrombolysis or thrombectomy within a relatively narrow time window of 4.5 - 7.3 h after ischemic onset. Neuroprotective strategies applied in the acute phase of ischemic stroke could improve patient recovery. Lactate is one of the most common metabolites in the body, and was considered as a waste product of metabolism. In the past three decades, growing evidence has highlighted the role of lactate as brain energy supply. It is well known that endogenous lactate increases dramatically after ischemia. Surprisingly, lactate administration

following reperfusion from ischemic stroke was found to provide neuroprotection in preclinical studies, reducing brain cell death and improving the neurological outcome by a mechanism involving both metabolism and signalling. The recently developed hyperpolarized (HP) magnetic resonance (MR) technique enables to investigate real-time in vivo metabolic transformations at a second time scale. In this study, we develop and apply this new HP MR technique in order to follow in real time the evolution of the therapeutic lactate bolus on a mouse model of transient ischemic stroke. Distinct metabolic kinetics in the acute phase of stroke were observed between ischemic and healthy brain, which could be linked to changes in the blood brain barrier permeability, rate of LDH exchange, as well as mitochondrial activity. This suggests that lactate could be used as a HP MR molecular imaging contrast for stroke. Combined with its neuroprotective effect, HP MR of lactate could be a promising future theranostic approach for stroke.

17. Identifying gene function and module connections by the integration of multi-species expression compendia

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The functions of many eukaryotic genes are still poorly understood. Here we developed and validated a new method, termed GeneBridge, which is based on two linked approaches to impute gene function and bridge genes with biological processes. First, Gene-Module Association Determination (G-MAD) allows the annotation of gene function. Second, Module-Module Association Determination (M-MAD) allows predicting connectivity among modules. We applied the GeneBridge tools to large-scale multi-species expression compendia—1,700 datasets with over 300,000 samples from human, mouse, rat, fly, worm, and yeast—collected in this study. G-MAD identifies novel functions of genes, for example WDFY4 in T cell activation, and also suggests novel components for modules, for example for cholesterol biosynthesis and mitochondria. By applying G-MAD on datasets from respective tissues, tissue-specific functions of genes were identified, for instance the roles of EHHADH in liver and kidney, as well as SLC6A1 in brain and liver. Using M-MAD, we identified a list of module-module associations, such as those between mitochondria and proteasome, mitochondria and histone demethylation, as well as ribosomes and lipid biosynthesis. The GeneBridge tools together with the expression compendia are available at systems-genetics.org, to facilitate the identification of connections linking genes, modules, phenotypes, and diseases.

18. Opposing action of NCoR1 and PGC-1$\alpha$ in mitochondrial redox homeostasis

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The ability to respond to fluctuations of reactive oxygen species (ROS) within the cell is a central aspect of mammalian physiology. This dynamic process depends on the coordinated action of transcriptional factors to promote the expression of genes encoding for antioxidant enzymes. Here, we demonstrate that the transcriptional coregulators, PGC-1$\alpha$ and NCoR1, are essential mediators of mitochondrial redox homeostasis in skeletal muscle cells. Our findings reveal an antagonistic role of these coregulators in modulating mitochondrial antioxidant induction through Sod2 transcriptional control. Importantly, the activation of this mechanism by either PGC-1$\alpha$ overexpression or NCoR1 knockdown attenuates mitochondrial ROS levels and prevents cell death caused by lipid overload in skeletal muscle cells. The opposing actions of coactivators and corepressors, therefore, exert a commanding role over cellular antioxidant capacity.

