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LIPIDS AS MEDIATORS OF CELLULAR AND ORGANISMAL FUNCTION
- POSTER ABSTRACTS -

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1 Aguettaz: A novel PLIN3 splicing variant reveals a conserved mitochondrial targeting of perilipin protein family members

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Perilipin3 (PLIN3) is a ubiquitous member of the Perilipins lipid droplet-coating protein family. Due to its lipid binding function, PLIN3 contributes to lipid droplet growth, lipophagy, phosphatidylcholine synthesis and to lipotoxicity cellular protection. PLIN3 is highly expressed in skeletal muscle, where its levels have been correlated with fatty acid oxidation and exercise training.

We identified a PLIN3 splicing variant, hence named PLIN3B. Expression of the canonical PLIN3A and PLIN3B in cells highlighted a specific mitochondrial targeting of the novel isoform. PLIN3B lipid droplet and mitochondria targeting is shared with zebrafish zPlin2 and zPlin3, suggesting a conserved double targeting feature.

PLIN3B expression in HeLa cells led to a reorganization of the mitochondrial network, with swollen mitochondria clustered in the perinuclear area. At the ultrastructural level, electron micrographs revealed alterations of the mitochondrial suborganellar organization. In accordance with the morphological phenotype, mass spectrometry analyses of PLIN3B interactors identified several mitochondrial partners, with a particular enrichment of intermembrane space proteins. zPlin2 and zPlin3 morpholino and CRISPR/Cas9 knocked down zebrafish presented lower spontaneous locomotion and reduced mitochondrial respiration.

We are currently investigating the role of PLIN3B in intraorganellar lipid trafficking and in the modulation of mitochondrial phospholipid balance. In addition, cellular and in vivo models are under development. The discovery of PLIN3B indicates the existence of a splicing-dependent regulation of PLIN3 targeting and a possible ancestral mitochondrial function of the perilipins family members.

2 Blanco-Fernandez: Discovering the immunometabolic reprogramming machinery during macrophage polarization

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Macrophages are sentinels of our innate immune system and express a variety of pattern recognition receptors. While pathogen-derived molecules, such as LPS, trigger the polarization of macrophages into inflammatory effectors (M1-type), other signals instead cause macrophages to dampen inflammation (M2-type). Intriguingly, macrophage polarization is accompanied by intense metabolic reprogramming: whereas M1 macrophages are largely glycolytic, M2 macrophages mainly rely on their mitochondria. To date, the mechanisms that lead to immunometabolic remodeling are not fully understood. However, it is clear that mitochondria play a key role in this process, not only by producing ATP, but also by synthesizing metabolites essential for epigenetic reprogramming and antibacterial molecules such as itaconate.

To discover the machinery that supports macrophage metabolic reprogramming, we performed quantitative proteomics on highly pure mitochondria from bone marrow-derived macrophages. These were either in their naïve state (M0) or polarized with pro- or anti-inflammatory molecules (LPS + IFNγ; IL4 + IL13, respectively). We found highly significant changes in the mitochondrial proteome depending on polarization, including the expression of many known factors such as the itaconate-producing enzyme Irg1, the nucleotide synthesizing enzyme Cmpk2 and the COX-like subunit Nmes1. Our work thus far not only highlighted several metabolic enzymes and transporters differentially expressed during macrophage polarization, but also allowed us to discover novel macrophage-specific mitochondrial proteins of unknown function.

3 Bohnacker: A helminth enzyme instigates a macrophage-mediated immune evasion via a p300-prostaglandin axis

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Bioactive metabolites of arachidonic acid (AA) are important derivatives of polyunsaturated fatty acids (PUFAs) with key roles in infection and inflammation. However, it remained unclear how helminths can affect the AA metabolism and whether this would lead to immune evasion in vivo. Here, we identified an immunoregulatory helminthic glutamate dehydrogenase (heGDH) in the larval extract of the parasite Heligmosomoides polygyrus bakeri (Hpb). We particularly assessed whether heGDH regulates type 2 immune responses by modulating immune cell metabolism. Effects of heGDH on the metabolism of monocyte derived macrophages (MDM) were quantified by mediator profiling via LC-MS/MS (eicosanoids, TCA metabolites). Furthermore, heGDH treated MDM were subjected to RNA and ChIP sequencing to assess effects on gene expression profiles and epigenetic changes. For characterization of immune regulatory effects in vivo, mice were treated with heGDH or neutralized during infection with Hpb. In macrophages, heGDH induced the production of prostanoids and 2-hydroxyglutarate (2-HG), which contributed to the suppression of pro-inflammatory cysteinyl leukotrienes. Moreover, heGDH treated MDM showed an induction of regulatory and type 2 suppressive genes, which depended on histone acetylation via p300 HAT. Treatment of mice with heGDH during Hpb infection resulted in an increased worm burden and PGE2 production of host macrophages, whereas neutralization revealed decreased worm counts. Our findings thus suggest that heGDH mediates immune evasion by affecting macrophage metabolism, particularly through induction of the p300-prostaglandin axis. Thus, anti-inflammatory modulation of macrophages by heGDH may be translated into new immunomodulatory strategies for immune evasion, lipid mediator regulation and chronic infections.

