Tuesday May 15, 2018
CHUV, César Roux Auditorium, BH 08
Rue du Bugnon 46, CH-1011 Lausanne

11th LIMNA Symposium

Organizing committee: Prof. Johan Auwerx, Prof. Bart Deplancke, Prof. Béatrice Desvergne, Prof. Lluis Fajas, Prof. Nelly Pitteloud, Prof. Bernard Thorens, Prof. Kei Sakamoto, Prof. Kristina Schoonjans and Dr. Laurence Descamps.

Guest organizers: Judit Castillo Armengol (UNIL), Caterina Collodet (NIHS), Mukul Girotra (UNIL), Mary Gonzalez Melo (CHUV), Elena Katsyuba (EPFL), Anita Nasrallah (UNIL), Olha Novokhatska (EPFL), Anna Surowska (UNIL), Laura Velazquez (EPFL), Vera van der Velpen (UNIL).

Invited Speakers

- **Prof. Béatrice Desvergne,** Center for Integrative Genomics, UNIL, Lausanne, Switzerland.

- **Prof. Katrien De Bock**, Laboratory of Exercise and Health, Institute of Movement Sciences, ETH Zürich, Switzerland.

Agenda

8h30-9h00 Welcome and distribution of badges

Opening

9h00
Welcome

Morning session

Chairman: Vera van der Velpen

9h10 **Béatrice Desvergne**
Why sex matters…

9h45 **Vasco Campos**
PhD, Laboratory of Prof. Olaia Naveiras, ISREC EPFL and CHUV
High-throughput, non-perturbing quantification of lipid droplets using Digital Holographic Microscopy

10h05 **Alessia Perino**
Post-Doc, Laboratory of Prof. Kristina Schoonjans, Laboratory of Metabolic signaling, EPFL
Regulation of extracellular matrix assembly by TGR5 is essential for normal bone homeostasis

10h25 Coffee Break

Chairman: Anita Nasrallah

10h45 **Mukul Girotra**
Post-Doc, Laboratory of Prof. George Coukos, Ludwig Institute for Cancer Research, UNIL
Specification of hematopoietic stem cell fate via modulation of mitochondrial activity

11h05 **Mathias Wenes**
Post-Doc, Laboratory of Prof. Pedro Romero, Department of oncology, UNIL, CHUV
Mitochondrial pyruvate carrier inhibition during CD8 T cell priming enhances central memory differentiation and anti-tumoral activity

11h25 **Laia Martinez Carreres**
PhD, Laboratory of Prof. Lluis Fajas, Center for Integrative Genomics, UNIL
The role of CDK4 in lysosomal biology

11h45 **Xu Wang**
Post-Doc, Laboratory of Prof. Johan Auwerx, Laboratory of Integrative Systems Physiology, EPFL
Systems phytohormone responses to mitochondrial proteotoxic stress

12h05 Lunch

Afternoon session

12h50 Poster session

Chairman: Caterina Collodet

14h30 **Katrien De Bock**
Metabolism in the muscle microenvironment

15h05 **Dogan Grepper**
PhD, Laboratory of Prof. Francesca Amati, Department of Physiology & Institute of Sport Sciences, UNIL
A novel human skeletal muscle cell line developed for metabolism investigations

15h25 **Nadège Zanou**
Post-Doc, Laboratory of Prof. Bengt Kayser, Department of Physiology & Institute of Sport Sciences, UNIL
High intensity interval training (HIIT)-induced Ca2+ leak through RyR1 channel is involved in mitochondrial plasticity in skeletal muscle

15h45 Coffee break

Chairman: Laura Velazquez

16h05 **Maria Marques de Lima**
Post-Doc, Laboratory of Marine Kraus, Nestlé Institute of Health Sciences
In vitro generation of functionally mature beta-cells from adult human iPSCs

16h25 **Alexandre Picard**
Post-Doc, Laboratory of Prof. Bernard Thorens, Center for Integrative Genomics, UNIL
Fgf15 neurons of the dorsomedial hypothalamus regulate glucose homeostasis

16h45 Concluding remarks and prizes distribution

ABSTRACTS SELECTED FOR A SHORT TALK

Campos Vasco

Affiliation: EPSL-SV-IBI

Title of presentation: High-throughput, non-perturbing quantification of lipid droplets using Digital Holographic Microscopy

Authors list: Vasco Campos, Benjamin Rappaz, Fabien Kuttler, Gerardo Turcatti, Olaia Naveiras

Abstract: Introduction: In vitro differentiating adipocytes are sensitive to liquid manipulations and have the tendency to float. Assessing adipocyte differentiation using current microscopy techniques involves cell staining and washing, while using flow cytometry involves cell retrieval in suspension. These methods induce biases, are difficult to reproduce and involve tedious optimizations. Methods and Results: In this study we present Digital Holographic Microscopy (DHM, Lyncée Tec) as a label-free, non-perturbing means to quantify lipid accumulation in differentiating adipocytes in a robust medium- to high-throughput manner. Taking advantage of the high refractive index of lipids, DHM can assess the production of intracellular lipid vesicles by differences in phase shift in a quantitative manner. The adipocytic differentiation together with other morphological features (cell confluency and cell death) of live, bone marrow-derived OP9 mesenchymal stromal cells was tracked over 6 days. We compared DHM with other currently available methods of lipid quantification and demonstrated its robustness with modulators of adipocytic differentiation in a dose-response manner. Moreover, we have successfully tested DHM-based lipid quantification on a medium-to-high throughput platform and have so identified novel inductors and inhibitors of bone marrow adipogenesis, which are currently under validation. Conclusion and Significance: Taken together, this work proposes DHM as a novel marker-free non-perturbing method to study lipid accumulation and may be envisioned for drug screens and mechanistic studies on adipocytic differentiation.

Perino Alessia

Affiliation: SV IBI-SV UPSCHOONJANS - EPFL

Title of presentation: Regulation of extracellular matrix assembly by TGR5 is essential for normal bone homeostasis.

Authors list: Alessia Perino, Lieve Verlinden, Laura Velazquez-Villegas, Andréane Fouassier, Giovanni Sorrentino, Vera Lemos, Ulrike Kettenberger, Dominique Pioletti, Geert Carmeliet, Kristina Schoonjans

Abstract: Age- and hormone-related osteoporosis is one of the primary causes of bone fracture and reduced mobility. In recent years, the bone has emerged from a structural component to a dynamic metabolic organ in which resorption and formation are maintained in a highly regulated equilibrium. Dysbalance of these mechanisms results in osteoporosis. Bile acids (BAs) are hormone-like molecules that regulate multi-organ homeostasis through the activation of both nuclear and membrane receptors. BAs are rhythmically released in the systemic circulation upon food intake and can virtually reach every tissue in the body, including the bone. Here, we investigated the role of the BA membrane receptor TGR5 in the regulation of bone homeostasis. Global TGR5 knockout (Tgr5-/-) mice displayed a striking loss of bone mass already at young age. This was associated with a reduction in osteoblast markers and bone mineralization, despite an increase in non-mineralized bone matrix. The osteopenic phenotype observed in Tgr5-/- mice was recapitulated in mice with a targeted deletion of Tgr5 in the osteoblast lineage (Tgr5-Runx2-/- mice). In line with these findings, differentiation of both neonatal and adult mesenchymal stem cells derived from Tgr5-/- mice failed to express the typical osteoblast signature and instead showed an increase in adipocyte markers. TGR5 seemed furthermore to be required for the formation of a proper extracellular matrix since collagen maturation was impaired in whole body Tgr5-/- and Tgr5-Runx2-/- bones. In summary, our findings indicate that TGR5 is required for the preservation of proper osteoblast function. Further studies are planned to evaluate the therapeutic value of BAs or synthetic TGR5 agonists in the intervention or prevention of hormone-related bone loss.