19. In vitro generation of functionally mature beta-cells from adult human iPSCs

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Islet transplantation has demonstrated that replacement of the beta-cell mass in diabetic patients is able to restore endogenous glycaemic control, but it is currently limited by the shortage of available donor tissue. Stem cell therapies hold great promise for generating a replenishable supply of insulin producing beta-cells for transplantation. Despite the progress achieved over the last decade, existing in vitro beta-cell differentiation methods require refinement regarding efficiency and cell maturation. In the present studies, we have used human iPSCs to generate functionally mature beta-cells in vitro. This population can be enriched by FACS sorting up to 50% of beta-cells in a scalable 3D culture system. The in vitro generated beta-cells display mature features including insulin content close to that of bona fide beta-cells, 95% proinsulin processing, Pdx1, Nkx6.1 and MafA expression, calcium-dependent insulin release and mature insulin granules. Furthermore, the in vitro differentiated beta-cells exhibit glucose regulated insulin secretion, displaying the first and second insulin release phases characteristic of adult islets. Following transplantation into immunocompromised mice, human C-peptide was detected 2 weeks post-implantation and graft functionality was sustained for 20 weeks. These findings pave the way for the generation of in vitro beta-cell models for personalised medicine strategies to improve metabolic health.

20. The role of glutamate dehydrogenase in hyperammonemia of HI/HA syndrome: study on the contribution by the liver

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Clinically, the hyperinsulinism/hyperammonemia syndrome (HI/HA) is characterized by elevated plasma ammonia levels accompanied by severe hypoglycaemia and epilepsy in about 40% of cases. It is a rare genetic disease caused by gain-of-function mutations in the GLUD1 gene encoding for the enzyme glutamate dehydrogenase (GDH). Mammalian GDH catalyses the reversible reaction of glutamate to $\alpha$-ketoglutarate ($\alpha$-KG) plus ammonia. HI/HA syndrome gives rise to increased (3-5 times) plasma ammonia levels, presumably due to systemic expression of mutant GDH. To explore the effects of GDH-S445L mutation on hepatic ammonia metabolism, we transduced in vivo liver-specific GDH knockout mice with adenoviruses carrying human mutant GDH. We performed challenges of gluconeogenic amino acids, both in vivo and ex vivo, in order to assess ammonia homeostasis. This study aims to elucidate the contribution of the liver in the elevated circulating ammonia levels associated with the GDH-S445L mutation. We used tamoxifen induced liver specific GDH null mice (Hep-Glud1-/-, Karaca et al. Diabetes 2018) for in vivo expression of human mutant GDH following retroorbital injection of 10^9 PFU of adenovirus (Ad-GDHmut). Control Glud1fl/fl floxed mice were injected with saline. The efficiency of transduction was controlled by immunoblotting on liver extracts. After an overnight fast, amino acid-induced gluconeogenesis was stimulated by i.p. glutamine and alanine challenges. To assess the systemic turnover of ammonia, blood samplings at different sites of the vasculature were performed (abdominal aorta, portal vein, hepatic vein, renal vein) and ammonia, urea and glutamate levels were determined. Immunoblotting indicated that the levels of Ad-GDHmut expression in the liver of Hep-Glud1-/- mice accounted for approx. 70% of endogenous Glud1fl/fl control mice. Alanine and glutamine are important substrates for gluconeogenesis. The ammonia production from glutamine is contributed by deamidation to glutamate by glutaminase and subsequent deamination to $\alpha$KG by GDH. However, alanine solely relies on GDH for ammonia production. Upon in vivo amino acid challenges, there was no difference in glucose production between Glud1fl/fl control and Hep-Ad-GDHmut. Although urea concentrations were stable throughout the study, circulating glutamate increased in Hep-Ad-GDHmut, up to 350% of the values of control mice. The efficiency of ammonia clearance from the portal vein was substantially decreased in Hep-Ad-GDHmut versus control mice (-83%, $p<0.05$). In parallel, ex vivo challenge with glutamine and alanine in perfused liver indicated ammonia intolerance in Hep-Ad-GDHmut mice at the expense of urea production. Our study shows that Hep-Ad-GDHmut livers were less efficient at ammonia disposal in vivo. Ex vivo, Hep-Ad-GDHmut livers acutely challenged with amino acid were ammonia intolerant. Overall, GDH-S445L mutation impaired the efficiency of hepatic nitrogen disposal.
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Generating an endogenous reporter mouse to study lipid droplets in neural stem cells
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Abstract: Lipid metabolism plays an important role in the regulation of neural stem cells (NSCs): Build-up of lipids through de novo lipogenesis seems to be crucial for NSC proliferation (Knobloch et al. 2013), whereas the break-down of lipids via fatty acid beta-oxidation has recently been shown to regulate NSC quiescence (Knobloch et al 2017). Lipid droplets (LDs) are the intracellular organelles that store lipids, mainly triacylglycerols and sterol esters. LDs have classically been seen as inert storage organelles, however studies over the recent years have revealed them as highly dynamic organelles that fulfil crucial metabolic functions. How LDs are involved in the regulation of adult NSCs is currently poorly understood. We are investigating the role of LDs in NSCs during development and in adulthood, both in vitro and in vivo. As LDs are very sensitive to the common staining methods used for visualization, we are using CRISPR/Cas9 to create an endogenous LD reporter mouse. This mouse will allow us to visualize LDs in a staining-free manner and will also enable us to follow LD dynamics using live imaging. Here we present the target selection, validation and tagging approach for this novel endogenous LD reporter mouse.