4 Brechet: Phosphatidylcholines from insect eggs reach the plant extracellular space

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Plants defend themselves against various biotic stresses. In Arabidopsis, the salicylic acid (SA) phytohormone is essential to respond against biotrophic pathogens. Interestingly, specific phospholipids called phosphatidylcholines (PC) from insect eggs also trigger the SA pathway. The fate of these PC following oviposition remains however unknown. Knowing that phosphatidic acid (PA) is a known defense signal, we hypothesize that PC may diffuse through the leaf natural barriers into the apoplast, where they would be hydrolyzed by PHOSPHOLIPASE D into PA. To determine the localization of PC upon oviposition, we follow PC tagged with different fluorophores overtime. Then, we aim at determining whether exogenous PC are transformed into PA using fatty acid chain-tagged PC in plants.

5 Domeniconi: Investigating phosphatidic acid signalling specificity through targeted elevation of native lipid species

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Lipids are a diverse group of essential biomolecules, who play a vital role in cells by maintaining structure and participating in signalling events. Phosphatidic acid, the simplest glycerophospholipid, has emerged as a key player in a plethora of cellular processes, both as a precursor for phospholipid synthesis and as a second messenger in central pathways such as MAPK, mTOR or HIPPO. Incidentally, these signalling functions have been shown to lead to very different outcomes depending on its localisation, precursor, or its membrane environment. To investigate phosphatidic acid signalling specificity, we use a chemical biology approach by delivering specific native phosphatidic acid species to live cells in a time-controlled manner using photocleavable 'cages' masking the lipid's function prior to release. Click-chemistry based modifications on the photocage allow sub-cellular targeting to organelles or membrane proteins, and fluorescent reporter proteins are used for lipid monitoring post-release. With this innovative chemical biology technique, we intend to gain new insights in phosphatidic acid signalling at an unprecedented level of specificity.

6 Drozdovska: Effects of exercise intensity and hypoxia on the expression of genes involved in mouse skeletal muscle metabolic pathways

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Exercise training may improve oxygen transport and skeletal muscle metabolism to a larger extent when performed in hypoxia than similar training in normoxia. The aim of the present study was therefore to investigate the expression of genes associated with muscle metabolic pathways in healthy mice, either sedentary or performing low or supramaximal exercise intensities in normoxia versus hypoxia. 8-week-old male C57BL/6J mice were randomly divided into six groups (n=6 mice/group): sedentary, low-intensity or supramaximal intensity training exposed to either normoxia or hypoxia (FiO2 =0.13). Exercise consisted in treadmill running 3 times/week for 4 weeks. Expression of genes involved in glucose, lipid and lactate metabolism, and in mitochondrial biogenesis was determined in the gastrocnemius muscle using real-time qPCR. The present study shows that 4 weeks of exercise training at low and supramaximal exercise intensities in normoxia and hypoxia have moderate effect on several transcriptional adaptations in healthy mouse muscles. However, supramaximal intensity training induced upregulation of genes involved in glucose and lactate transport pathways. Therefore, the present study confirms a specific effect of supramaximal intensity exercise performed in hypoxia on muscular adaptations, when compared to low intensity or to supramaximal intensity in normoxia.

7 Đukanović: ACOT11 links lipid metabolism and sleep wake regulation

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Sleep loss is linked with a higher risk for developing metabolic disorders. Using a system genetics approach in mice, we discovered that a polymorphism in Acot11 (Acyl-CoA Thioesterase 11) affected the Acot11 expression in liver as well as the homeostatic regulation of time spent in NREM sleep. Acot11 encodes an enzyme known to be involved in lipid homeostasis and regulating energy expenditure in tissues with high metabolic rates. Acot11 knockout (KO) mice have been shown to be protected from developing metabolic disorders such as insulin resistance. We deprived Acot11 KO mice of sleep for the first 6h of their main rest phase, which led to transcriptional alterations in cerebral cortex and liver. Many of the dysregulated transcripts relate to metabolic pathways and synaptic function. Moreover, KO mice failed to compensate for the sleep-deprivation (SD) incurred loss of NREM sleep. To assess possible metabolic differences, we performed in vivo calorimetry. The preliminary results indicate that energy expenditure during SD and recovery is higher in KO mice. Lipidomic analysis in liver, cortex, and blood after SD and recovery showed that while SD acutely dysregulated lipids in all three tissues, the largest genotype effects were observed in cortex and concerned mostly cell-membrane associated lipids. To gain further insight into the molecular mechanisms underlying these differences we now study the SD-associated changes in membrane properties in vitro.

8 El Atab: The effect of ApoE rare polymorphisms on ApoE lipid-loading properties and on lipoprotein particles composition

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Apolipoprotein E (ApoE) plays a major role in lipid homeostasis in the brain and periphery. In the brain, ApoE is mainly secreted by astrocytes in form particles that feed the neurons. In return, hyperactive neurons release excessed fatty acids in the form of ApoE-particles that are taken up by astrocytes for detoxification. Humans express three common isoforms of ApoE: ApoE2, ApoE3 and ApoE4, which differ in two amino acids at positions 112 and 158. APOE2 has two cysteines, APOE3 has a cysteine at position 112 and an arginine at position 158, whereas APOE4 has arginine at both positions. ApoE contains two domains: an amino terminal domain that contains the LDL receptor-binding region and a phosphatidyl inositol biphosphates-binding site, and a carboxy terminal domain that have lipid loading properties. ApoE polymorphism combined with aging and environmental stress affect the risk of developing Alzheimer's disease (AD). While ApoE4 allele presents the strongest genetic risk factor for developing AD, ApoE2 and some rare variant are protective. In this project, we study the effect of ApoE rare polymorphism on its recruitment to artificial membranes with different lipid compositions. For this, we purified different GFP tagged ApoE isoforms, full length and truncated domains: ApoE2, E3 and E4, as well as some rare mutations: ApoE3 Christchurch (R136S), APOE3 Jacksonville (V236E) and APOE4-R251G. We study the way these variants handle lipids using a variety of in vitro biochemical assays. Interesting mutants will be expressed in astrocytes to study the effect of these mutations on ApoE-lipoprotein particles composition.