Girotra Mukul

Affiliation: LICR, UNIL

Title of presentation: Specification of hematopoietic stem cell fate via modulation of mitochondrial activity

Authors list: Mukul Girotra, Marcela Rincon-Restrepo, Caroline Monnard, George Coukos, Serge Rezzi, Nicola Vannini

Abstract: A tight control of hematopoietic stem cell (HSC) fate is crucial for lifelong blood production. Therefore, a fine balance of quiescence, self-renewal, and differentiation is key to maintain the HSC pool and blood homeostasis. In recent years cellular metabolism has emerged as a crucial regulator of HSC fate. HSCs differ from their committed progeny by relying primarily on anaerobic glycolysis rather than mitochondrial oxidative phosphorylation for energy production. However, whether this change in the metabolic program is the cause or a consequence of the unique function of HSCs remains unknown. We previously demonstrated that modulation of mitochondrial metabolism affects HSC fate, by chemically uncoupling the electron transport chain we were able to maintain HSC function in culture conditions that normally induce rapid differentiation (Vannini N, Girotra M. et al., Nat Comm 2016). Moreover, we demonstrated that modulation of mitochondrial activity in ex-vivo cultured human HSCs, via NAD+ boosting agent Nicotinamide Riboside (NR), results in better long-term blood production in serially transplanted humanized mice (Under Revision). Here we proceeded to carry out a mini screen, using mitochondrial activity as readout, to identify novel metabolic modulators that can be used to enhance HSC activity and function. Our data thus reveal a causal relationship between mitochondrial metabolism and fate choice of HSCs, and also provide a valuable tool to identify optimal ex vivo conditions for HSC expansion and improve the outcome for patients suffering from bone marrow insufficiency.

Wenes Mathias

Affiliation: Department of fundamental oncology

Title of presentation: Mitochondrial pyruvate carrier inhibition during CD8 T cell priming enhances central memory differentiation and anti-tumoral activity

Authors list: Mathias Wenes; Lianjun Zhang; Nina Dumauthioz; Ping-Chih Ho; Pedro Romero

Abstract: T cell fate is tightly linked with specific metabolic characteristics. We have found that inhibiting the mitochondrial pyruvate carrier with the small molecule UK5099 during CD8 T cell priming, unexpectedly led to an increase in mitochondrial oxygen consumption, driven by fatty acid oxidation. This metabolic adaptation was accompanied by an increased surface expression of the central memory marker CD62L. Adoptive transfer of UK5099-treated CD8 T cells into melanoma tumor-bearing mice, resulted into a better tumor control compared to DMSO-treated cells. A much higher proportion of adoptively transferred UK5099-treated CD8 T cells formed central memory cells. Furthermore, upon mitochondrial pyruvate carrier inhibition, T cells infiltrating the tumor were characterized by a reduced PD-1 expression and increased cytokine production following in vitro restimulation. Thus, this study shows that metabolic adaptations induced during early CD8 T cell priming can lead to long-lasting central memory T cell differentiation, resulting in an increased anti-tumor control.

Martinez Carreres Laia

Affiliation: CIG-UNIL

Title of presentation: The role of CDK4 in lysosomal biology.

Authors list: Laia Martinez Carreres, Isabel-Cristina Lopez Mejía, Meritxell Orpinell, Julien Puyal, Lluis Fajas

Abstract: Cyclin Dependent Kinase 4 (CDK4) is a serine/threonine kinase that belongs to the CDK kinase family, which only exerts its function when associated to a Cyclin partner. When CDK4 forms a complex with the D-type cyclin in G1 phase of the cell cycle, is able phosphorylate protein targets such as the Retinoblastoma (Rb) protein. Phosphorylated Rb releases the transcription factor E2F1, which is then able to activate its target genes and hence drive the transition from the G1 to S phase of the cell cycle. Despite the role of CDK4 in the cell cycle progression has been extensively studied, work from our lab has provided extensive proof that CDK4 plays alternative but crucial roles in Insulin signaling pathways as well. In the present study, using MDA-MB-231 breast cancer cell line as a model, we show that CDK4 have a direct impact on mTORC1 pathway. CDK4 inhibition or depletion leads not only to mTORC1 inactivation, but also leads to an increase of lysosomal biogenesis, one of the processes that mTORC1 is regulating. We are currently identifying potential CDK4 phosphorylation targets in mTORC1 pathway. The discovery of this novel regulatory role for CDK4 will bring a better understanding of cancer metabolism, which in turn will help to open new perspectives for cancer therapies.

Wang Xu

Affiliation: LISP-SV-EPFL

Title of presentation: Systems phytohormone responses to mitochondrial proteotoxic stress

Authors list: Xu Wang, Johan Auwerx

Abstract: Mitochondrial function is controlled by two separate genomes. This feature makes mitochondria prone to proteotoxic stress in case a stoichiometric imbalance occurs in the protein complexes that perform oxidative phosphorylation (OXPHOS), which consist of both nuclear- and mitochondrial-encoded proteins. Such a proteotoxic stress is known to induce the mitochondrial unfolded protein response (UPRmt) in animals. It is unknown whether UPRmt occurs in plants. Here, we repressed mitochondrial translation in Arabidopsis, through chemical or genetic interference. Mitochondrial proteotoxic stress activated a plant-specific UPRmt and impaired plant growth and development. The retrograde signal is initiated by a fast oxidative burst, resulting in MPK6 activation, culminating in a systemic hormone response mainly reliant on ethylene signaling (but also involving auxin and jasmonate). This will activate an anterograde response that aims to repair mitochondrial translation (through the induction of the mitochondrial ribosomal proteins—MRPs) and mitochondrial protein import/folding, which will ultimately compensate for the decreased level of OXPHOS components that triggered the response. Our data not only highlight the universal nature of key features of mitonuclear stress signaling pathways, but also indicate specific effectors (mitokines vs. phytohormones) and transcriptional circuits (ATF4/5 vs. ERFs) that are divergent between the plant and animal kingdoms. In summary, our study ascertains that the network of mitochondrial protein quality control pathways is conserved in plants and that hormone signaling is an essential mediator that regulates mitochondrial proteostasis. Reference: Wang, X. & Auwerx, J. Molecular Cell 68: 540-551 (2017).

