Thursday November 8, 2018
Olympic Museum - Quai d'Ouchy 1, 1006 Lausanne

LIMNA Symposium: Central Regulation of Metabolism and Feeding

Organizing Committee: 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.

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Invited Speakers

- **Daniela Cota, INSERM Neurocentre Magendie, Bordeaux University, France**
- **Sabrina Diano, Yale University School of Medicine, USA**
- **Ted Dinan, University College Cork, Ireland**
- **Ana I. Domingos, University of Oxford, UK**
- **Miguel Lopez, CiMUS, University of Santiago de Compostela, Spain**
- **Serge Luquet, University Paris Diderot, France**
- **Martin G. Myers, University of Michigan Medical school, Ann Arbor, USA**
- **Sophie Steculorum, Max Planck Institute for Metabolism Research, Cologne, Germany**

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Agenda

8h30-9h00 Welcome and distribution of badges

Opening

9h00 **Bernard Thorens**
Welcome

Session I

Chair: Lluis Fajas

9h10 **Martin G. Myers**
"The CNS control of blood glucose-roles for VMN subpopulations"

9h45 **Sabrina Diano**
"Central mitochondria dynamics and metabolism"

10h20 Coffee Break

Session II

Chair: Bart Deplancke

10h40 **Miguel Lopez**
"Targeting hypothalamic AMPK for the treatment of obesity"

11h15 **Daniela Cota**
"Bile acid signaling as novel mechanism in the central control of energy balance"

11h50 **Laura Velazquez Villegas**
"Bile acid-TGR5 Axis in the control of central energy Homeostasis"

12h05 **Sophie Croizier**
" Role of guidance proteins in synapse formation on hypothalamic neurons : influence of postnatal overnutrition"

12h20 Lunch
13h05 Poster session

Session III

Chair: Bernard Thorens

14h05 **Ted Dinan**
" Gut microbe to brain signalling: implications for depression and co-morbid obesity"

14h40 **Ana I. Domingos**
"Sympathetic Neuroimmunity in Obesity"

15h15 **Laia Morató Fornaguera**
" Stress-induced alterations in the adipokine eNAMPT reduce sociability via impairment of the NAD+/SIRT1 pathway in the nucleus accumbens"

15h30 Coffee Break

Session IV

Chair: Kristina Schoonjans

15h50 **Jürgen Ripperger**
" Impact of the Suprachiasmatic nuclei (SCN) on the feeding behavior of mice"

16h05 **Sophie Steculorum**
"Novel regulators of the central control of feeding and systemic insulin sensitivity"

16h40 **Serge Luquet**
"Triglyceride sensing in the mesolimbic system and the control of food reward"

17h15 Concluding remarks and prizes distribution

ABSTRACTS TALKS

The CNS control of blood glucose-roles for VMN subpopulations

Martin G. Myers
University of Michigan Medical school, Ann Arbor, USA

While the ventromedial hypothalamic nucleus (VMN) plays an essential role in the control of glucose homeostasis, including by mediating the counter-regulatory response (CRR) to hypoglycemia, the VMN contains multiple subpopulations of neurons that play distinct roles in the control of hypoglycemia, and the neurons that control hepatic glucose output, including during the CRR have not previously been molecularly defined. Since we previously showed that cholecystokinin (CCK) neurotransmission plays an important role in the VMN-mediated CRR, we generated CCK receptor b (*Cckbr*)-cre mice to study the potential role of VMN^{Cckbr} neurons in the control of blood glucose. $Cckbr^{Cre}$ reporter mice identified a substantial population of VMN^{Cckbr} neurons in the dorsomedial VMN. The optogenetic activation of VMN^{Cckbr} neurons increased blood glucose, consistent with a role in the control of glucose production. Furthermore, while tetanus toxin-mediated silencing of the entire VMN (using $SF1^{cre}$) promoted obesity and hyperglycemia, silencing VMN^{Cckbr} neurons did not alter body weight, but reduced blood glucose at baseline and impaired the CRR (as well as the hyperglycemic response to other stimuli). Thus, VMN^{Cckbr} neurons play a crucial role in the control of glucose production, including during the CRR.

Central mitochondria dynamics and metabolism

Sabrina Diano1,2,3,4,5
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Our research has been focusing on deciphering intracellular mechanisms that enable cells in the Central Nervous System (CNS) to sense and respond to changes in circulating nutrient and hormone levels in the control of systemic energy and glucose metabolism. Our recent findings have unmasked CNS mitochondria as critical intracellular organelle in detecting changes in peripheral metabolism. By altering their size, shape and function, mitochondria enable cells to adjust their activity, which in turn alter behavior and peripheral tissue functions to fine tune systemic metabolism. This presentation will highlight these cellular biological process in the CNS regulation of energy and glucose homeostasis.

Targeting hypothalamic AMPK for the treatment of obesity

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Besides the classical neuropeptide and neurotransmitter-based theory for feeding and energy expenditure regulation, recent data demonstrate that AMP-activated protein kinase (AMPK) plays a major role in the modulation of energy balance. At central level, the AMPK pathway is a canonical route regulating energy homeostasis, by integrating peripheral signals, such as hormones and metabolites with neuronal networks. Current evidence has linked hypothalamic AMPK with feeding brown adipose tissue (BAT) thermogenesis and browning of white adipose tissue (WAT), both through modulation of the sympathetic nervous system, as well as muscle metabolism, hepatic function and glucose homeostasis a process in with hindbrain AMPK has been also involved. The relevance of these data is interesting from a therapeutic point of view since several agents with potential anti-obesity and/or antidiabetic effects, currently being clinically used, such as nicotine, metformin and liraglutide are known to act through AMPK, at peripheral or central level. Furthermore, the orexigenic and weight-gain effect of worldwide used antipsychotic drugs, like olanzapine, are also mediated by hypothalamic AMPK. Overall, this evidence makes hypothalamic AMPK signaling an interesting target for drug development. This talk will summarize the role of hypothalamic AMPK on whole body energy metabolism and its potential as target for obesity treatment.

Bile acid signaling as novel mechanism in the central control of energy balance

Daniela Cota, MD 1,2
1. Team leader, Team Energy Balance and Obesity, INSERM U1215, Neurocentre Magendie, Bordeaux, France
2. University of Bordeaux, France

Previous studies have shown that bile acids are critical regulator of metabolic responses in peripheral organs. However, no investigation has so far evaluated the role of bile acids and its receptor TGR5 in the hypothalamic control of energy balance. Data that we have generated support the existence of a hypothalamic bile acids-TGR5 signaling system, which is relevant for the maintenance of energy homeostasis. Pharmacological stimulation of central TGR5 reduces food intake and body weight, while decreasing adiposity and improving insulin sensitivity in diet-induced obese mice. Conversely, hypothalamic deletion of TGR5 in adult mice causes hyperphagia and obesity. Investigations currently ongoing will help define the cellular and molecular mechanisms involved, thus providing further evidence supporting a beneficial role for bile acids in obesity and type 2 diabetes.