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A role for AMPK in hypothalamic glucose sensing
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Abstract: Glucose homeostasis relies on accurate and continuous assessment of glycemia. This is accomplished in part through glucose sensing mechanisms in specific neuronal populations in the central nervous system. One brain region containing neurons capable of glucose sensing is the ventromedial hypothalamic nucleus (VMN). In that region, a subpopulation of neurons expressing the steroidogenic factor 1 (SF1) is mostly composed of glucose sensing cells : 47% of them are glucose-excited (GE), 25% are glucose-inhibited (GI) and 28% are non-responders. It was hypothesized that in these neurons, AMP-activated protein kinase (AMPK), a kinase activated upon metabolic stress, is responsible for glucose sensing. In this work, we show that removal of AMPK activity leads to the loss of glucose sensing ability in GI neurons but not in GE neurons. We developed a Translating Ribosome Affinity Purification assay (TRAP) in order to specifically purify RNAs from SF1 neurons and evaluate the transcriptional consequences of abolishing AMPK activity. Upon removal of AMPK, several genes are altered. Notably, a gene implicated in ROS detoxification, Txn2, was downregulated. We show that even the in absence of AMPK activity, reestablishment of Txn2 expression is sufficient to restore GI function in SF1 neurons of the VMN.

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Investigation of a Physiological Role of the Skeletal Muscle-Selective AMPK $\gamma 3$ isoform
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Abstract: Background : AMPK consists of the catalytical $\alpha$-subunit and two regulatory subunits ( $\beta$, $\gamma$ ) with seven existing isoforms ( $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$ and $\gamma 3$). AMPK heterotrimers show a tissue-specific distribution with some $\beta 2$ complexes such $\alpha 2 \beta 2 \gamma 1$, $\alpha 1 \beta 2 \gamma 1$ and $\alpha 2 \beta 2 \gamma 3$ being predominantly expressed in skeletal muscle. The $\gamma 3$ subunit appears to be selectively expressed in skeletal muscle fibres and was shown to have a major impact on carbohydrate as well as lipid metabolism. Moreover, $\alpha 2 \beta 2 \gamma 3$ was shown to be primarily activated through exercise in humans. Despite this unique and key role for $\gamma 3$ in metabolic processes in skeletal muscle, molecular mechanism by which $\gamma 3$ isoform-containing complexes is regulated by exercise and drugs is elusive. Methods: To gain more insight on the $\gamma 3$ isoform-specific regulation on glucose metabolism, we used a $\gamma 3$ KO mouse model. We used ex vivo incubation of isolated skeletal muscles (glycolytic EDL and oxidative soleus muscles) to assess glucose uptake and AMPK activity/signaling in response to small-molecule AMPK activators (991 and AICAR). Results: We found that AICAR- and 991- stimulated glucose uptake was robustly blunted in EDL, but not in soleus muscle, and that this was at least partially associated with decreased $\alpha 2$ activity. Notably, downstream phosphorylation of TBC1D1, a RabGAP known to be involved in GLUT4 traffic, was decreased in drug-stimulated EDL, which may add another layer of understanding of the role of AMPK $\gamma 3$ in glucose uptake. Conclusions: We found that decreased drug-stimulated glucose uptake in EDL muscles may be linked to TBC1D1 phosphorylatation, making TBC1D1 one of the key targets for investigation of AMPK-mediated glucose uptake. Further insights into phosphorylation sites and other targets will shed light onto the physiological functions of skeletal muscle AMPK in glucose homeostasis.