Grepper Dogan

Affiliation: Department of Physiology

Title of presentation: A novel human skeletal muscle cell line developed for metabolism investigations

Authors list: Dogan Grepper, Sylviane Lagarrigue, Sonia Conde Alonso, Francesca Amati, Aging and Muscle Metabolism Lab, University of Lausanne

Abstract: Cultured human skeletal muscle cells are an excellent model to study physiological and biological properties of human muscle. These cells provide the most relevant genetic background to investigate human disease and allow the study of pathologic mechanisms on molecular levels. Working with primary human skeletal muscle cells has its limitations. Donors for human muscle tissue are scarce and once the muscle cells are cultured their proliferative potential decreases within passaging due to telomere shortening, which leads to cellular senescence. To remove the restriction of the limited amount of human muscle tissue, the aim of this study was to develop an immortalized human skeletal muscle cell line. De-identified primary muscle cells of six healthy volunteers were transformed with the simian virus 40 (SV40). Transformed SV40 cells were compared to the primary muscle cells (D6 cells) to reveal the consequences of the virus transformation. Results show that the passaging number increased in SV40 cells compared to D6 cells. SV40 cells lost their capability to differentiate into multinuclear myotubes and are preserved as myoblasts. While SV40 cells are not mature in terms of contractile proteins (myosin heavy chains) after 8 days of differentiation, specific physiological and molecular properties are conserved such as insulin responsiveness and modifications of the electron transport chain. We propose this human muscle myoblast cell line as interest for metabolism investigations requiring human skeletal muscle models.

Zanou Nadège

Affiliation: DP/UNIL

Title of presentation: High intensity interval training (HIIT)-induced Ca2+ leak through RyR1 channel is involved in mitochondrial plasticity in skeletal muscle

Authors list: Nadège Zanou, Chris Donnelly, Haikel Dridi, Pau Gama Perez, Steve Reiken, Pablo Garcia-Roves, Andrew Marks, Bengt Kayser, Nicolas Place

Abstract: We recently found that, in recreationally trained volunteers, a single session of HIIT, High intensity interval training, (6 x 30s all-out cycling) induced fragmentation of the sarcoplasmic reticulum Ca2+ release channel (RyR1, ryanodine receptor type 1). In this study, we tested the hypothesis that Ca2+ leak through modified RyR1 (the Ca2+ release channel) in response to high intensity interval training (HIIT) exercise may trigger mitochondrial plasticity. We mimicked HIIT and endurance exercises in C2C12 myotubes using the C-Pace electrical device and observed RyR1 phosphorylation and release from calstabin (the small molecule stabilizing RyR1) and a lower sarcoplasmic reticulum Ca2+ content in response to HIIT, indicating that HIIT induced leaky RyR1. This was accompanied by greater mitochondrial biogenesis (NRF1, Tfam1, PGC1 expression), dynamics (OPA1 and DRP1 modifications) and respiration (O2 consumption in O2k Oroboros device). We confirmed these results on muscle biopsies from healthy humans submitted to HIIT exercise, as compared to a classical endurance exercise. Taken together, our results provide, for the first time, into insight of the mechanisms by which HIIT exercise is so efficient.

Maria Marques de Lima

Affiliation: Nestle Institute of Health Sciences

Title of presentation: In vitro generation of functionally mature beta-cells from adult human iPSCs

Authors list: Corinne Haller*, Maria João Lima*, Laura Sanchez Burgos, Thomas Robert, Umberto de Marchi, Isabelle Chareyron, Andreas Wiederkehr, Federico Sizzano, El Hadji Dioum, Daniel Pipeleers and Marine R-C Kraus

Abstract: Islet transplantation has demonstrated that replacement of the beta-cell mass in diabetic patients is able to restore endogenous glycaemic control. 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 beta-cell differentiation methods require refinement regarding efficiency and cell maturation. In the present studies, we report the generation of functionally mature beta-cells from human iPSCs. This population can be enriched to 50% of beta-cells in 3D culture. These newly 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 human islets. Following transplantation into immunocompromised mice, human C-peptide can be detected in the mice serum 2 weeks post-implantation. These findings pave the way for the generation of in vitro beta-cell models for personalised medicine strategies to improve metabolic health.

Picard Alexandre

Affiliation: CIG

Title of presentation: Fgf15 neurons of the dorsomedial hypothalamus regulate glucose homeostasis

Authors list: Alexandre Picard, Salima Metref, David Tarussio, Gwenaël Labouebe and Bernard Thorens Center for Integrative Genomics, University of Lausanne, 1015 Lausanne, Switzerland

Abstract: We identified through an unbiased genetic screen hypothalamic Fgf15 as a regulator of glucagon secretion (Picard et al. 2016). We have shown that i.c.v. delivery of Fgf15 human ortholog FGF19 decreased 2-deoxy-D-glucose (2DG) -induced glucagon secretion. That could be explained by a blunted vagal activation in response to 2DG. Moreover, shRNA-mediated silencing of Fgf15 in the dorsomedial hypothalamus (DMH) induced an increased in 2DG-induced glucagon secretion. These data demonstrate a role for Fgf15 in the DMH in the regulation of glucagon secretion through the regulation of parasympathetic activity. To specifically adress the role of Fgf15 expressing neurons in the regulation of glucose homeostasis, we generated a knock-in mouse model allowing for the expression of cre recombinase within the Fgf15 locus (Fgf15 cre mice). Stereotactical delivery of AAV-EF1a-DIO-EYFP within the DMH of Fgf15 cre mice revealed the presence of Fgf15 postive neurons in this nucleus. Further, we injected AAV-HSYN-DIO-HM3DQ-MCHERRY in Fgf15 cre mice, allowing the specific neuronal activation of Fgf15 neurons. Chemogenetic activation of Fgf15 neurons of the DMH impaired glucose intolerance with no change in insulin secretion. Further, activation of Fgf15 neurons of the DMH impaired both vagal activation and glucagon secretion in response to i.p. insulin. Electrophysiological analysis showed that a subset of these Fgf15 neurons were glucose sensing, with their activity beeing increased (glucose excited) or decreased (glucose inhibited) after a rise in extracellular glucose concentration. These first results show that Fgf15 neurons of the DMH are glucose sensing neurons that regulate glucose tolerance and hypoglycemia-induced glucagon secretion through the control of vagal nerve activity.

POSTERS

1.Wang Xu

Affiliation: LISP-SV-EPFL

Title of presentation: Systems phytohormone responses to mitochondrial proteotoxic stress

Authors list: Xu Wang, Johan Auwerx

Abstract: Mitochondrial function is controlled by two separate genomes. This feature makes mitochondria prone to proteotoxic stress in case a stoichiometric imbalance occurs in the protein complexes that perform oxidative phosphorylation (OXPHOS), which consist of both nuclear- and mitochondrial-encoded proteins. Such a proteotoxic stress is known to induce the mitochondrial unfolded protein response (UPRmt) in animals. It is unknown whether UPRmt occurs in plants. Here, we repressed mitochondrial translation in Arabidopsis, through chemical or genetic interference. Mitochondrial proteotoxic stress activated a plant-specific UPRmt and impaired plant growth and development. The retrograde signal is initiated by a fast oxidative burst, resulting in MPK6 activation, culminating in a systemic hormone response mainly reliant on ethylene signaling (but also involving auxin and jasmonate). This will activate an anterograde response that aims to repair mitochondrial translation (through the induction of the mitochondrial ribosomal proteins—MRPs) and mitochondrial protein import/folding, which will ultimately compensate for the decreased level of OXPHOS components that triggered the response. Our data not only highlight the universal nature of key features of mitonuclear stress signaling pathways, but also indicate specific effectors (mitokines vs. phytohormones) and transcriptional circuits (ATF4/5 vs. ERFs) that are divergent between the plant and animal kingdoms. In summary, our study ascertains that the network of mitochondrial protein quality control pathways is conserved in plants and that hormone signaling is an essential mediator that regulates mitochondrial proteostasis. Reference: Wang, X. & Auwerx, J. Molecular Cell 68: 540-551 (2017).