Acknowledgements: INSERM, Aquitaine Region, ANR, FFRD, Mexican ConaCyt.

Bile acid-TGR5 Axis in the control of central energy Homeostasis

Laura Velazquez-Villegas, *Alessia Perino, Andréane Fouassier, Daniela Cota, Kristina Schoonjans*
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Bile acids (BAs) are not only steroid lipid emulsifiers but also signaling molecules able to drive critical cellular responses in physio-pathological processes. After feeding, BAs can reach every tissue in our body through enterohepatic recirculation and spill-over in the systemic circulation. Circulating BAs can cross the blood brain barrier and their concentrations in the brain correlate with circulating levels. However, the physiological role of BAs in the central nervous system (CNS) is still largely unknown. In this study, we show that the G protein-coupled receptor TGR5, one of the main mediators of BA signaling, is expressed in the CNS. Immunohistochemistry analysis confirmed the presence of TGR5 in hypothalamic neurons. Mouse models with whole body (Tgr5-/-) or neuron specific (Tgr5Syn-/-) TGR5 deletion, showed a significant increase in food intake compared to their wild-type littermates. Accordingly, the mRNA levels of the orexigenic peptides agouti-related peptide (Agrp) and neuropeptide Y (Npy) in the arcuate nucleus (ARC) were not reduced upon feeding and remained significantly higher in Tgr5-/- and Tgr5Syn-/- mice compared to their controls. Administration of the specific TGR5 agonist (INT-777) exerted an anorexigenic effect, resulting in a decrease in food intake and Agrp and Npy expression in the ARC of Tgr5+/+ and Tgr5Syn+/+ but not in Tgr5-/- and Tgr5Syn-/- mice. Finally, TGR5 activation in the hypothalamic N41 cell line resulted in a decrease of Agrp secretion and expression, an effect that was lost in TGR5 silenced cells. Taken together, these data identify BA-TGR5 as a novel pathway implicated in the regulation of eating behavior. A better understanding of these mechanisms may lead to novel therapeutic approaches to prevent obesity-related diseases.

Role of guidance proteins in synapse formation on hypothalamic neurons : influence of postnatal overnutrition

Manon Gervais, Marc Lanzillo, ***Sophie Croizier***
Center for Integrative Genomics, UNIL

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. Epidemiological and mice studies showed that maternal obesity and postnatal overnutrition predispose the offspring to develop metabolic diseases. Decades of studies showed that the central nervous system, particularly the hypothalamus, was 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) and the dorsomedial nucleus (DMH). To build a functional neuronal network, growing axons travel to reach the appropriate area and make synapses with neurons. The axon growth and the synapse formation are

orchestrated by guidance molecules including Ephrin cell-surface signalling molecules. The ontogenesis of the PVH->ARH and DMH->ARH projections and the guidance cues involved in their development remain unknown. We then, aim to determine when the inputs to the POMC and AgRP/NPY neurons develop and to identify the guidance cues involved. Finally, we will determine whether postnatal overnutrition influences the development of these neurocircuits.

Gut microbe to brain signalling: implications for depression and co-morbid obesity

Ted Dinan MD, PhD
Department of Psychiatry and APC Microbiome Institute, University College Cork, Ireland

Evidence is accumulating to suggest that gut microbes may be involved in neural development and function, both peripherally in the enteric nervous system and centrally in the brain. There is an increasing and intense current interest in the role that gut bacteria play in maintaining the health of the host. Altogether the mass of intestinal bacteria represents a virtual inner organ with 100 times the total genetic material contained in all the cells in the human body. However, a disordered balance amongst gut microbes is now thought to be an associated or even causal factor for many chronic medical conditions as varied as obesity and inflammatory bowel diseases. While evidence is still limited in psychiatric illnesses, there are rapidly coalescing clusters of evidence which point to the possibility that variations in the composition of gut microbes may be associated with changes in the normal functioning of the nervous system, especially in patients with major depression. Studies in germ-free animals indicate aberrant development of the brain monoaminergic system together with memory deficits and autistic patterns of behaviour. These deficits can be partially normalised if there is early gut colonisation. Recent pre-clinical studies suggest that certain *Bifidobacteria* may have anxiolytic or antidepressant.

Major depression is associated with an increased risk of metabolic disturbance. Our studies have demonstrated that depressed patients have increases in visceral fat content, which is a risk factor for heart disease. Such patients also have increases in circulating pro-inflammatory cytokines, notably IL-6 and TNF-alpha. Furthermore, there are now three separate studies showing that the gut microbiota is altered in depression. When rodents undergo a microbiota transplant from patients with depression they show behavioural and immune changes consistent with depression. These observations have led to the conclusion that depression is a disorder of the brain-gut-microbiota axis.

Metchnikoff was the first to observe the fact that those living in a region of Bulgaria who consumed fermented food in their diet tended to live longer. He first published his observations in 1908 and this gave rise to the concept of a probiotic or bacteria with a health benefit. That bacteria might have a positive mental health as well as physical benefit is now becoming clear. Such bacteria may influence the capacity to deal with stress, reducing anxiety, perhaps positively impacting on mood and are now called psychobiotics. Some strains of probiotics/psychobiotics may have the capacity not just

to treat stress related disorders but also the ability to impact co-morbid obesity. Prebiotics, which are indigestible fibres, have been shown to positively impact the metabolic syndrome and our recent studies indicate that the prebiotics FOS and GOS also impact stress responses.

The mechanisms through which gut microbes influence mood and co-morbid metabolic disturbance are not entirely clear. The production of short chain fatty acids such as butyrate, propionate and acetate may be important. *Lacobacillus rhamnosus* has potent anti-anxiety effects in animals and does so by producing major changes in the expression of GABA receptors in the brain. GABA is the most important inhibitory transmitter in the human brain and these are the receptors through which benzodiazepines such as diazepam and various anaesthetic agents act. The changes in these receptors are mediated by the vagus nerve which connects the brain and gut. The vagus nerve has been shown to have potent anti-inflammatory potential which may be important in counteracting the pro-inflammatory effects of co-morbid obesity frequently observed in depression.

Communication between the brain and gut is bidirectional and complex. Increased understanding of this axis and the role of the gut microbiota may aid the development of therapies not just for mood disorders but related metabolic disturbance.

Sympathetic Neuroimmunity for Obesity

Ana Domingos, PhD [1,2]
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The brain controls adiposity via central and peripheral neural circuits. We used molecular genetic tools such as *optogenetics* to probe the connection between peripheral sympathetic neurons and adipocytes. Further, we found this neuro-adipose junction to drive lipolysis via norepinephrine (NE) signaling (1) and that the SNS is necessary and sufficient for fat mass reduction (1,2). As obesity is a chronic inflammatory state, we set to define neuroimmune mechanisms that link inflammation to SNS neurons (3). We report the discovery of *S*ympathetic neuron-*A*ssociated *M*acrophages (SAMs) that directly regulate the extracellular availability of norepinephrine (NE). We identified the molecular mechanism by which SAMs import and metabolize norepinephrine (NE). Abrogation of the mechanism for the uptake of NE by SAMs increases NE availability, which in turn promotes thermogenesis and browning, and long-term amelioration of obesity independently of food intake (3). The role of SAMs at steady state and obesity will be discussed.