9 Hartung & Haimerl: Extracellular vesicle miRNAs drive aberrant macrophage responses in NSAID-exacerbated respiratory disease

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Background: Extracellular vesicles (EVs) have been implicated in the pathogenesis of asthma, however how EVs contribute to immune dysfunction and type 2 airway inflammation remains incompletely understood. We aimed to elucidate roles of airway EVs and their miRNA cargo in the pathogenesis of NSAID-exacerbated respiratory disease (N-ERD), a severe type 2 inflammatory condition.
Methods: EVs were isolated from induced sputum or supernatants of cultured nasal polyp or turbinate tissues of N-ERD patients or healthy controls by size-exclusion chromatography and characterized by particle tracking, electron microscopy and miRNA sequencing. Functional effects of EV miRNAs on gene expression and mediator release by human macrophages or normal human bronchial epithelial cells (NHBEs) were studied by RNA sequencing, LC-MS/MS and multiplex cytokine assays.
Results: EVs were highly abundant in secretions from the upper and lower airways of N-ERD patients. N-ERD airway EVs displayed profoundly altered immunostimulatory capacities and miRNA profiles compared to airway EVs of healthy individuals. Airway EVs of N-ERD patients, but not of healthy individuals induced inflammatory cytokine (GM-CSF and IL-8) production by NHBEs. In macrophages, N-ERD airway EVs exhibited an impaired potential to induce cytokine and lipid mediator production, while enhancing M2 macrophage activation. Let-7 family miRNAs were highly enriched in sputum EVs from N-ERD patients and mimicked suppressive effects of N-ERD EVs on macrophage activation.
Conclusions: Aberrant airway EV miRNA profiles may contribute to immune dysfunction and chronic type 2 inflammation in N-ERD. Let-7 family miRNAs represent targets for correcting aberrant macrophage activation and lipid mediator responses in N-ERD.

10 Francisco: Highly sensitive and comprehensive LC-MS/MS method for bile acid quantification across tissues and biofluids

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Bile acids are key players in lipid metabolism. Importantly, they don't serve only as "detergents" to facilitate lipid absorption but also as potent signaling molecules or enterohepatic-derived hormones which regulate lipid metabolism and global metabolic health. Furthermore, the bile acids can be chemically modified (through the process of dehydroxylation, deconjugation and dehydrogenation) by the gut microbiome to yield "secondary bile acids", mediators of host-microbiome interactions.
Here, we present a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of 83 species of bile acid in a single run. A solid-phase extraction with internal standard spike warrants a high sensitivity, accuracy and precision for bile acid measurement. Bile acid signatures or composition "barcodes" of different biofluids (e.g., plasma, feces) and brain tissue were acquired, and biofluid- and tissue-associated differences are discussed in the context of tissue-specific metabolic roles. This highly sensitive and quantitative targeted method represents a state-of-the-art strategy for comprehensive and straightforward assessment of bile acid metabolism in biomedical and clinical research.

11 Geller: Cyclin-dependent Kinase 4 (CDK4) is involved in the myelin sheath maintenance of hypothalamic neurons by modulating lipid biosynthesis

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The hypothalamus is the control center for many homeostatic mechanisms. Recent data obtained by our group and others suggested that Cyclin-dependent Kinase 4 (CDK4) could be a key factor in the regulation of hypothalamic regulation. Inhibition of CDK4 expression and/or activity in the hypothalamus altered fat mass gain as well as cold resistance in mice. CDK4 is a critical cell cycle regulator, cell differentiation, and regulator of metabolic processes. However, the role(s) and function(s) of CDK4 in the brain, even more in the hypothalamus, are not well known. The objectives of this study are to determine the cell types that expressed CDK4 in the adult hypothalamus, as well as the role of this protein in hypothalamic functions.
We found that CDK4 is mainly expressed by glial cells in the adult hypothalamus such as oligodendrocytes progenitors (OPC), oligodendrocytes, and astrocytes. These cell types are mainly involved in myelin synthesis and maintenance. Adult male mice deleted for CDK4 (CDK4-KO) were used to explore the role of this protein in hypothalamic myelin maintenance. Hypothalami of CDK4-KO mice not only exhibit lower expression of genes involved in myelin compound/synthesis but also less myelin content. CDK4-KO mice displayed fewer percentage of axons myelinated at the level of projection of oxytocin and vasopressin neurons in the median eminence (ME). In contrast, we do not show differences in the number of axons in the ME or the number of OPC and oligodendrocytes. However, the ultrastructure of myelin differs between both genotypes (i.e higher myelin g-ratio); suggesting that myelinated axons should display reduced condition velocity compared to control littermate. However, the lipidomic study revealed a lower percentage of hexosylceramide content in of hypothalami of CDK4-KO (i.e. Glucosylceramide and Galactosylceramide). Moreover, the expression of the enzyme involved in final step of galactosylceramide named Ugt8a(CGT), is specifically down-regulated in the hypothalamic of these mice.
Mouse deleted for CDK4 present default of myelin sheath of hypothalamic neurons. The alteration of myelin sheath does not seem due to a default of oligodendrocytes ontogenesis but seems due to a default of specific lipid biosynthesis such as Galactosylceramide. Knowing this lipid is the quantitative most significant lipid in myelin, involved in myelin stabilizer, and Ugt8a mutant mice display myelin abnormalities, we can propose that deletion of CDK4 induces a default of hexosylceramide synthesis resulting in impairment of myelin content. We are currently exploring the cellular role of CDK4 in this process.