2. Luan Peiling

Affiliation: LISP/SV

3. Berdous Dassine

Affiliation: UNIL CIG

Title of presentation: A genetic screen in recombinant inbred mice identifies novel regulators of insulin secretion

Authors list: Dassine Berdous1, Xavier Berney1, Ana Rodriguez Sanchez-Archidona1,2, Maxime Jan1,2, and Bernard Thorens1 1 Center of integrative genomics, University of Lausanne, Switzerland. 2 Vital-IT, Swiss Institute of bioinformatics, Lausanne, Switzerland.

Abstract: Aim /Hypothesis: Type 2 diabetes is characterized by insulin resistance and insulin secretion impairment. The disease has a multifactorial and polygenic origin. Here, we aimed at identifying, in an unbiased way, novel regulators of glucose stimulated insulin secretion and gluco-incretin action. Methods: We isolated pancreatic islets from a panel of thirty-six recombinant inbred BXD mice (issued from the cross of C57BL/6J and DBA/2J mice) and measured insulin content and secretion in basal condition (2.8mM Glucose) and following stimulation with 16.7mM Glucose or 16.7mM Glucose plus Exendin-4. Islets from the same mice were also utilized for RNA extraction and RNA sequencing. The secretion data were used for QTL mapping and the genes within the QTL responsible for the secretion response were identified by combining phenotypic and gene expression data. Results: We found a QTL for insulin secretion in response to high glucose plus exendin-4, in chromosome 2, and a QTL for insulin secretion in response to high glucose and high glucose plus exendin-4 in chromosome 7. These genomic regions contain 173 and 221 genes, respectively. We identified six genes on chromosome 2: five are positively and one is negatively correlated with insulin secretion. One of those genes is already known to regulate insulin secretion. Preliminary results indicate that two others also regulate insulin secretion. On chromosome 7, we found one gene that is negatively correlated to insulin secretion phenotype. Conclusion/Interpretation: Our unbiased genetic screen identifies 7 novel regulators of glucose and gluco-incretin stimulated insulin secretion. Further investigations are ongoing such as: effect of each gene knockdown on insulin secretion, in cell lines and dispersed islets.

4. Piccand Julie

Affiliation: Nestle Institute of Health Sciences - Stem Cells

Title of presentation: Generation of humanized models recapitulating human liver and/or pancreatic functions

Authors list: Julie Piccand, Umberto De Marchi, Claudio Franceschi, Diana van Heemst and Marine Kraus

Abstract: Over the years, invertebrate animal models have been very useful to identify genes and pathways involved in basic biological mechanisms. However, their evolutionary distance from humans and highly different metabolism and poor knowledge of their pathology, represent a major limitation to study human age-related diseases. Even within the vertebrate, the differences observed between mouse and human physiology are frequently so disparate that data gained from experimentation with mice are not directly relevant to human metabolism, gene regulation or pathology. To overcome these limitations, the European FP7 project HUMAN aim at developing humanized mouse models recapitulating liver and pancreatic human function. We have adapted established methods to generate macro-encapsulated functional beta-cells in vivo from human induced pluripotent stem cells (iPSCs). This technology is combined with a model of “humanization” of the liver developed by the Karolinska Institute in which most hepatocytes are human cells deriving from a single human donor , . These animals are recapitulating human liver and pancreas functions at similar levels than observed in human donors, supporting the notion that mice with humanized liver and pancreas will be unique and innovative tools to study human metabolic diseases and human aging. This innovative approach offers the unique possibility of studying metabolism in an integrated living system (the mouse body), but in human-derived organs, i.e. liver and pancreas. Our ultimate goal is to improve understanding of chronic metabolic disorders such as diabetes with the aim to develop biomarkers of health and disease and science-based nutritional interventions.

5. Lee Umji

Affiliation: NIHS_EPFL

Title of presentation: Investigation of Transcriptional Regulation of Apelin to Reprogram Skeletal Muscle Aging by Accelerating Apelinergic Nexus

Authors list: Umji Lee, Julie Russeil, Maria Deak, Pascal Stuelsatz, Laura Lukjaneko, Bart Deplancke, Jerome Feige

Abstract: Apelin is an anti-aging circulating peptide partially produced and secreted by muscle fibers during exercise. It is an endogenous ligand of the G protein-coupled receptor APJ, that can act both at the paracrine and endocrine level as a myokine. Apelinergic system gradually dysfunctions during aging and the restoration of apelin, both endogenously and pharmacologically, in mice counteracts phenotypes of muscle aging and aging-related impairment of cardiovascular functions (1)(2). Although the reversal of murine aging by young circulation has been well documented, very little is known about the mechanisms how muscle fibers produce the rejuvenator at the molecular and cellular levels. Thus, this project focuses on determining which specific pathways control apelin expression with the goal to discover new strategies to reverse sarcopenia and aging associated muscle diseases.

6. Schiffrin Mariano

Affiliation: CIG

Title of presentation: Pparg null mice show a sex dimorphism in Nonalcoholic Fatty Liver Disease

Authors list: Mariano Schiffrin, Carine Winkler, Laure Quignodon, Aurélien Naldi, Bao Khanh Trang, Harald Köfeler, Hugues Henry, Paolo Parini, Federica Gilardi and Béatrice Desvergne

Abstract: Peroxisome proliferator-activated receptor gamma (Pparg is required for adipocyte differentiation. Pparg null mice are totally deprived of any form of adipose tissue and develop non-alcoholic fatty liver disease (NAFLD) with massive fat storage in the liver due, at least in part, to the inability to store lipids. Interestingly, this chronic hepatic lipid accumulation is gender-related and is characterized by a higher content of lipid droplets, hepatic triglycerides (TGs) and neutrophil infiltration in females compared to males at 20 weeks. Exploration of the lipid synthesis, storage and oxidation pathways revealed that genes involved in lipid droplet synthesis were induced in Pparg null females, whereas genes involved in microsomal w-oxidation were induced in Pparg null males. Lipidomic analyses showed that females had a higher hepatic content of short chain highly saturated TG species compared to males. Sex dimorphism of hepatic TG and of neutrophil infiltration was abolished by gonadectomy in Pparg null mice, indicating that sex hormones are involved in the liver phenotype. Hepatic gene expression is physiologically sex-biased in control mice. Importantly, we found that the sex-dependent expression of many sex-related genes was strongly altered in Pparg null mice. This was also found in another independent mouse model of NAFLD, the Ob/Ob mice. We further demonstrated that the growth hormone/STAT5b pathway, which controls hepatic sex-biased gene expression, is dysregulated in NAFLD in mice. Interestingly, meta-analysis revealed that humans with NAFLD have also an altered expression of sex-biased genes. Our study emphasizes the need of considering gender specific studies for NAFLD consequences.