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3. Roksana M Pirzgalska*, Elsa Seixas*, Jason S Seidman, Verena M Link, Noelia Martínez Sánchez, Inês Mahú, Raquel Mendes, Vitka Gres, Nadiya Kubasova, Imogen Morris, Bernardo A Arús, Chelsea M Larabee, Miguel Vasques, Francisco Tortosa, Ana L Sousa, Sathyavathy Anandan, Erin Tranfield, Maureen K Hahn, Matteo Iannacone, Nathanael J Spann, Christopher K Glass & **Ana I Domingos ψ** (2017). Sympathetic neuron–associated macrophages contribute to obesity by importing and metabolizing norepinephrine. *Nature Medicine.* ***Feature:*** Conor A. Bradley (2017). Specialized macrophages contribute to obesity. *Nature Reviews Endocrinology.*

Stress-induced alterations in the adipokine eNAMPT reduce sociability via impairment of the NAD+/SIRT1 pathway in the nucleus accumbens

Laia Morató*, Ioannis Zalachoras, Simone Astori, Sriparna Ghosal, Marie-Isabelle Guillot de Suduiraut, Jocelyn Grosse, Olivia Zanoletti and Carmen Sandi*
Behavioral Genetics Laboratory, EPFL

Early-life exposure to stressful experiences has been described as a predisposing factor to develop alterations both in metabolism and behavior. Here, we show that exposure to a paradigm of unpredictable stress during the peripubertal period reduces sociability in mice together with alterations in peripheral metabolism, such as increased percentage of fat mass. Remarkably, the protein levels of NAMPT in the adipose tissue of stressed mice are decreased and the blood levels of the cytokine eNAMPT -the extracellular form of the enzyme- predict the behavioral deficits displayed later in life. Aiming at understanding whereby eNAMPT differences can cause alterations in the brain, we analyzed NAD+ levels, expression of mitochondrial genes and neuronal excitability in the nucleus accumbens (NAc), a key brain region for the stress response. We observed that the NAc of stressed mice exhibits an impairment in the NAD+/SIRT1 pathway together with reduced neuronal excitability. Systemic treatment with the NAD+ booster nicotinamide mononucleotide (NMN) or adipose-specific overexpression of Nampt prevents sociability and neuronal excitability deficits in the NAc. Moreover, genetic and pharmacological modulation of SIRT1 expression in the NAc rescues sociability deficits. These findings strengthen the idea of eNAMPT as a mediator of fat-brain communication and pave the way for the use of NAD+ boosters to treat stress-related disorders such as depression.

Impact of the Suprachiasmatic nuclei (SCN) on the feeding behavior of mice

***Jürgen Ripperger**, Iwona Olejniczak, Urs Albrecht*
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Daytime feeding of mice causes phase adaptation of peripheral circadian oscillators such as the one of liver. Consequently, the metabolic and detoxification programs of liver remain in phase with the food challenge. By contrast, the phase of the circadian clock in the Suprachiasmatic (SCN) nuclei, which represent the master clock of an organism, stays synchronized to the external light-dark phase. Hence, it was concluded that food is a dominant Zeitgeber over the SCN signals for the liver circadian oscillator. Nevertheless, signals from the SCN can still regulate the time of food uptake. However, so far it is unknown, how the SCN would affect this time of food uptake under normal conditions. Here, we analyzed the feeding behavior of mice to understand the impact of the circadian clock. We used Nestin-driven CRE expression to abolish the function of the circadian regulator Period2 mainly in the neurons (NPer2 mice). As an experimental system, we gave every day sufficient food to the mice 4h after the lights were switched on to maintain ad libitum conditions. Surprisingly, the NPer2 mice but not their control mice were using more food during the light phase, while keeping the same overall food consumption. A similar phenotype was observed by injecting a CRE-expressing Adeno-Associated Viral (AAV) vector directly into the SCN neurons. Daytime feeding similar to NPer2 mice could be induced in the control mice by feeding more palatable food. As conclusion, the data suggest that the SCN output does not affect the homeostatic component of feeding, but rather the hedonic component and suppresses food uptake behavior during the day.

Novel regulators of the central control of feeding and systemic insulin sensitivity

Sophie M. Steculorum
Max Planck Institute for Metabolism Research

Over the last decades, our understanding of the fundamental processes governing energy balance and glucose homeostasis has largely evolved and pinpointed a pivotal role of the central nervous system and more particularly of the arcuate nucleus of the hypothalamus (ARH). Notably, activation of orexigenic AgRP-expressing neurons located in the ARH potently promotes feeding. We demonstrate that in addition to its orexigenic effects, chronically altering AgRP-neurons activity also affects peripheral glucose homeostasis. Acute activation of AgRP-neurons causes insulin resistance through impairment of insulin-stimulated glucose uptake into brown adipose tissue (BAT) and decreased sympathetic nerve activity. Optogenetic circuitry mapping reveals that feeding and insulin sensitivity are controlled by both distinct and overlapping AgRP-projections. We notably find that activation of AgRP→aBNSTvl (ventro lateral part of the anterior bed nucleus of the stria terminalis) projections mediates the effect of AgRP-neuron activation on insulin sensitivity and BAT gene expression. Our results thus reveal a mechanism by which these neurons rapidly coordinate hunger states with glucose homeostasis. Along this line, we discovered a novel AgRP-neurons' stimulatory

pathway able to modulate both feeding and insulin sensitivity. We show that AgRP-neurons express the purinergic receptor 6 (P2Y6) and that activation of P2Y6 by its endogenous ligand uridine-diphosphate increases AgRP-neuron's action potential firing and promotes feeding. Further, selectively abrogating P2Y6-signaling in AgRP-neurons alleviates obesity-associated adiposity, hyperphagia, and insulin resistance. Our work therefore reveals that modulating AgRP-neurons by targeting P2Y6-signaling improves obesity-associated metabolic outcomes.