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WeCare Project: Development of a new generation of cognitive and non-invasive wearable devices for continuous sweat biomonitoring





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Abstract: Wearable sensors have recently met considerable interest, with promising applications, particularly with biofluids like sweat. WeCare project aims to develop a new generation of non-invasive wearable sweat biomonitoring device to improve our knowledge about thermoregulation physiology and hydration management during exercise. WeCare is a Swiss National Science Foundation (SNF) granted project (Sinergia) over a period of 4 years. It brings together the Institute of Neuroinformatics from the University of Zurich and ETH Zurich, expert in the field of neuromorphic engineering and event-driven Deep Neural Networks; the Soft Transducers Lab of EPFL, specialized in printed electronics; the Instituto de Microelectrónica de Barcelona, a reference European micro and nano technology facility for the design, integration and characterization of biochemical smart systems, and finally the Sports Medicine Center of the University Hospital of Lausanne (CHUV). To achieve goals of the project, the four main partners (representing a total of 16 investigators) work closely together according to their expertise through meetings and multidisciplinary exchanges. The CHUV Sports Medicine Center elaborates different study protocols with healthy athletes, to assist in the development of the device and for scientific purposes. Many challenges exist to get an effective monitoring tool, such as sweat collection and analysis, wearability, real-time transmission of information to a mobile with friendly interface, machine learning models and obviously interpretation of data. In conclusion, WeCare fosters an innovative and collaborative line of research and technology development to construct personalized models of physiological states in sports practice by use of the developed wearable device for continuous sweat biomonitoring.

25.
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The role of Agpat5 in mitochondrial function and glucose sensing in murine hypothalamic neurons
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Abstract: Glycaemia is maintained in a very narrow range by a series of hormonal and neuronal responses. In particular, the counterregulatory response to hypoglycemia, which is triggered by hypothalamic nuclei, restores blood glucose concentrations by stimulating glucagon secretion and hepatic glucose release. While hypoglycaemia is rare in healthy individuals, it is a frequent adverse effect of insulin therapy in diabetes mellitus. The increased risk of hypoglycaemia is related to impaired glucagon secretion due to abnormal counterregulatory response. The molecular basis for impaired hypoglycaemia sensing in not known. Our laboratory has previously performed a genetic screen in recombinant inbred BDX mice aimed at identifying quantitative trait