7. Croizier Sophie

Affiliation: CIG Thorens group

Title of presentation: Development of hypothalamic neural projection into the arcuate nucleus : role of guidance proteins

Authors list: Gervais Manon and Sophie Croizier

Abstract: The worldwide obesity epidemic is reaching an alarming rate, particularly among children. There is an urgent need to better understand the mechanisms underlying the early onset of this pathological condition. Energy balance is essential to maintain normal body weight and prevent obesity. The central nervous system, particularly the hypothalamus, is crucial for balancing energy intake and expenditure. In the arcuate nucleus of the hypothalamus (ARH), two main antagonistic populations have been observed: anorexigenic neurons that produce pro-opiomelanocortin (POMC) and orexigenic neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP). These neurons receive inputs from several hypothalamic areas, including the paraventricular nucleus (PVH), the dorsomedial nucleus (DMH) and the lateral hypothalamic area (LHA). However, the ontogenesis of these projections and the guidance cues involved in their development remain unknown. We, then studied when the inputs to ARH neurons develop and identified the relevant guidance cues involved. To adress this question, we used DiI axonal labeling to examine the development of projections from PVH and DMH to ARH in neonatal mice. The results indicated that PVH projections reach the ARH at postnatal day 14 (P14) and the DMH projections develop later to reach the ARH at P16. In order to identify guidance cues involved in the development of projections into POMC and NPY neurons, Pomc-cre ;Td-Tomato and NPY-GFP-expressing cells were sorted at P14 and P16 based on the fluorescence. RNA-seq experiments were then performed to identify guidance cues. Identifying guidance signals will help us to better understand how hypothalamic connections develop and identify developmental mechanisms underlying early onset of obesity.

8. Bourdillon Nicolas

Affiliation: ISSUL, Université de Lausanne

Title of presentation: Detection of overreaching using photo-plethysmography

Authors list: Nicolas Bourdillon, Masih Nilchian, Grégoire P. Millet

Abstract: Introduction: controlling the onset of functional and non-functional overreaching is of prior importance to ensure adequate training plan and performance improvement. Objective: Assess photo-plethysmography (PPG) in athletes and test whether 1) it would be affected differently in functional overreached athletes (FOR) compared to non-overreached (AF) and 2. specific responses would yield an immediate diagnosis of FOR. Design: Fifteen athletes performed 2-wk baseline (BSL) training followed by 3-wk overload (+45%; OVL) and 2-wk recovery (-20%; RCV). Methods: PPG was measured overnight, every third night throughout BSL, OVL and RCV. Augmentation index (diastolic over systolic amplitude ratio, AI), pulse width and inflection point area ratio (IPA) are given as examples of PPG characteristics that change specifically in overreached athletes. All values are normalised to baseline (nu). Results: AI, pulse width and IPA significantly decreased during OVL in the FOR group but not in the AF group (all $p < 0.05$). Those three parameters returned to BSL values during RCV. AI, FOR: 1.00 ± 0.02, 0.97 ± 0.01, 0.99 ± 0.02; AF: 1.00 ± 0.02, 1.01 ± 0.02, 1.00 ± 0.002. Pulse width: FOR: 1.00 ± 0.02, 0.98 ± 0.02, 0.99 ± 0.02, AF: 1.00 ± 0.02, 1.00 ± 0.02, 1.00 ± 0.02, IPA: FOR: 1.00 ± 0.01, 0.98 ± 0.01, 0.99 ± 0.02; AF: 1.00 ± 0.01, 1.00 ± 0.01, 1.00 ± 0.01 for BSL, OVL and RCV respectively. Conclusion: PPG is an efficient tool for the diagnosis of overreaching as it shows specific changes in simply accessible parameters prior performance decrement. It is also fast responding so that training plan can be adjusted daily to control FOR onset and avoid non-functional overreaching.

9. Civiletto Gabriele

Affiliation: NIHS

Title of presentation: A transgenic zebrafish model for whole-organism monitoring of autophagy dynamics in skeletal muscle

Authors list: Gabriele Civiletto, Guillaume Jacot, Benjamin Brinon, Joy Richard, Alice Parisi, Joris Michaud, Denis Barron, Jerome Feige, Philipp Gut

Abstract: Autophagy is an intracellular catabolic process that promotes the recycling of organelles and cytoplasmatic components, acting as regulator of homeostasis and cellular metabolism. Several pathways can either positively or negatively regulate different steps of autophagosome formation and maturation, making autophagy a highly dynamic process. Due to its critical role in cellular quality control and metabolism, autophagy modulation has raised interest as possible therapeutic target for various human conditions ranging from age related diseases, such as neurodegeneration and muscle frailty, to immunity and cancer. Despite the great potential, no molecules specifically targeting autophagy have been used for human interventions so far. In addition The limited number of autophagy modulators known to date have limitations: for example some of them target key metabolic sensors that control energy status and cell growth such as AMPK and mTOR, showing low specificity and off-target effects. In addition most of the known autophagy modulators have been identified by in vitro screening, which is not sufficiently predictive and informative for in vivo implementation. Here, we describe a zebrafish transgenic autophagy reporter, which expresses ZsGreen-tagged map1lc3 protein specifically in skeletal muscle: Tg(actc1b:map1lc3-ZsGreen;cryaa:TdTomato), hereafter termed actc1b:lc3-ZsGreen. Treatment of transgenic zebrafish larvae with spermidine clearly induced autophagic flux quantified by confocal microscopy. To further automate the analysis of autophagy dynamics in vivo, we developed a method for high-content image acquisition and of actc1b:lc3-ZsGreen larvae. This new platform represents a high-throughput technology for the identification of new autophagy inducers.

10. Romani Mario

Affiliation: EPFL - LISP

Title of presentation: Enhancing mitochondrial proteostasis reduces amyloid-β proteotoxicity

Authors list: Mario Romani, Vincenzo Sorrentino, Laurent Mouchiroud, John S. Beck, Hongbo Zhang, Davide D'Amico, Norman Moullan, Francesca Potenza, Adrien W. Schmid, Solène Rietsch, Scott E. Counts & Johan Auwerx

Abstract: Amyloidosis are proteotoxic disorders that can affect the nervous system, which is the case in Alzheimer's disease (AD), the most common form of dementia. To date, no efficient therapy is available for AD, a disease with a strong component of amyloid-β (Aβ) aggregation. Clinical trials for AD have focused primarily on counteracting Aβ aggregation in the brain, which is considered the key pathogenic mechanism. However, AD is a complex, multifactorial disease and mitochondrial dysfunction has been shown to be a common pathological hallmark. Mitochondrial abnormalities in AD include decreased mitochondrial respiration and activity and alterations in mitochondrial morphology. Yet, the relevance of other aspects of mitochondrial homeostasis, such as mitochondrial proteostasis, to the pathogenesis of AD is still mostly unknown. We identified a cross-species mitochondrial stress response signature that implicates mitochondrial proteostasis as a key mechanism in response to Aβ proteotoxic stress. Our work also provided evidence that mitochondria have an active role in the pathogenesis of Aβ diseases. In fact, reducing mitochondrial homeostasis via atfs-1 depletion in an AD nematode model aggravated the hallmarks of the disease. Conversely, boosting mitochondrial proteostasis by increasing the UPRmt and mitophagy decreased protein aggregation, delayed disease progression, ultimately translating to increased cognitive function in an AD mouse model.