Triglyceride sensing in the mesolimbic system and the control of food reward

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Circulating triglycerides (TGs) normally increase after a meal but are altered in pathophysiological conditions, such as obesity in both human and rodent. TG-hydrolyzing enzymes are expressed in the mesolimbic dopaminergic system suggesting that central TG-sensing might regulate dopamine neurons activity and ultimately reward-driven behaviour.
Using brain-specific TG delivery (BTGD), we show nutritional TG access mesocorticolimbic (MCL) structures where they modulate dopamine (DA) neurons activity and signalling. Central TG sensing is mediated, at least in part, by the lipoprotein lipase (LPL) whose transcript is specifically found onto DA and medium Spiny Neuron (MSN) neurons. TG detected centrally create positive reinforcement in a conditioned

place preference paradigm (liking) but lead to reduced motivation to work for reward (wanting) as assessed by both operant conditioning and self-administration. Finally, we find that TG action on reward seeking behaviour primarily rely on the indirect Dopamine receptor DRD2 pathway.
Using functional magnetic resonance (fMRI) we found that in human, post-prandial TG excursions modulate brain response to food versus non-food cues. The response of the ventromedial prefrontal cortex (vmPFC) was specific to TG and independent of other energy-related signals. Finally, the action of TG onto brain response was driven by the genetic polymorphism TaqAI, a mutation known to affect D2DR signaling and susceptibility to addiction.

Collectively, these findings reveal new mechanisms by which dietary TG alter mesolimbic circuit function and reward seeking behaviour, and provide a novel hypothesis by which energy-rich diet might lead to dopamine circuitry adaptation and ultimately addictive behaviour.

POSTER ABSTRACTS

1.
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Cdk4 regulates non-shivering thermogenesis

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Brown adipose tissue (BAT) is a highly specialized tissue that functions to maintain body temperature during cold challenge through the non-shivering thermogenesis, and contributes to the whole body energy balance. Numerous genes and pathways that regulate brown adipocyte biology have now been identified. However, the role of cell cycle regulators in the development and the function of this oxidative depot has not been thoroughly studied yet. We now aim to determine the role of cyclin-dependent kinase 4 (Cdk4) in the function of BAT. Interestingly, Cdk4 inactivation in vivo led to a marked metabolic phenotype in BAT, pointing towards a participation of Cdk4 in non-shivering thermogenesis.
First, in vitro, BAT cells treated with a chemical inhibitor of Cdk4 show increased oxygen consumption. In vivo, 3 days of treatment with the Cdk4 inhibitor significantly increased the expression of numerous mitochondrial genes, like PGC1a, TFAM and NRF1 in BAT.
Second, BAT from mice lacking Cdk4 (Cdk4-/-) are smaller, and exhibit increased mitochondrial number and decreased number of lipid droplets. Moreover, the expression of canonical thermogenic genes is increased in the BAT of Cdk4-/- mice. Interestingly, Cdk4-/- mice have increased resistance to cold exposure. Overall, our findings demonstrate that Cdk4 has a major role in brown adipose tissue biology and in temperature regulation.

2.

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Behavioural and metabolic effects of low mitochondrial respiration in the Nucleus accumbens

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Aberrant brain mitochondrial function has been linked with a number of psychopathologies (including depression and anxiety) and metabolic disturbances. Previously, it has been shown that pharmacological inhibition of mitochondrial complex I in the Nucleus accumbens (NAc) results in increased passive copying behaviour and impaired performance in a social dominance test. The activity of the two prominent types of medium spiny neuron subpopulations in the NAc, which are defined by the expression of D1 or D2 receptors, often exerts opposite effects on behaviour. Therefore, to study the whole range of effects of mitochondrial dysfunction in the NAc, cell-type-independent pharmacological manipulations are not adequate. Here, using viral delivery of cre-recombinase under the control of specific promoters in the NAc of NDUFS4Flox mice, we induced virally-mediated mitochondrial complex I deficiency, specifically in D1 or D2 medial spiny neurons. Subsequently we examined the effects of this manipulation in a wide range of anxiety- and depression-like behaviours, as well as metabolic parameters. Our results showed that NDUFS4 deletion in D1 cells in the NAc resulted in decreased saccharine preference, reduced body weight gain and increased activity in the metabolic cages. On the other hand, NDUFS4 deletion in D2 cells in the NAc resulted in increased food consumption and respiratory exchange ratio in the metabolic cages. Our data suggest a pivotal role for mitochondrial respiration in the NAc in the control of behaviour and metabolism, highlighting the importance of brain bioenergetics in health and disease.

3.

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RXR ChIP-seq on mouse liver profiles heterodimers activities modulation during circadian and nutrient response cycles

Khanh B. Trang, Federica Gilardi, Micheal Baruchet, Vivian Praz, Eugenia Miglivacca, Aurelien Naldi, Nicolas Guex, Beatrice Desvergne

The expression of most members of the nuclear receptors superfamily is dynamic and, in key metabolic tissues, follows a circadian pattern. Their activation regulates the expression of specific genes, controlling the development, homeostasis, metabolism of the organism. Their interplaying roles have been under scrutiny. One can use intersection from multiple arrays established for each nuclear receptor. Alternately, Retinoid X receptor (RXR) appears to be a perfect candidate to characterize this global regulatory landscape, as RXR is a common heterodimerization partner for several other nuclear receptors. We found that RXR bindings cluster together to promoter-rich regions, and this colocalization increases transcription activity in relation to the abundance of sites, rather than to the intensity of RXR binding affinity. Analysis of the sequence motifs underneath RXR binding sites (which suggestively mirror the heterodimer partner) showed that half of RXR target genes present only one type of binding motif, which suggests that they are regulated by one heterodimer partner. The other half are regulated by multiple motifs, inferentially multiple co-regulators. Interestingly, the set of RXR target genes bearing multiple type of motifs correspond to those that carry out the main metabolic functions and circadian rhythm maintenance, while the monotypic-motif genes are mainly involved in the cell cycle checkpoint function. Only 10% of RXR bindings are circadian or ultradian, and affect the circadian expression of a total of 162 genes. This disproportion implied that the circadian response in transcription requires more than the circadian binding of RXR, but also the synchronization with other factors.

4.

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The impact of mitochondrial fission on metabolic adaptation

Miriam Valera-Alberni, Magali Joffraud, Carles Canto

Mitochondrial dysfunction is a hallmark for multiple metabolic and age-related diseases. Mitochondrial quality control mechanisms are largely influenced by mitochondrial fusion and fission events, referred to as mitochondrial dynamics, which also influence mitochondrial bioenergetics properties. Acute changes in mitochondrial morphology can be controlled through the phosphorylation of the mitochondrial fission orchestrator, Drp1. In cultured cell models, phosphorylation of Drp1 mouse protein at Ser643 (Ser637 in humans) is believed to impair mitochondrial fission, whereas phosphorylation of Ser622 (Ser616 in humans) promotes it. Our results indicate that this functional exclusivity in the phosphorylation of both residues, however, does not occur in differentiated tissues, where the two phosphorylations coexist. Moreover, the induction of Ser643 phosphorylation consistently led to the downstream phosphorylation of Ser622 in mouse tissues. To further explore the relevance of these phosphorylation events in Drp1 function, mitochondrial physiology and organismal metabolism, we developed a knock-in S643A (Drp1 S643A KI) mouse model. Our results indicate that Drp1 S643A KI mice display enhanced lipid utilization and increased maximal respiration in brown adipose and liver tissues. These effects were further accentuated when mice were challenged with a high fat diet. Overall, we demonstrate that the regulation of Drp1 phosphorylation differs between cultured cell models and in differentiated tissues, and that the inability to phosphorylate S643 enhances mitochondrial respiratory capacity and bias mice towards a higher use of lipids as energy substrate.