12 Lagarrigue: LACTB, a new player in lipid modulation

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Mitochondria evolved from alpha-proteobacteria through endosymbiosis. Several mitochondrial proteins are evolutionarily related to bacterial proteins although not always keeping similar functional properties. A good example of this is Lactamase B (LACTB). LACTB, derived from the penicillin-binding/beta-lactamase protein family involved in peptidoglycan synthesis of bacteria, is in eukaryotic cells localized in the mitochondrial intermembrane space. Since mitochondria do not synthesize peptidoglycan, our working hypothesis is that LACTB may have gained one or more novel function(s).

Using the CRISPR-Cas9 technology, we generated a LACTB knock-out zebrafish and developed further LACTB KO fibroblasts. LACTB deletion alters proteins of the mitochondrial electron transport chain, particularly complex I, and decrease mitochondrial function. In parallel, changes in skeletal muscle lipidomics, show modifications of specific glycerophopholipids. In LACTB KO cells, we observe increases in lipid droplets, changes in lysosomal size and function, as well as altered LC3B flux.

Taken together, our results reveal that LACTB plays a potential role in autophagy, mitochondrial function and phospholipids homeostasis. We pursue the effort to understand the physiological and pathophysiological functions of this endosymbiotic protein.

13 Landaluce-Iturriria: Macronutrition and signal-induced regulation of RNA splicing in adipose tissue depots

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The increase of food availability and consumption of high energy dense diets is leading to a worldwide increment in obesity. Obesity is a risk factor for the development of insulin resistance (IR) and several chronic disorders, making its high global incidence a major public health concern. RNA splicing is an important gene expression regulation mechanism in eukaryotic cells that brings transcriptome and proteome diversity. Alterations in alternative splicing (AS) are known to be implicated in a wide range of human diseases, but the role of macronutrient and signaling-controlled RNA splicing in the WAT, and whether AS alterations contribute to the development of metabolic disorders remains unknown. RNA sequencing analysis from white adipose tissue (WAT) from C57BL/6 mice fed with a high fat diet (HFD), replicating conditions of obesity and IR, has shown an enrichment of the spliceosome pathway, alterations in the expression levels of RNA binding proteins (RBPs) and AS alterations. We identified alterations in the expression of Lgals3, Fmr1 and Rbfox2, amongst other RBPs; as well as hundreds of AS alterations, including decreased inclusion of Slc22a17 exon 4 and decreased inclusion of Insr exon 11.To study RBPs-AS networks in adipocytes at a molecular level, we established an in vitro model using 3T3L1 mature adipocytes treated with different stimuli to mimic some of the conditions found in IR and obesity patients: TNFα (inflammation), high levels of FFAs, 25mM glucose (hyperglycemia), 100nM Insulin (hyperinsulinemia). Our results show that, out of the conditions tested, TNFα treatment recapitulates more of the alterations observed in vivo, functionally and at the level of gene expression. Indeed, insulin-induced glucose uptake experiments show a decrease in 2-NBDG uptake in response to insulin in mature adipocytes treated with TNFα, showing that our model replicates insulin resistance. In addition, mRNA and protein levels of Lgals3 were increased in response to TNFα and insulin, while Fmr1 and Rbfox2 showed decreased mRNA expression in response to TNFα. Moreover, in response to TNFα we see a decrease in the inclusion of Insr exon 11 and Slc22a17 exon 4, replicating the alterations observed in vivo. Our in vitro model will be used to analyze RBP-AS networks in order to better understand how macronutrient and signaling-regulated AS can be key modulators of cellular and tissue function in the context of metabolism and investigate whether RBPs or specific AS evets can be targeted pharmacologically to treat obesity.

Keywords: RNA, RNA Splicing, Obesity, Insulin Resistance, Adipocytes, RNA Binding proteins (RBPs), alternative splicing (AS).

14 Landoni: Illuminating elusive mitochondrial dynamic events using smart super-resolution microscopy.

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The highly dynamic nature of the mitochondrial network and its complex inner membrane ultrastructure are crucial for its function and cellular health. Recent advances in live imaging have uncovered numerous events and organellar interactions involved in this process. However, many of these events occur stochastically and on time and length scales that challenge the limits of conventional light microscopy, hindering discovery and quantitative research.

Adaptive event-driven imaging enables us to circumvent the trade-offs of light microscopy by shifting imaging modes based on live event identification using trained neural networks. Thus, we can obtain event-enriched datasets by maximizing the temporal and spatial resolution during the event, while minimizing cellular damage otherwise. In combination with high-throughput super-resolution microscopy, we can systematically map and characterize elusive mitochondrial and organellar events in large numbers and super-resolved dynamic detail.

Using "smart" microscopy and other super-resolution approaches, we identified an uncharacterized mitochondrial behavior, and we structurally and functionally dissect its putative role as a novel content management and quality-control mechanism.