11. Mashinchian Omid

Affiliation: Ageing of skeletal muscle

Title of presentation: 3D-Derivation of Uncommitted Human Muscle Stem Cells from iPSCs

Authors list: Omid Mashinchian, Filippo De Franceschi, Nagabhooshan Hegde, Pascal Steiner, Sylke Hoehnel, Nathalie Brandenberg, Matthias P. Lutolf, Jerome N Feige and C. Florian Bentzinger

Abstract: One of the most fundamental problems associated with stem cell therapy of skeletal muscle is the limited availably of cells that can robustly engraft into the stem cell compartment. It has extensively been attempted to isolate adult muscle stem cells (MuSCs) and expand them in culture to obtain sufficient cell numbers for such treatments. The challenge associated with this approach is that, once isolated from their niche and maintained in culture, MuSCs become terminally committed to myogenic differentiation and show a dramatically reduced engraftment potential. However, the recent discovery of induced pluripotent stem cells (iPSCs) has opened new avenues for the in-vitro derivation of cell types that are more suitable for transplantation. Here, we report a highly efficient approach for the scalable derivation of uncommitted MuSCs from human iPSCs in a biologically faithful 3D environment. We employed human iPSCs and a spectrum of immortalized cell lines to generate 3D aggregation conditions promoting mesoderm formation and subsequent specification to the myogenic lineage without the parallel upregulation of myogenic commitment markers. Taken together, our work reveals a novel highly efficient derivation protocol for the generation uncommitted MuSCs from human iPSCs that can easily be scaled up to the bioreactor level. Our novel protocol has fundamental implications for cell therapy of muscular dystrophy and will inspire future preclinical studies that will pave the way towards trails in human patients.

12. Roujeau Clara

Affiliation: Group B. THORENS, Center for Integrative Genomics, UNIL, Lausanne, Switzerland

Title of presentation: Pancreatic GLUT2 as a signaling receptor ? An approach to resolve its interaction network

Authors list: Clara Roujeau, Patrice Waridel, Manfredo Quadroni, Bernard Thorens

Abstract: The facilitative glucose transporter GLUT2, highly expressed in murine pancreatic β cells, is required for glucose uptake and glucose-induced insulin secretion (GSIS). In human β cells, GLUT2 has very low levels, compared to rodents islets and compared to GLUT1 and GLUT3. Interestingly, several GLUT2 variants are strongly associated with transient neonatal diabetes, type 2 diabetes (T2D) risk and β cell failure in humans, suggesting a crucial role of GLUT2 in the physiology of human β cell despite its low level of expression. In addition to its function of glucose transporter, GLUT2 was proposed to trigger a specific signaling cascade, defining GLUT2 as a signaling receptor. In order to assess the dual role of GLUT2 as a glucose transporter and receptor in human β cell, we aim to (i) characterize the interaction network of GLUT2; (ii) focus on the candidates relevant to transduce intracellular signaling; (iii) study the impact of GLUT2 mutations associated with T2D on this signaling network. Here, we describe an approach to identify GLUT2 partners in the EndoCBH1 human β cell line. The method is based on proximity labeling mediated by the engineered peroxidase APEX2, in combination with isobaric tagging for quantitative mass spectrometry. This technique resolved 3 new candidates for the interaction network of GLUT2: ADCK4, ETHE1 and CDK5RAP1. These proteins are involved in several signaling pathways, and may therefore contribute to transduce a GLUT2-dependent intracellular signal required for the physiology of human β cell. The functional impact of these 3 candidates on GSIS is currently under investigation in EndoCBH1 cells.

13. Langlet Fanny

Affiliation: UNIL-CIG

Title of presentation: Tanycyte/neuron interactions in the regulation of glucose homeostasis

Authors list: Fanny Langlet

Abstract: The brain senses glucose and regulates glucose metabolism using unique glucose-sensing neurons. However, neurons do not act alone but also require the support of glial cells to perform as an integrated metabolic sensing unit. Among glial cells, specific hypothalamic ependymoglial cells called tanycytes are able to detect variations of glucose levels, and play a role in the regulation of energy balance as “sensors” of the metabolic state. In this study, we seek to understand the mechanisms underlying tanycyte/neuron crosstalk and the physiological impact of this dialogue on the regulation of glucose homeostasis. By stereotactically infusing TAT-CRE in the lateral ventricle of adult Rosa26-floxed stop tdTomato mice, we induced the expression of tdTomato in tanycytes, in order to analyze cell morphology. Tanycytes are polarized cells that line the lateral walls and the floor of the third ventricle. They are directly in contact with the cerebrospinal fluid at their apical surface, and send a single basal process into the brain parenchyma. Our approach allowed us to identify new structural features in particular spines on tanycyte process and at least two different type of endfeet: 1- sleeves, what is typically observed around vessels, or 2- boutons, what is typically observed in contact to neurons. For the first time, we also observed "en passant" boutons. We then confirmed that around 30% of these boutons contact neurons, some of them being involved in the regulation of energy balance. The elucidation of tanycyte fuel-sensing functions within the hypothalamus will contribute to explain the glucostatic regulation of food intake and allow the development of new therapeutic strategies for obesity and associated metabolic syndromes.

14. Zanella Claudia

Affiliation: LIFMET EPFL

Title of presentation: Mapping Lactate Concentrations via CEST Magnetic Resonance Imaging

Authors list: Claudia Zanella [1] Elise Vinckenbosch [2] Rolf Gruetter [1] [1] Laboratory for Functional and Metabolic Imaging (LIFMET), Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne [2] Haute école de santé Genève, HES-SO, Geneva

Abstract: A method for non-invasively studying cerebral lactate via Chemical Exchange Saturation Transfer (CEST) at high field is proposed. CEST is an approach in Magnetic Resonance Imaging (MRI) that takes advantage of the hydrogen exchange between a lactate pool and a water pool to amplify the signal for lactate concentration measurements. As a consequence, the abundance of lactate can be mapped which is useful for studying glycolysis-related metabolic disorders[1]. Lactate overproduction, known as the Warburg effect, is for example observed in many tumors and would thus be expected to show in CEST imaging[2]. The feasibility of imaging lactate using CEST was shown using a 9.4T MR scanner[1]. The small chemical shift difference between lactate and water make the measurements challenging, especially at low concentrations such as the ones found in vivo [3,4]. We therefore propose to go to higher magnetic fields, namely 14.1T, which is expected to be favorable in terms of resolution and sensitivity. We optimized the CEST effect in sodium lactate solutions (2mM–100mM) using a train of Gaussian pulses for saturation (B1 = 1uT–4uT, saturation time = 0.5s–15s) coupled with a segmented gradient echo sequence. Preliminary results have shown that a CEST effect up to 18% can be achieved for a 50mM lactate solution at physiological temperature, which corresponds to a higher CEST effect than the one reported previously[1]. Furthermore, we are currently able to detect lactate concentrations as low as 5mM, which is close to brain lactate levels expected during lactate overproduction. CEST MRI is a promising tool to monitor brain lactate levels non-invasively and thereby contributes to therapeutic evaluations.