5.

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The Role of Novel Kinases in Adipose Tissue Biology

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Recently, the occurrence rate of obesity, which is the excessive accumulation of body fat, has dramatically increased. Adipose tissue dysfunction is a primary defect, linking obesity to numerous health problems, including insulin resistance type 2 diabetes, hypertension, dyslipidemia, atherosclerosis, cancer, etc. However, not all obese individuals develop cardiovascular or obesity-related metabolic disorders. This might be due to maintained normal adipose tissue function and architecture. It is well established that pro-inflamatory cytokines, such as IL-6 and TNF-α, affect insulin signalling, which in turn is essential to maintain glucose homeostasis and to regulate its metabolism in the liver, muscle, and adipose tissues. This leads to the stimulation of downstream protein kinases, thus activating and crosslinking numerous pathways, potentially resulting in insulin resistance. Consequently, insulin resistance status is determined by the type of activated inflammatory pathways, abnormalities of lipid metabolism, as well as in the type of activated kinases and their downstream targets. The second part of our project revolves around the role of novel kinases in SAT and VAT of patients that are insulin resistant (IR) or insulin sensitive (IS). Several of the known protein kinases involved in the onset of insulin resistant are AMP-activated protein kinase (AMPK), IκB kinase (IKK), protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), etc. Identifying new and specific protein kinases involved in obesity-induced chronic inflammation may help in developing the targeted drug therapies to minimize insulin resistance in patients.

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CDK7 is critical for brown adipose tissue thermogenesis

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Brown adipose tissue (BAT) dissipates energy through Ucp1-mediated uncoupled respiration and its activation may represent a therapeutic strategy to combat obesity. Cyclin-dependent kinase 7 (CDK7) is a member of the cyclin-dependent protein kinase (CDK) family, which is an important regulator of cell cycle progression and more recently, metabolism. Here we show that CDK7 controls BAT non-shivering thermogenesis in mice. We generate brown adipose tissue-specific CDK7 knockout mice and show that disruption of CDK7 in BAT does not predispose mice to systemic metabolic dysfunction. However, compared with wild-type littermates, these mice have decreased BAT mass. When the mice were challenged with cold exposure, the knockout mice develop hypothermia, which was accompanied by a marked reduction in blood glucose and in stores of triglyceride in BAT. Thus, CDK7 is required for preparing mice for acute cold exposure during fasting state. Mechanistically, we found the mitochondrial over nuclear DNA ratio (mtDNA/nDNA) was significantly reduced in BAT from CDK7 bKO mice, suggesting that CDK7 is implicated in the regulation of mitochondrial biogenesis. Moreover, high-resolution respirometry of BAT homogenates demonstrated that oxygen consumption rates (OCRs) in BAT of CDK7bKO were lower compared with WT BAT. Thus, CDK7 deficiency leads to mitochondrial dysfunction in BAT. Furthermore, RNAseq analysis indicated that loss of CDK7 disrupts the expression of metabolic genes (like PGC-1a, UCP1) in response to cold. Currently, CHIP-seq is employing to decipher how CDK7 co-activates thermogenesis related genes in BAT. Our research demonstrated CDK7 is important to orchestrate nutrient homeostasis and thermogenesis to survive in life-threatening challenges, such as cold and starvation.

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A knock-in rat model for glutaric aciduria type I confirms cerebral ammonium accumulation

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Glutaric aciduria type I (GA-I) is caused by deficiency of glutaryl-CoA dehydrogenase (GCDH). Most untreated patients are asymptomatic at birth and then develop encephalopathic crises most often triggered by a catabolic stress, which lead to irreversible neurological impairment. Despite numerous in vitro and in vivo studies, the pathogenesis of neurological damage in GA-I remains poorly understood. R411W, the rat homologue of the frequent human mutation R402W, was introduced into the Gcdh gene of Sprague-Dawley rats by CRISPR/Casp9-mediated genome engineering. Homozygous Gcdhki/ki rats showed a normal growth and were fertile. They revealed a biochemical phenotype typical for GA-I including elevations of 3-OHGA, GA and glutarylcarnitine in tissues and body fluids. Further, a significant increase of ammonium (NH4+) was found in plasma accompagnied by glutamine decrease and glutamate increase, suggesting that NH4+ was produced by the enzyme glutaminase (Gls). Histologically, Gcdhki/ki rats developed the typical diffuse spongiform myelinopathy as known from autopsies of GA-I patients. However, Gcdhki/ki rats did not present any signs of an encephalopathic crisis. Proteomic analyses on brain tissues revealed dysregulated genes in Gcdhki/ki rats that are implicated in synaptic signal transmission and synapsis organization. Further, Gls was found 12-fold up-regulated in brain tissues of Gcdhki/ki rats. We successfully created the first transgenic rat model for GA-I. The characterization of this new model showed further evidence for a role of cerebral NH4+ production by Gls in the neuropathogenesis of GA-I. New therapeutic strategies targeting cerebral NH4+ accumulation have to be developed and can be tested in the Gcdhki/ki rat.

8.

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Impact of the Suprachiasmatic nuclei (SCN) on the feeding behavior of mice

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Daytime feeding of mice causes phase adaptation of peripheral circadian oscillators such as the one of liver. Consequently, the metabolic and detoxification programs of liver remain in phase with the food challenge. By contrast, the phase of the circadian clock in the Suprachiasmatic (SCN) nuclei, which represent the master clock of an organism, stays synchronized to the external light-dark phase. Hence, it was concluded that food is a dominant Zeitgeber over the SCN signals for the liver circadian oscillator. Nevertheless, signals from the SCN can still regulate the time of food uptake. However, so far it is unknown, how the SCN would affect this time of food uptake under normal conditions. Here, we analyzed the feeding behavior of mice to understand the impact of the circadian clock. We used Nestin-driven CRE expression to abolish the function of the circadian regulator Period2 mainly in the neurons (NPer2 mice). As an experimental system, we gave every day sufficient food to the mice 4h after the lights were switched on to maintain ad libitum conditions. Surprisingly, the NPer2 mice but not their control mice were using more food during the light phase, while keeping the same overall food consumption. A similar phenotype was observed by injecting a CRE-expressing Adeno-Associated Viral (AAV) vector directly into the SCN neurons. Daytime feeding similar to NPer2 mice could be induced in the control mice by feeding more palatable food. As conclusion, the data suggest that the SCN output does not affect the homeostatic component of feeding, but rather the hedonic component and suppresses food uptake behavior during the day.

9.