15 Li: Cholesterol Depletion Sensitizes CD4+ T Cell Cytotoxicity in the Context of Cancer Immunotherapy

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Cancer cell progression is often associated with biochemical changes, such as downregulating tumor antigen presentation, secreting immunosuppressive soluble factors, or upregulating immune checkpoints. However, the biomechanical changes are often overlooked during cancer progression and in the context of cancer immunotherapy. Indeed, cancer cells constantly undergo biomechanical changes during cancer progression and metastasis, which can be sensed and responded to by immune cells. Emerging evidence shows that lymphocytes could sense biomechanical cues and translate them into intracellular biochemical signals, a process called "mechanotransduction". However, the implications of mechanosensing in pathological conditions including cancer remain elusive. CD4+ T cells are traditionally known as T helper cells. Recently, a new subset of CD4+ T cells was found to have direct cytotoxicity against tumor cells. Previously, we found that cellular softness could be one of the means that cancer cells use to evade CD8 T cell-mediated immunosurveillance. We also extend this finding to CD4+ T cells. Here, we report found that plasma membrane cholesterol depletion in cancer cells is associated with increased cortical stiffness and underlying cytoskeleton remodeling, immune synapse formation, and immune cell cytotoxicity. We showed that stiffening tumor cells via chronic cholesterol depletion is associated with enhanced CD4+ T cell-mediated killing in vitro. Therefore, we hypothesize that cellular softness could be one of the means that cancer cells use to evade immunosurveillance,

Since cancer cell softening during malignant progression is universal across different cancer cell lines, mechanical engineering of CD4 cells could provide a fundamental understanding on stiffness-mediated CD4+ T cell cytotoxicity and it could also be extremely useful in eradicating tumors that evade immunosurveillance by downregulating their MHC-I molecules.

16 Lisci: A nutrient- and genetic-based approach to modulate cancer proliferation

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The fundamental ability of mammalian cells to survive nutrient restriction enables their survival upon malnutrition, infection and even tumorigenesis. While it is known that cells can adapt to fluctuations in nutrient availability in different tissues, the pathways that enable them to do so have not been fully elucidated. Here, we employed a systematic nutrient screen and CRISPR/Cas9 screen to unravel the molecular mechanisms supporting proliferation in glutamine-depleted conditions. Our results indicate that in glutamine-starved cells, pyruvate addition fuels anaplerosis and enhances the production of aspartate and asparagine, thus promoting nucleotide and protein synthesis. Based on the results of our CRISPR/Cas9 screen, we highlight the malate-aspartate shuttle and biotin metabolism as critical mediators of this process. Taken together, our results provide the first comprehensive analysis of nutrients and molecular mechanisms enabling mammalian cell proliferation upon glutamine restriction.

17 Medina: Omic-Scale High-Throughput Quantitative LC–MS/MS Approach for Circulatory Lipid Phenotyping in Clinical Research

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Lipid analysis at the species level represents a valuable opportunity for clinical applications due to the essential roles that lipids play in metabolic health. However, a comprehensive and high-throughput lipid profiling remains challenging given the lipid structural complexity and diversity. Herein, we present an 'omic-scale targeted LC–MS/MS approach for high-throughput quantification of a broad panel of complex lipid species across 26 lipid (sub)classes. The workflow involves an automated single-step extraction with 2-propanol, followed by analysis using hydrophilic interaction liquid chromatography in a dual-column setup coupled to tandem mass spectrometry with acquisition in the timed- selective reaction monitoring mode. The workflow consists of an initial screen of 1903 species, followed by high-throughput quantification of the detected species. Lipid quantification is achieved by a single-point calibration with 75 isotopically labeled standards from different lipid classes, covering lipid species with diverse acyl/alkyl chain lengths and unsaturation degrees. When applied to human plasma, 795 lipid species were measured with median intra- and inter-day precisions of 8.5 and 10.9%, respectively, evaluated within a single and across multiple batches. The concentration ranges measured in NIST plasma were in accordance with the consensus intervals. Finally, to benchmark our workflow, we characterized NIST plasma materials with different clinical and ethnic backgrounds and analyzed a sub-set of sera (n = 81) from a clinically healthy elderly population. Our quantitative lipidomic platform allowed for a clear distinction between different NIST materials and revealed the sex-specificity of the serum lipidome, highlighting numerous statistically significant sex differences.

18 Panfilova: Exploring the Role of Lipid Droplets in Neural Stem Cells and Their Progeny: A Proteomic Analysis

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Neural stem and progenitor cells (NSPCs) are crucial for brain development and maintenance as they give rise to various brain cell types, including neurons and glial cells. In our recent studies we have demonstrated that lipid droplets (LDs) play an important role for proper NSPC metabolism and proliferation (Ramosaj, Madsen et al., Nature Communications 2021), and that LDs are present in various brain cell types to a much larger extent than previously thought (Madsen et al., BioRxiv 2022). However, the functional significance of LDs in brain cells remains elusive, and it is not known if LDs differ in their composition between different brain cell types. To investigate the potential role of LDs in cellular identity, we established an LD isolation protocol using primary mouse derived NSPCs. We are comparing LD protein profiles among different cellular states, including proliferative NSPCs, quiescent NSPCs, and NSPC-derived astrocytes. Through isolation of LDs from each cell type and subsequent liquid chromatography-mass spectrometry (LC-MS) analysis of the extracted proteins and lipidomic analysis of the lipids, we want to obtain a comprehensive LD proteome dataset spanning different states of NSPCs, ranging from quiescence to differentiation. This will allow us to shed light on the functional role of LDs in NSPCs and their progeny.