loci (QTL) and hypothalamic genes that control glucagon secretion in response to insulin-induced hypoglycaemia. Combining QTL analysis and RNASeq analysis of the hypothalamus of the BXD mice, we identified Agpat5, located on Chr 8, as a potential novel regulator of glucagon secretion. Agpat5 codes for an enzyme that catalyses the conversion of lysophosphatidic acid (LPA) into phosphatidic acid (PA) in mitochondria and endoplasmic reticulum. PA has been previously shown to alter mitochondrial dynamics by inhibiting mitochondrial fission. Previous studies have shown the balance between mitochondrial fission and fusion constitutes an important factor in regulating glucose sensing in hypothalamic neurons. Moreover, Agpat5 is a part of lipid synthesis pathway that was previously implied to be competing with fatty acid beta-oxidation (FAO) in mitochondria. Analysis in cell lines showed that siRNA-mediated Agpat5 knockdown (KD) increases mitochondrial respiration and glycolytic rate in GT1-7 cells (murine neuronal cell line) without altering total mitochondrial mass or mitochondrial reactive oxygen species production. Moreover, Agpat5 KD increased ATP levels in GT1-7 cells incubated with 0.1mM glucose, 1mM sodium pyruvate and 2mM L-glutamine serum-free DMEM without fatty acids or sodium pyruvate, suggesting that Agpat5 KD pyruvate utilisation and/or endogenous FAO in mitochondria are increased. In addition, when incubated with 5mM glucose DMEM medium Agpat5 KD showed a trend towards an elevated lactate secretion in culture medium. Increased mitochondrial respiration observed in Agpat5 KD model seems to be partially due to an increased FAO, as this effect was abolished by etomoxir (FAO inhibitor) treatment. We hypothesise that Agpat5 regulates hypothalamic glucose sensing through altering mitochondrial dynamics and/or altering mitochondrial FAO in neurons. In vivo, insulin-induced hypoglycaemia activates (c-fos immunodetection) neurons of the paraventricular nucleus of the hypothalamus (PVN) and NPY neurons of the arcuate nucleus (ARC), suggesting that these areas are involved in hypoglycaemia-stimulated glucagon secretion. Therefore, in order to investigate the role of Agpat5 in neuronal activity in vivo, we are generating Sim1-Cre; Agpat5flox/flox and AgRP-Cre; Agpat5flox/flox mice to knockout Agpat5 in the PVN and ARC nuclei, respectively.

26.
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Biomimetic models recapitulate bone marrow adipocyte subtypes relative to lipid composition and hematopoietic support

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Abstract: Two distinct types of adipocytes have been identified in the bone marrow (BM). The labile BM Adipocytes (BMAds) interspersed within the hematopoietic marrow of the proximal





skeleton are small in size and high in saturated lipids, whereas the stable BMAds in the non-hematopoietic marrow of the distal skeleton are larger in size with a high proportion of unsaturated lipids. Since mass spectrometric analysis revealed triacylglycerols to be the major component of primary human and murine BMAds, we hypothesized that detailed analysis of their lipid profiles would reveal further insight in the elusive relationship between stable and labile BMAds. We have been able to quantify BMAd skeletal distribution in relationship to hematopoietic activity with MRI in vivo, however due to their small size the resolution in mouse is limiting. Raman microspectroscopy represents a non-invasive and label-free method providing information on molecular composition at the micrometer scale that we applied to BM derived adipocytes in vitro. Lipid composition was assessed through either a classical induction of adipogenesis or through spontaneous adipogenesis after 17 days in culture, with molecular imaging that allowed for acquisition of spectra at the single droplet level. We found that classical adipogenic induction led to preferential accumulation of unsaturated lipid species as opposed to high saturation ratios in spontaneously differentiated BMAds, which corresponded to HPLC analysis. Moreover, in a biomimetic co-culture model we were able to reproduce the nuances of the labile and stable BMAds in terms of differential hematopoietic support capacity. Thus we conclude that raman microspectroscopy is a powerful tool for single cell analysis of BMAds, and we postulate that the observed saturation differences in BMAds may reveal a stepwise adipogenic maturation that could be important in the context of hematopoiesis.

27.
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Exploring Metabolome & Lipidome: From model systems to human population studies

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Abstract: Metabolomics (comprising Lipidomics) represents the final piece of the ‘omics puzzle. The metabolome (including lipidome) encompasses small molecule - metabolites that in addition of being downstream products of gene and protein activity also have a far-reaching activity in the regulation of gene and protein expression and biological processes in general. Information contained in metabolites provides a direct, sensitive and dynamic read out of the cellular activity, i.e. phenotype at the molecular level. Metabolomics has evolved into the high-throughput technology to complement the multi-scale data acquisition afforded by genomics, transcriptomics and proteomics, towards systems biology approach. Here, we present approaches that the platform offers for the analysis of model systems to clinical, human population studies.

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