[1]DeBrosse et al. 2015 [2]Heiden et al. 2009 [3]Dienel et al. 2012 [4]Zhang et al. 2018

15. Dumayne Christopher

Affiliation: CIG-UNIL

Title of presentation: Can Krüppel Cripple? Role of the Transcription Factor Krüppel-like Factor 6 (KLF6) in Beta-Cell Function

Authors list: Christopher Dumayne, Mark Ibberson, Bernard Thorens

Abstract: The transcription factor Krüppel-like factor 6 (Klf6) controls the expression of several target genes regulating various cellular processes such as apoptosis and metabolism. However, its function in the pancreatic β-cell has not yet been established. The aim of the study was to investigate the impact of Klf6 in the regulation of β-cell function whose dysregulation is central in the pathogenesis of diabetes.Here we assessed the regulatory contribution of Klf6 in glucose-stimulated insulin secretion (GSIS) upon its silencing in Min6B1 cells, an insulinoma cell line. Knockdown (KD) of Klf6 reduced insulin secretion upon glucose stimulation but was restored in presence of amino acids. Klf6-silenced cells displayed reduced glucose-induced oxygen consumption rates (OCR) and increased extracellular levels of lactate reflecting impaired oxidative phosphorylation and glycolysis, respectively. Nevertheless α-ketoglutarate stimulation resulted in equivalent OCR values between experimental groups further supporting Klf6's specificity to glucose sensing. This also suggests that Klf6 affects glucose signalling by interfering in step(s) upstream of the Krebs cycle. mRNA expression analyses exhibited an up-regulation of genes involved in glucose uptake, glycolysis, pyruvate conversion to lactate, and lactate export in Klf6-silenced cells. A preference for glycolysis over oxidative phosphorylation upon Klf6 silencing may be triggered by an increase in pyruvate dehydrogenase kinase 1 (Pdk1) expression. Collectively, our data confirms a decline in GSIS capacity upon Klf6 KD that may be explained by a reduction of the amount of pyruvate required for proprer insulin secretion. This effect may be brought on by Klf6's transcriptional regulation of genes involved in pyruvate metabolism.

16. Lavier Jessica

Affiliation: UNIL-CHUV

Title of presentation: Effects of three exercise training intensities on endothelial function in healthy mice: a preliminary study.

Authors list: Jessica Lavier, Manon Beaumann, Steeve Menetrey, Lucia Mazzolai, Anne-Christine Peyter, Maxime Pellegrin, Grégoire P. Millet

Abstract: Purpose: Exercise training (ET) has been shown to improve vascular reactivity in individuals with impaired endothelial function (EF). However, it remains unclear whether ET also improves EF in healthy subjects. Moreover, the effects of exercise intensity, i.e. low intensity continuous training (Low), maximal intensity interval training (Max) and supra-maximal intensity repeated sprint training (Supra), have never been investigated so far in healthy individuals. Aim: To Compare the effects of Low, Max and Supra ET on vascular responses in healthy mice. Design/Methods: C57BL/6J male mice performed running ET three times per week over a period of 4 weeks at three different intensities on a motorized rodents treadmill: Low (40min at 40% of maximal aerobic speed (MAS); n=5 mice), Max (8x1min at 90% of MAS; n=5 mice) and Supra (4 series of 5 bouts of sprints at 150% of MAS; n=5 mice). Total work was matched between groups. MAS was determined prior ET using an incremental treadmill test to exhaustion. After ET, lower abdominal artery (LAA) was harvested and placed in organ chambers for vascular reactivity assessment. Vessels were pre-contracted with phenylephrine (Phe; 10-4 M) and cumulative dose-response curves to the endothelium- and nitric oxide- (NO) dependent vasodilator acetylcholine (Ach; 10-9 to 10-4M) and to the endothelium-independent NO donor (DEA)/NO 10-9 to 10-4M) were performed. Results: No significant differences were observed in Ach- or (DEA)/NO-induced relaxation in LAA at any of the concentrations between Low, Max and Supra groups. Conclusion: Our preliminary underpowered findings showed that ET 3 times a week for 4 weeks does not enhance EF in healthy mice. Exercise intensity might not be a key determinant of EF response.

17. Lê Thanh Phong

Affiliation: Haute école de santé de Genève, HES-SO Genève - Laboratory for functional and metabolic imaging (LIFMET), EPFL

Title of presentation: Probing in-vivo Real-Time Lactate Metabolism and Neuroprotection in a Mouse Model of Ischemic Stroke using Magnetic Resonance Spectroscopy with Hyperpolarized L-[1-^{13}C]-Lactate

Authors list: Thanh Phong Lê, Lara Buscemi, Mario Lepore, Lorenz Hirt, Jean-Noël Hyacinthe, Mor Mishkovsky

Abstract: Lactate is known as a neuroprotector in a mouse middle cerebral artery occlusion (MCAO) model of stroke (Berthet et al. 2012). It is suggested that it could provide energy to the deprived neurons or increase the HCA1 receptor expression (Castillo et al. 2015). The tremendous sensitivity of magnetic resonance spectroscopy (MRS) with hyperpolarized ^{13}C hyperpolarized (HP) lactate probe allows detecting the real-time subsequent metabolism of lactate (Chen et al. 2008). The feasibility of probing HP lactate to pyruvate turnover in MCAO after injection of HP lactate was previously assessed (Mishkovsky et al. 2016). In the present work, a new HP lactate sample providing higher MR signal aims to probe its metabolism more in detail, not only by detecting the transformation of lactate to pyruvate, but also by probing the conversion of the latter into bicarbonate and alanine. A frozen sample of L-[1-^{13}C]-Lactate doped with OX63 radical was hyperpolarized by dynamic nuclear polarization at 7T/1K. Transient stroke was induced in C57BL6 male mice by inserting a silicon filament in the MCA during 30 min before reperfusion. The animal was then placed in a 9.4T MRI scanner. At 1h or 2h after reperfusion, 325uL of 0.1M HP lactate solution was injected intravenously and immediately followed by ^{13}C MRS acquisitions of the brain every 3s. Preliminary results of this ongoing work suggest that the real-time evolution of hyperpolarized lactate, pyruvate, alanine, bicarbonate can be followed with a resolution of 3s. This could contribute to improving understanding of the activities of lactate dehydrogenase, pyruvate dehydrogenase, alanine aminotransferase and monocarboxylate transporters, as well as the neuroprotective role of lactate in ischemic stroke. Supported by CIBM and FNS 310030_170155

18. Bernini Adriano

Affiliation: SMIA

Title of presentation: Cerebral ketone metabolism at the acute phase of traumatic brain injury in humans: implications for glycemic and nutritional control.