19 Parashar: Deciphering the role of Cyclin-Dependent Kinase 4 (CDK4) in cytoskeleton remodeling and its impact on cell metabolism.

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CIG-UNIL

The cell cycle is a highly regulated and controlled series of events that allows proper division of the cell into two daughter cells. Cyclin-dependent kinases (CDKs) are highly conserved regulators of cell cycle progression and phosphorylate multiple substrates to synchronize cell cycle events. Beyond CDKs' known role in cell cycle regulation, it recently emerged as a key metabolic regulators, modulating biological activity of various organelles, such as mitochondria, lysosomes, and lipid droplets. Organelle functioning within the cells is contributed to by organelle distribution, which depends on cytoskeleton filament arrangement. Cyclin-Dependent Kinase 4 (CDK4) is a crucial cell-cycle regulator involved in the early G1 phase, but its importance in the regulation of cytoskeleton filaments is barely understood. Apart from strict cell cycle regulation, some cyclin-CDK complexes, including CDK1, CDK2, CDK4/6, were shown to phosphorylate many cytoskeleton, adhesion, and migratory proteins (Bendris et al., 2015). Based on preliminary morphometrics and phospho-proteomics data in CRISPR-based CDK4 knockout cells, we found that loss of CDK4 induces both morphological changes and alterations of phosphorylation of cytoskeleton-related proteins. Interestingly, these CDK4 knockout cells display increased cell size cell, enhanced migratory properties, but also disruption of mitochondrial distribution. Overall, this study aims to tackle the role of CDK4 in cytoskeleton filament organization and subsequently in these cytoskeleton-mediated phenotypes and metabolic processes by taking advantage of advanced imaging techniques and methods.

20 Petrelli: The role of astrocytic fatty acid beta-oxidation in health and in Alzheimer's disease.

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The breakdown of lipids, called fatty acid beta-oxidation (FAO), is an important metabolic pathway in many tissue. However, the role of this metabolic pathway in the brain remains poorly understood. Recent studies suggest that astrocytes, the most abundant glial cells of the brain, use FAO as a detoxifying mechanism to free stressed neurons from excess fatty acids. These studies have opened up interesting perspectives on how FAO in astrocytes might be relevant for postnatal brain development and normal brain function as well as in the context of Alzheimer's Disease (AD). However, functional studies to prove such connections are still lacking. Using a transgenic reporter GFP mouse line, we show that carnitine palmitoyl transferase (CPT1a), the rate limiting mitochondrial FAO enzyme, is mainly expressed in astrocytes during postnatal brain development and in adult brain. In addition, we show that early postnatal deletion of Cpt1a in immature astrocytes affects their proliferation and maturation, and decreases the number of surrounding glutamatergic synapses. Interestingly, deletion of Cpt1a in mature astrocytes also decreases synapse numbers, but astrocyte morphology is not affected. Taken together, these findings suggest that astrocytic FAO is required for both normal postnatal brain development and adult astrocytic functions. Finally, we also found that deletion of Cpt1a in astrocytes in an AD mouse model significantly increased the number of beta-amyloid plaques and lead to increased astrocyte reactivity. These data highlight the importance of astrocytic FAO in the development of the disease.

21 Pourcel: Enzyme and transporter annotation in UniProtKB using Rhea and ChEBI

Lucille Pourcel and the UniProt Consortium[1,2,3,4]

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The UniProt Knowledgebase (UniProtKB, at www.uniprot.org) is a reference resource of protein sequences and functional annotation. Here we describe a broad ranging biocuration effort, supported by state-of-the-art machine learning methods for literature triage, to describe enzyme and transporter chemistry in UniProtKB using Rhea, an expert curated knowledgebase of biochemical reactions (www.rhea-db.org) based on the ChEBI ontology of small molecules (www.ebi.ac.uk/chebi/). This work covers proteins from a broad range of taxonomic groups, including proteins from humans, plants, fungi, and microbes, and both primary and secondary metabolites. It provides enhanced links and interoperability with other biological knowledge resources that use the ChEBI ontology and standard chemical structure descriptors, and improved support for applications such as metabolic modeling, metabolomics data analysis and integration, and the use of advanced machine learning approaches to predict enzyme function and biosynthetic and bioremediation pathways.

22 Reeves: Highly sensitive and comprehensive liquid chromatography-tandem mass spectrometry method for quantification of steroids within a limited volume of plasma

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Steroids are key regulators in multiple biological processes and are at the origin of sex differences in metabolism (i.e. energy storage, utilization, metabolic signaling). Derived from cholesterol, they are produced in several tissues and transported throughout the body via carrier proteins in the plasma, with biologically active concentrations varying from micromolar to picomolar. Fluctuations in their levels with reproductive aging (age-related androgen decline and "loss" of estrogen in menopause) have an important impact on lipid metabolism and predisposition to non-communicable, cardiometabolic diseases (CMDs). Measuring the global steroid profile is crucial to inform about the hormonal status and allow for accurate stratification of a population with reproductive aging.
Here, we present a high throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of 26 steroids from a single preparation, from a limited plasma volume of 100µL or less. An internal standard spike, followed by solid-phase extraction, warrants a high sensitivity, accuracy and precision for steroid measurement. This analysis includes progestogens (pregnenolone, progesterone, 17α-hydroxyprogesterone, pregnenolone sulfate), androgens (dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androsterone, androstene-3,17-dione, 5α-dihydrotestosterone, epitestosterone, testosterone, etiocholanolone), estrogens (estrone, estradiol, estriol, estrone sulfate, ethynylestradiol), glucocorticoids (cortisol, 11-deoxycortisol, 21-deoxycortisol, cortisone), and mineralocorticoids (aldosterone, corticosterone, 11-deoxycorticosterone). This highly sensitive and quantitative targeted method represents a state-of-the-art strategy for comprehensive and straightforward assessment of steroid metabolism in biomedical and clinical research.