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Stress-induced alterations in the adipokine eNAMPT reduce sociability via impairment of the NAD+/SIRT1 pathway in the nucleus accumbens

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Early-life exposure to stressful experiences has been described as a predisposing factor to develop alterations both in metabolism and behavior. Here, we show that exposure to a paradigm of unpredictable stress during the peripubertal period reduces sociability in mice together with alterations in peripheral metabolism, such as increased percentage of fat mass. Remarkably, the protein levels of NAMPT in the adipose tissue of stressed mice are decreased and the blood levels of the cytokine eNAMPT -the extracellular form of the enzyme- predict the behavioral deficits displayed later in life. Aiming at understanding whereby eNAMPT differences can cause alterations in the brain, we analyzed NAD+ levels, expression of mitochondrial genes and neuronal excitability in the nucleus accumbens (NAc), a key brain region for the stress response. We observed that the NAc of stressed mice exhibits an impairment in the NAD+/SIRT1 pathway together with reduced neuronal excitability. Systemic treatment with the NAD+ booster nicotinamide mononucleotide (NMN) or adipose-specific overexpression of Nampt prevents sociability and neuronal excitability deficits in the NAc. Moreover, genetic and pharmacological modulation of SIRT1 expression in the NAc rescues sociability deficits. These findings strengthen the idea of eNAMPT as a mediator of fat-brain communication and pave the way for the use of NAD+ boosters to treat stress-related disorders such as depression.

10.

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Studying cancer cells phenotypes integrating omics data in human reduced genome-scale metabolic models

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Cancer is a leading cause of death in the world, and the mechanisms underlying this disease are still not completely understood. In the last decades, altered tumour metabolism has been recognized as a hallmark of cancer. This has created a resurgence of interest in the field of systems biology and metabolic modelling to analyse and understand the metabolic changes occurring in cancer cells. Modelling the different phenotypes of healthy and cancer cells will help to make predictions to create effective therapies to prevent, diagnose and treat cancer. The reconstruction of genome-scale models (GEMs) enables the computation of phenotypic traits based on the genetic composition of a target organism. Therefore, we use the human genome-scale model Recon 2 to build cancer cell-specific human GEMs by integrating experimental data (fluxomics, metabolomics, genomics, transcriptomics) and thermodynamic data. To overcome the well-known challenges when working with large networks, we generate systematically reduced models around specific subsystems, considering the composition and usage of the extracellular medium metabolites, and the biosynthesis of the biomass precursor metabolites. Furthermore, we apply pathway enrichment to study the regulation of the pathways under study. The reduced models can be used for a broad range of applications ranging from omics data integration to kinetic models. The proposed pipeline will enhance the comparison and understanding, at the stoichiometric and kinetic level, of the main metabolic differences that emerge in cancer development and progression. Furthermore, predicting the network responses will help to design experiments to find new targets for therapies and drugs.

11.

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Glucose-sensing neurons of the PVT exert opposite control of motivated sucrose-seeking behaviour.

Gwenaël Labouèbe, Sébastien Kessler Sophie, Bernard Thorens

In pancreatic beta-cells, the glucose transporter Glut2 and the enzyme Glucokinase (Gck), that facilitates the phosphorylation of glucose to glucose-6-phosphate, are critical regulators of glucose-induced insulin secretion. Indeed, glucose uptake occurs through Glut2, followed by its phosphorylation by Gck which controls the subsequent increase in intracellular ATP/ADP ratio, membrane depolarization, Ca2+ influx, and insulin secretion by beta-cells. It has been proposed that central glucose-sensing mechanisms could also utilize similar mechanisms to detect and signal glucose variations in the brain via specialized neurons. There are two types of glucose sensing neurons, glucose-excited (GE) and glucose-inhibited (GI) neurons whose firing rate is increased by a rise or a fall in extracellular glucose concentrations, respectively. GE and GI neurons can be found in numerous brain regions, notably in hypothalamic and brainstem nuclei. Here, we describe a brain area, the paraventricular thalamic nucleus (PVT), that contains Glut2 or Gck-expressing neurons. Interestingly, PVT has recently emerged as an important structure in the regulation of motivated feeding through its interaction with the dopamine reward system and notably the nucleus accumbens (NAc). We are here investigating the ability of Glut2 and Gck neurons to sense glucose variations in the PVT and their putative role in physiological functions related to motivated feeding behavior.

12.

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Gene expression profiling and analysis identify a key metabolic mediator of the master energy sensor, AMP-activated protein kinase

Caterina Collodet, Marc Foretz, Maria Deak, Laurent Bultot, Sylviane Metairon, Benoit Viollet, Frederic Raymond, Kei Sakamoto, Patrick Descombes

AMPK is a master metabolic regulator playing a critical role for maintenance of cellular energy homeostasis. AMPK activation through exercise or pharmacological means has beneficial effects in various tissues, balancing their carbohydrate and lipid metabolism. Consequently, there has been keen interest in developing AMPK-stimulating drugs for therapeutic use in the treatment of metabolic disorders. We have compared the effect of two different AMPK activators targeting the enzyme with distinct mechanisms of action (i.e. AICAR, an AMP-mimetic and 991, an allosteric activator), on global gene expression from wild-type and AMPK α1/α2-null primary hepatocytes and mouse embryonic fibroblasts. We demonstrated that although AICAR has been widely used as a pharmacological AMPK activator, it regulates a vast number of genes in an AMPK-independent fashion, whereas 991 has an effect exclusively AMPK dependent. Gene ontology on the dataset has clarified that AMPK primarily regulates genes related to energy metabolism. To predict which transcription factor(s) could mediate AMPK's action, we performed an in silico promoter analysis on the identified AMPK-regulated genes. The prediction revealed that TFEB, the master regulator of lysosomal biogenesis, is a mediator for AMPK-dependent metabolic gene regulation. Our study offers a first comprehensive AMPK-dependent gene signature in fibroblasts and hepatocytes. Moreover, we have identified a novel AMPK-TFEB cascade as a potential mechanism responsible for AMPK-mediated metabolic adaptations. Further investigation of the AMPK-TFEB axis will be required to determine whether this mechanism may be a valid and valuable target for treating metabolic diseases.

13.

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Implication of the Mitochondrial Fusion Protein Mitofusin 1 in the Nucleus Accumbens in Anxiety-and Depression-Like Behaviors.

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Mitochondrial function in the nucleus accumbens (NAc) has been recently implicated in the regulation of complex social behaviors, and impairment in mitochondrial functions in the NAc is observed in highly anxious animals. Mitofusin 1 (Mfn1) is a mitochondrial fusion protein, and its mutation has been shown to cause mitochondrial fragmentation and defects in mitochondrial motility. Here, we investigated the role of accumbal Mfn1, a major modulator of mitochondrial dynamics, for the regulation of anxiety and depression-like behaviors. We demonstrate that neuronal loss of Mfn1 in the NAc increases anxiety- and depression-like behaviors. Specifically, we show that Mfn1 floxed mice injected with AAV-Synapsin-Cre into the NAc spent less time in the open arms of the elevated plus maze (EPM) and in the lit part of the light-dark box than controls (littermate Mfn1 floxed mice receiving AAV-GFP virus into the NAc). In addition, Mfn1 NAc knockdown (KD) mice displayed less time interacting with an unfamiliar CD1 mouse in a social interaction test. In the forced swim test, Mfn1 NAc KD mice exhibited a lower latency to immobility than controls. Notably, Mfn1 NAc KD mice did not differ from controls in measures of locomotion in the open field test or distance traveled in the EPM. Collectively, these results identify a protective role for Mfn1 in the NAc against anxiety-and depression-like behaviors, and suggest that targeting mitochondrial function may be an important avenue for developing new mood stabilizing agents.