Authors list: A. Bernini, M. Masoodi, D. Solari, L. Carteron, N. Christinat, P. Morelli, M. Beaumont, JP. Miroz, S. Abed-Maillard, M. Hartweg, F. Foltzer, B. Cuenoud, M. Oddo

Abstract: Introduction: Adaptive metabolic response to injury includes utilization of alternative energy substrates, such as ketones, to protect the brain against further damage. Here, we investigated cerebral ketone metabolism at the acute phase of traumatic brain injury (TBI) in humans, focusing on the impact of caloric intake, glycemic targets and endogenous ketosis. Methods: This was an observational cohort study conducted in TBI subjects (N=34; mean age 49 years, mean GCS 7) who underwent cerebral microdialysis for the measurement of total brain interstitial tissue ketone bodies (KBs). We first analyzed (Study 1; N=24) changes of brain KBs from fasting (0 Kcal; mean 37 h from hospital admission) to initial caloric intake (500 Kcal; mean 16 h from nutrition start) and the influence of glycemic targets (categorized as tight 4-6 mmol/L vs. moderate 6.1-10 mmol/L) in this setting. We further examined (Study 2; N=10) the relationship between brain and endogenous circulating plasma KBs. Results: Initial caloric intake was associated with a significant decrease in total fasted brain KBs (from 32.9 [IQR 19.8-45.1] to 13.3 [8.8-25.9]µmol/L, p=0.0002, paired t-test), which was paralleled by a concomitant increase in brain and blood glucose (both p<0.01). Brain KBs were higher at tight vs. moderate glycemic targets (41.2 [32.3-83.7] vs. 17.3 [9.4-32.5]µmol/L, p=0.001). Endogenous circulating plasma KBs reached markedly elevated levels (mean 1.14 [range 0.17-3.82]mmol/L), and correlated strongly with brain KBs (r=0.83, p<0.0001). Conclusions: Our data at the acute phase of TBI demonstrate increased endogenous production and transfer of KBs to the injured human brain, where they may act as supplemental cerebral energy substrate in conditions of limited carbohydrate availability.

19. Pujol Julien

Affiliation: NIHS

Title of presentation: Gprin1 Regulates Actin Remodeling and Insulin Secretion in Pancreatic Islets

Authors list: Julien Pujol, Eija Heikkila, El-Hadji Dioum

Abstract: G protein regulated inducer of neurite outgrowth 1 (Gprin1) was originally identified in the brain as an effector of activated Gi protein subfamily (Gαi, Gαo and Gαz) and was shown to regulate neurite outgrowth and cytoskeleton remodeling. We have identified for the first time the expression of Gprin1 in rodent and human pancreatic islets. Gprin1 was expressed in α and β cells and regulated glucagon and insulin secretion. Isolated islets from Gprin1 knockout (KO) mice showed a reduced first and second phase of glucose stimulated insulin secretion with impaired Ca2+ dynamics and a reduced number of plasma membrane-docked insulin vesicles. These functional changes were associated with a denser F-actin network, increased stress fiber formation and dysregulation of focal adhesion dynamics during glucose stimulation in Gprin1 KO β cells. Furthermore, Gprin1 interacted with GTP-Gαo, regulated glucose-induced Cdc42 activation and formed dynamic complexes with SNARE proteins involved in insulin exocytosis, Munc18-1a and SNAP25. The Gprin1 KO mice have normal glucose tolerance and insulin sensitivity, but display significantly higher fasting glycemia compared to wild type (WT) littermates. However, under high-fat diet feeding, Gprin1 KO mice became glucose intolerant and showed decreased compensatory insulin secretion. Overall, we identified a novel signaling pathway regulated by Gprin1 in β cells that coordinates the activities of Gαo and Cdc42 and regulates actin cytoskeletal dynamics and insulin vesicle docking and release. Our data indicate that Gprin1 modulates both glucagon and insulin secretion and might be targeted.

20. Renaud Cédric

Affiliation: Service of Endocrinology, Diabetology and Metabolism, Lausanne University Hospital

Title of presentation: Nrf2 couples antioxidant defense with thyroglobulin production and iodination in the thyroid gland

Authors list: PG Ziros, IG Habeos, DV Chartoumpekis, E Ntalampyra, E Somm, CO Renaud, M Bongiovanni, IP Trougakos, M Yamamoto, TW Kensler, P Santisteban, N Carrasco, C Ris-Stalpers, E Amendola, X-H Liao, L Rossich, L Thomasz, GJ Juvenal, S Refetoff, GP Sykiotis

Abstract: While generation of oxidative substances is part of normal iodide metabolism during thyroid hormone synthesis, the thyroid must also defend itself against excessive oxidation in order to maintain normal function. Although a number of antioxidant and detoxification enzymes presumably aid thyroid cells to maintain homeostasis by ameliorating oxidative insults, including under conditions of exposure to excess iodide, the factors that coordinate their expression with the cellular redox status are not well known. The ubiquitously expressed transcription factor Nrf2 is required to defend tissues against oxidative stress. Using ubiquitous and thyroid-specific Nrf2 knockout (Nrf2-KO) mice and Nrf2- or Keap1-KO thyroid follicular cell lines, we show that Nrf2 mediates antioxidant transcriptional responses in thyroid cells and protects the thyroid from oxidation induced by iodide overload. Surprisingly, we also found that Nrf2 has a dramatic impact on both the basal and the thyrotropin-inducible intra-thyroidal abundance of thyroglobulin (Tg), the precursor protein of thyroid hormones. This effect is mediated by cell-autonomous regulation of Tg gene expression by Nrf2 via its direct binding to two evolutionarily conserved antioxidant response elements in an upstream enhancer. Yet, despite upregulating Tg levels, Nrf2 activity also limits Tg iodination both under basal conditions and in response to excess iodide.

21. Chareyron Isabelle

Affiliation: NIHS - Mitochondrial Function

Title of presentation: Chronic high glucose impairs mitochondrial energy metabolism and glucose responsiveness in human islet beta cells

Authors list: Isabelle Chareyron, Andreas Wiederkehr

Abstract: Metabolic alterations in Type 2 diabetic patients lead to hyperglycemia and dyslipidemia, which exert a stress on the pancreatic β-cell. Hyperglycemia may cause β-cell dysfunction by interfering with mitochondrial energy metabolism, which is central for β-cell nutrient sensing. Here we have studied mitochondrial function in human islets from healthy individuals cultured for 4 days in 5.6mM glucose (control) or 11.1mM glucose (chronic high glucose). The elevated glucose concentrations was selected to mimic glucose concentrations in the plasma of individuals with impaired glucose tolerance. In human islets maintained in high glucose concentrations, resting respiratory rates were elevated compared to controls. In response to glucose, control islets increased their respiratory rate by about 40-50%. Strikingly, treated islets were much less glucose responsive, increasing their respiration by only 15-20%. The failure of human islet beta-cell mitochondria to respond to glucose also caused a dramatic loss of glucose-induced ATP production, calcium signaling and insulin secretion. When calcium signaling was restored using tolbutamide during glucose stimulation, the respiratory response was markedly improved highlighting the important role of calcium in the activation of β-cell mitochondria. The observed mitochondrial phenotype was partially reversible. Within 24 hours of culture in control medium (5.6mM glucose) the β-cells recovered a large fraction of their respiratory response to glucose and calcium signaling was partially normalized. We demonstrate that high glucose levels alone have an important negative effect on β-cell function. Impairment of the mitochondrial responsiveness to glucose leads to a cascade of downstream defects lowering the insulin secretory response.