23 Rumpler: The role of a novel visceral IGFBP2+ stromal population in regulating adipose tissue expansion

Marie Rumpler, Radiana Ferrero, Pernille Rainer, Julie Russeil, Carles Canto, Bart Deplancke

EPFL

Given the worrying increase in the worldwide prevalence of obesity, studying the onset of this debilitating syndrome is of great interest. However, our knowledge of the developmental origin of adipocytes that make up the bulk of fat tissue is still very limited. Using single cell transcriptomics (scRNA-seq), the Deplancke Lab (EPFL) has recently examined the composition of adipose stem and progenitor cells (ASPCs) within the stromal vascular fraction (SVF) of mouse and human adipose tissue depots. Interestingly, one subpopulation in mouse subcutaneous adipose tissue (SAT) exhibited a remarkable capacity to inhibit mammalian adipocyte formation in vitro and in vivo, hence why these cells were termed Adipogenesis Regulators (Aregs). While Aregs have so-far mainly been characterized in murine and human SAT, visceral adipose tissue (VAT) is the one associated with metabolic complications. Visceral ASPCs display very limited adipogenic potential when cultured ex vivo, and the contribution of specific VAT ASPC subpopulations to metabolic disbalance is unclear. Using scRNA-seq, we have recently characterized the cells in the SVF of human omentum fat biopsies. This led us to the identification of a mesothelial-like new population displaying an "Areg-like function". This omental population is identified by high expression of the insulin growth factor binding protein 2 (IGFBP2). Similar to Aregs, these cells are able to inhibit adipogenesis which likely occurs through the action of secreted IGFBP2 itself. Importantly, preliminary analyses hint to the existence of an IGFBP2+ mesothelial population also in murine epididymal adipose tissue.

24 Sudria-Lopez: The Role of Fatty Acid Beta-Oxidation in Human Brain Development

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Neural stem/progenitor cells (NSPCs) are the stem cells that give rise to the entire brain and even continue to form new neurons throughout life. Understanding what regulates NSPC behavior is thus important for development and adulthood. Recently, metabolism has been shown to have an important role in the regulation of stem cell activity/fate in different tissues. Previously, Knobloch and colleagues specifically described the importance of lipid metabolism for NSPC quiescence, proliferation and integration of their progeny in the mouse brain. However, whether lipid metabolism plays a similar role in the regulation of human NSPCs remains poorly understood.
Therefore, we aim to assess the importance of lipid metabolism, specifically the breakdown of lipids, for human NSPCs and during human brain development.
To do so, we are targeting carnitine palmitoyl transferase 1a (CPT1A), the rate-limiting enzyme of fatty acid beta-oxidation (FAO) using the pharmacological FAO inhibitor etomoxir and shRNAs against CPT1A. We are using monocultures of in vitro derived humans NSPCs to assess the effect of FAO blockage for NSPC proliferation, apoptosis, quiescence and differentiation capacities. To better understand the effect of FAO inhibition during brain development, we are also using cerebral organoids and neural rosettes, which model early brain development.
Our results show that CPT1A is highly expressed in NSPCs during brain development. While blocking FAO in human NSPC monocultures only showed subtle effects, blocking FAO in cerebral organoids strongly reduced NSC proliferation and increased cell death, suggesting that FAO is indeed an important metabolic pathway for human NSPCs.

25 Tabasso: "Oh my DAG", unveiling the specific link between human insulin resistance and lipotoxicity

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Obesity and type 2 diabetes have important consequences on morbidity and mortality. Ectopic lipid depositions, defined as an excess accumulation of lipids in non-adipose tissues, are positively associated with obesity and are key determinants of insulin resistance (IR). Among other lipids, diacylglycerols (DAG) have been incriminated in driving a large part of the lipotoxicity theory, but which DAG moieties are responsible of this negative effect is not yet known.
In this project, we will describe the distribution of DAG moieties in the different organelle of muscle from donors with and without IR to unveil which DAG species are associated with IR. To this aim, organelle fractions will be extracted from muscle biopsies of lean and obese volunteers. Custom isomer specific standards will be used as reference for the quantification of organelle-specific DAG in a precision medicine approach, permitting determination of fatty acid chains position, chain length and saturation degree.
After the identification of target DAG moieties, we will characterize their mechanisms of actions. For this, specific caged-DAG will be specifically delivered to organelles, i.e. mitochondria, lipid droplets (LD) and plasma membranes, to allow a local increase of the targeted DAG species. This will be performed in human primary muscle cells of insulin resistant and insulin sensitive individuals. Phosphorylation pathways activated by DAG, as well as insulin sensitivity and mitochondrial capacity will be measured as main outcomes.
This project will allow to describe the causal paradigm and identify those DAG species that are "lipotoxic".