14.

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Functional Role of Yin-Yang 1 (YY1) Acetylation in Brown Adipose Tissue Thermogenesis

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Aims. Obesity is the result of an imbalance between energy intake and energy expenditure. In recent years, it was found that promoting brown adipose tissue (BAT) activation might be a promising alternative to treat for obesity. BAT is the main tissue for non-shivering thermogenesis in response to the increase in cAMP during cold exposure. This process is mediated mainly by uncoupling protein 1 (UCP1) in the mitochondria. UCP1 uncouples the oxidative phosphorylation in the ATP synthesis with heat being produced, leading to the increased consumption of stored energy. The transcriptional co-activator PGC1a is the main regulator of thermogenesis by activation of different transcription factors, leading to UCP1 expression. Our recent studies have shown that Yin-Yang 1 (YY1), an ubiquitously expressed transcription factor, is also involved in the transcription of key thermogenic genes including UCP1. By mass spectrometry, we have recently identified two post-translational modifications (PTM) sites in YY1, K286 and K288, that undergo acetylation when cAMP level increases. The goal of this project is thus to further investigate the functional role of YY1 acetylation in thermogenesis.
Methods. Point mutations of lysine residues at 286 and 288 were generated by either arginine (RR) substitutions to block the acetylation or glutamine (QQ) to mimic the acetylation. Four mutant pBAT cell-lines which stably expressed GFP, FLAG-YY1 (wild type, wt), FLAG-YY1 (K286/288R, RR) or FLAG-YY1 (K286/288Q, QQ) were generated.
Results. Pull down of FLAG-tagged YY1 demonstrated that wt-YY1 and YY1-QQ could interact with PGC1a in response to cAMP signalling, but not YY1-RR, indicating that the interaction between YY1 and PGC1a seemed to be dependent on YY1 acetylation at these two PTM sites. Interestingly, wt-YY1 and YY1-QQ significantly stimulated the expression of both PGC1a and UCP1 at protein levels, further confirming that YY1 might played an important functional role in thermogenesis. This increase in expression was abolished when YY1-RR was over-expressed, indicating that YY1 acetylation might be crucial in the activation of YY1 function in thermogenesis.
Conclusions. Taking together, these data suggest that YY1 acetylation at K286 and K288 is required by PGC1a interaction and for the function of YY1 in activating UCP1-dependent thermogenesis mediated by PGC1. Further studies are needed to investigate if YY1 can be used as a novel therapeutic target for obesity treatment.

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Identification of novel regulators of adiposity and lipid metabolism, role of INMT in metabolic homeostasis.

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Whole body energy balance is maintained through complex regulatory systems which preserve an equilibrium between energy intake and energy expenditure. Disruptions in this equilibrium result in an excess of energy storage, expansion of adipose tissue mass and increase triglyceride content in liver, causing obesity. The molecular mechanisms that maintain energy balance involve multiple levels of regulation, but they are not fully understood. Mouse models have been widely used in the research of obesity. The fact that multiple genetically modified mouse models are protected against diet induced obesity reveals the complexity and multifactorial nature of this condition. In order to identify novel targets of adiposity and lipid metabolism, we correlated the adipose tissue gene expression of 20 inbred mouse strains (on high fat diet) to their total fat content (measured by MRI). We identified Indolethylamine-N-methyltransferase (Inmt) as the most strongly correlated gene, whose adipose tissue gene expression level inversely correlates with adipose tissue mass in mice. INMT is an enzyme that methylates indole rings using S-adenosylmethionine (SAM) as a methyl donor, particularly in the tryptophan metabolism pathway. However, the role of INMT in physiology is poorly understood. Our preliminary data indicates, that Inmt knockdown in mice targeting liver and adipose tissue by antisense oligos (ASOs), leads to a reduction of fat mass, increased hepatic fatty acid oxidation, and improved glucose sensitivity. Interestingly, this is associated with increased phosphorylation of AKT in liver. In conclusion, our results suggest that INMT expression may be detrimental in lipid and glucose homeostasis. The identification of the molecular function of INMT in adipose tissue and liver will help understand the role of specific metabolites in energy homeostasis and could provide alternative ways to target energy imbalance and metabolic disorders.

16.

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The Role of SR proteins SRSF1 and SRSF2 in Adipose Tissue during Metabolic Syndrome

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The serine- and arginine-rich (SR) family of RNA binding proteins is composed of 12 members in humans. Two of those members: SRSF1 and SRSF2 play roles in splicing and transcription. A respectable amount of studies have been carried out to understand and reveal their implication in various types of diseases. However, the same cannot be said regarding the role of SR proteins in metabolic syndrome, especially in adipose tissue. Thus, we set out to determine the role of the SRF1 and SRSF2 during metabolic syndrome in adipose tissue. Preliminary results of in vitro models show changes in SRSF1 and SRSF2 protein expression during the differentiation of white and brown adipocytes. Both genes were increased with β3-adrenergic and Insulin stimulation. Furthermore, 72-hours treatment using IL-6 and TNFα, as insulin resistance models, separately lowered the increase of SRSF1 in response to Insulin. However, there was no significant difference in the expression of SRSF2 between insulin resistant conditions and normal condition. In SRSF1 adipose tissue-specific knockout mice (Srsf1 ATKO), the perigonadal and subcutaneous white adipose tissue (WAT) depots were significantly smaller compared to ones of WT. As for the liver and the brown adipose tissue (BAT), the sizes of these organs were bigger in the Srsf1 ATKO than those of WT mice. At the same time, SRSF2 adipose tissue-specific knockout mice (Srsf2 ATKO) present promising early phenotypes which include impaired insulin sensitivity, higher lean mass, lower fat mass, and smaller perigonadal and subcutaneous WAT depots. Similar size differences were observed in BAT. Srsf2 ATKO mice, gain significantly less weight with high-fat diet (HFD), but show a worse fasting glycemia profile when compared to the wild type (WT) mice.

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DIRECT MEASUREMENT OF GLUCOSE METABOLISM IN VIVO IN HUMAN GBM MICE MODELS BY HYPERPOLARIZED [2H7,13C6]GLUCOSE

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13C MRS of hyperpolarized endogenous compounds via dissolution DNP (dDNP)(Ardenkjaer-Larsen et al., 2003) provides metabolic information in real-time, and has been employed to study tumor metabolism in a variety of animal models (Brindle et al., 2011). Glioblastoma (GBM) are the most malignant primary brain tumor in adults, they exhibit high metabolic activity and are notorious for their resistance to multimodal therapy. Aberrant glucose metabolism is considered a hallmark of cancer, via the so called 'Warburg Effect' , however recent ex vivo studies show evidences for active glucose oxidation in human GBM(Maher et al., 2012; Marin-Valencia et al., 2012). Direct detection of tumor glycolysis can provide new evidences on this debate. Thus the present work relates to the optimization of hyperpolarized 13C glucose experiment for direct detection of cerebral glycolysis in mouse brain(Mishkovsky et al., 2017) and its application in human GBM mouse model. To account for the different compartments of the human GBM, measurements were performed on two types of GBM cell line xenografts, i.e. U87MG tumor that represents core of GBM lesion, and LN-3708GS patient-derived GBM sphere line (Sciuscio et al., 2011) that forms diffuse tumour and represents the infiltrative compartment of GBM. We demonstrate the feasibility to detect tumor glycolysis in real time in GBM mouse model with hyperpolarized [2H7,13C6]glucose. We report that larger amount of hyperpolarized [1-13C]lactate is produced in the focal tumor compared to the infiltrative one after the infusion the hyperpolarized [2H7,13C6]glucose during our measurement time (ca. 50 s), that implies on higher glycolysis rate in the focal tumor compared to the infiltrative one.

18.

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Deciphering the role of a new endothelial protein

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The development of a functional vascular network is a complex process requiring a coordinated response of endothelial and perivascular cells, adjusted to the organ needs. Aberrant formation of blood vessels is associated with several human diseases such as obesity, diabetes and cancer. In this project we investigate the role of a novel endothelial gene Myc target 1 (Myct1). To study Myct1 function in the vasculature, we generated a conditional knockout mouse model for its endothelial-specific ablation. We studied the effects of Myct1 deletion in developmental angiogenesis, using the mouse retina model, and in pathological angiogenesis, using a subcutaneous tumor model. Ongoing experiments investigate the role of Myct1 in adult mice, whose vasculature is mostly quiescent. Our data suggest that targeting Myct1 may represent a novel strategy for regulation of vessel function in several common diseases.

19.

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Cerebral creatine deficiency and lower weight gain in a new KI rat model of creatine transporter deficiency

Lara Duran-Trio, Marc Loup, Cristina Cudalbu, Olivier Braissant

Creatine (Cr) is a nitrogenous organic acid essential for recycling ATP. Cr is synthesized in a 2-step pathway by the enzymes AGAT and GAMT, and specifically transported by SLC6A8. Cerebral Cr deficiency syndromes (CCDS), 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 the first characterization of a new rat model of SLC6A8 deficiency. The knock-in Slc6a8Y389C/y rat strain is based on one same missense point mutation described in human abolishing completely the Cr transporter activity (c.1166A>G; p.Tyr389Cys). Mutant male rats showed absence of Cr peaks in central nervous system by 1H-MRS, and a 40% decrease in body weight gain at 14-18 weeks (as compared to age-matched WT). The homozygous females had the same pattern as mutant males, while heterozygotes were indistinguishable from WT. Astrocytic fibers (GFAP) appeared disorganized, in particular in the cerebellar cortex. Our first results validate this rat model as a promising tool to better understand SLC6A8 deficiency. In particular, morphological alterations of brain structures in our Slc6a8Y389C/y rats, and their loss of weight gain, may help to comprehend and treat human pathology.

20.

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Toward understanding cellular adaptive responses to mitochondrial respiratory chain dysfunction

Christopher Wall, Jaime Santo-Domingo and Andreas Wiederkehr.

The term “mitochondrial disease” describes a broad range of clinical conditions with a very diverse underlying pathophysiology. Disease exacerbations are highly tissue specific, hinting at different cellular adaptations in response to mitochondrial disease. These adaptive responses have been proven effective targets for therapies. We are interested in discovering novel adaptive responses and using these to moderate the mitochondrial disease phenotype in cells. To study this we have developed three different pharmacological models of mitochondrial respiratory chain dysfunction. We treated primary human dermal fibroblasts (HDF) with the respiratory chain (RC) inhibitors rotenone, antimycin A and oligomycin A to repress the function of complex I, III and V of the mitochondrial electron transport chain, respectively. We optimised the models for maximal effects on respiration and cellular proliferation and minimal toxic side effects. In order to identify cellular adaptations to complex I, III and V deficiency, we performed QuantSeq 3’ mRNA sequencing on our models of mitochondrial disease. GO process enrichment analysis revealed differential expression of genes associated in processes previously associated with mitochondrial disease. We obtained information on a common transcriptional fingerprint by exploring enrichment in genes consistently changed in all treatment groups. This provided evidence of a strong downregulation of lipid homeostasis and biosynthesis. There are literature references between dysregulated cholesterol or lipid metabolism and mitochondrial defects. We are now focussed on assessing the contribution of these adaptive responses to the progression of mitochondrial disease.

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mTORC2 inhibition in adipose tissue promotes pancreatic β-cells to secrete insulin

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Abstract: Adipose tissue not only stores energy, but also interacts with other organs to maintain whole body energy homeostasis. Adipocytes secrete messenger molecules, so-called adipokines, which act on brain, liver and pancreas to establish organ communication. Increasing evidence suggests that dysregulation of adipokines leads to the development of metabolic disorders such as Type II Diabetes. However, the regulation of adipokine secretion is poorly understood. Mammalian target of rapamycin complex 2 (mTORC2) is a serine/threonine protein kinase complex that initiates a metabolic switch upon activation by insulin. Here, we show that acute and adipocyte-specific inhibition of mTORC2 causes hyperinsulinemia with no change in blood glucose level. This increase in insulin is due to elevated secretion from pancreatic β-cells. Interestingly, isolated pancreatic islets from adipose-specific mTORC2 knockout mice showed no difference in glucose-stimulated insulin secretion in vitro compared to islets isolated from control mice. Thus, we propose that adipose tissue mTORC2 controls insulin secretion via an adipokine.

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Bioluminescent Assays for Investigating Insulin Action and Steatosis

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Steatosis is the abnormal accumulation of lipids within a cell. A small degree of lipid accumulation can be protective, but an excess of lipids for a prolonged period of time can lead to inflammation, fibrosis, cirrhosis, and potentially carcinoma. Although it can occur in any organ, e.g. the kidneys or heart, the liver is the primary site of lipid metabolism and thus most often associated with steatosis. Nonalcoholic fatty liver disease (NAFLD), and its more advanced form nonalcoholic steatohepatitis (NASH), are the most prominent causes of chronic liver disease worldwide. Here we describe the development of robust, sensitive and easy to use bioluminescent plate-based detection assays for measuring markers of metabolic syndrome and other related biochemical pathways.

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