26 Teav: Leading-edge mass spectrometry platform for deep annotation and quantification of polar and lipid metabolome

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Metabolomics, along with lipidomics, has emerged as a high-throughput technology to complement the comprehensive omics data acquisition generated through genomics, transcriptomics, and proteomics, to facilitate a systems biology approach. Here, we present technological approaches and methodologies offered by our mass-spectrometry based platform for polar and lipid metabolite measurement across diverse biological matrices, collected in model systems (e.g., cell, organoid, tissue lysates) as well as human population and clinical intervention studies. The approaches comprise High Resolution Mass Spectrometry (HRMS) untargeted, quantitative targeted analysis, and isotopic profiling. Untargeted screening serves as a discovery strategy for qualitative characterization of matrix composition and deep annotation of polar and lipid metabolome. Targeted analysis can be divided in two categories: single-pathway and multiple-pathway targeted. Single pathway targeted analysis implies the quantification of metabolites (relative or absolute) in one specific pathway of interest (e.g., glycolysis, TCA cycle, amino acid metabolism, ß-oxidation, bile acid metabolism, etc.). Multiple-pathway targeted analyses were implemented to bridge the gap between traditional targeted quantification (focus on one pathway) and untargeted, aiming to detect as broad range of metabolites as possible. The multiple-pathway or high-coverage targeted analysis can be performed with a focus on polar metabolites (involved in multiple pathways of central carbon metabolism) or complex lipids (e.g., glycerolipids, glycerophospholipids, sphingolipids, cholesterol esters). Finally, the isotopic profiling approach is used to track the fate of labelled nutrient and deduce the pathway activity in different conditions associated with the investigated phenotype. The applied modes of analysis and coverage of each method will be presented.

27 Van Gijn: An epigenetic analysis to uncover the roles of transcription factors in the inhibitory function of adipogenesis regulators (Aregs)

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Adipose stem and progenitor cells (ASPCs) play an important role in the expansion of adipose tissue during obesity via hyperplasia. Recently, we discovered a distinct murine ASPC subpopulation in mouse subcutaneous adipose tissue, characterized by high F3 expression, encoding for CD142. These CD142+ ASPCs are refractory to adipogenesis. Additionally, they provide paracrine signals capable of inhibiting adipogenesis in neighboring adipogenic cells. Hence, these cells were named adipogenesis regulators (Aregs). The secretory factors expressed specifically by Aregs also shift CD142− ASPCs into a non-adipogenic Areg-like state. These observations suggest that the transcriptional identity of Aregs is precisely regulated and dynamic. However, thus far it remains unclear how epigenetics regulate and result in the Areg specific transcriptome and which transcription factors TFs play a role in their transcriptional regulation. We will knock down Areg specific TFs and using bulk RNA- and ATACseq determine what effect this has on a chromatin accessibility and transcriptional level. Additionally, we will investigate the effects of recombinant CD142 protein on a chromatin accessibility level of the adipogenic ASCPS population. Since, we already know they become transcriptomically more like Aregs when treated with CD142. Collectively, these results help our understanding of the molecular mechanisms governing the unique Areg phenotype and open new therapeutic possibilities for the manipulation of fat expandability.

28 Vonaesch: Stunted child growth is associated with small intestinal colonization by oral bacteria driving lipid malabsorption and inflammation in vitro

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Environmental enteric dysfunction (EED) is an inflammatory disease postulated to contribute to stunted child growth and to be associated with intestinal dysbiosis and nutrient malabsorption. To characterize the microbiota in EED we performed a cross-sectional study in two African countries recruiting roughly 1'000 children aged 2-5 years. We assessed the microbiota in the stomach, duodenum and feces and investigated the role of clinical isolates in EED pathophysiology using tissue culture and animal models. We find overgrowth by ectopically colonizing, oral bacteria in the small intestine, leading to increased permeability and inflammation and to replacement of classical small intestinal strains. Most importantly, these oral isolates directly decrease lipid absorption in both cultured enterocytes and mice, providing a mechanism by which they may exacerbate EED and stunted child growth. These findings have important therapeutic implications for modulating the microbiota through microbiota-targeted interventions.

29 Wang: Microbiota mediated fat whitening enhances anti-tumor immune response under warm exposure

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Background and Aims: Change of environmental temperature induces immune responses in both cellular and humoral aspects in humans and animals. However, whether cancer occurrence and tumor progression could be associated with environmental temperature, and how the whole-body metabolism alterations due to temperature change influence the tumor microenvironment, remains unappreciated. We have shown that the mouse gut microbiota composition is dramatically changed following cold temperature exposure and that, reversely, warmer temperatures also drive microbiota changes, accompanied by suppressed adipose browning and increased intestinal absorption. Specific lipids such as saturated fatty acids, mainly produced by gut microbiota fermentation, have been linked to an increased risk of certain types of cancer development. In addition, adipose tissue plays a vital role in regulating the tumor microenvironment by storing and generating lipids, which provide energy for cancer cell survival. This study addresses the role of the gut microbiota and the adipose tissues in the response of tumors to warm exposure. Moreover, using intestine-specific knock-out of lipid metabolism, we elucidate the metabolic requirements for gut growth during tumor development and address the intestine's role in overall adaptation to warm exposure. Together, our study provides multiple insights into the mechanisms that regulate anti-tumor immune response through the temperature-mediated interaction between adipose tissue, the microbiota, and the host, which may lead to development of anti-tumor immunotherapies.

30 Wedemann: Tracking the secrets of diacylglycerol transport and metabolism in the cell

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Diacylglycerols are important secondary messenger lipids involved in many cellular processes, leading to a wide range of signaling outcomes from cellular proliferation or mobility to apoptosis. However, cells produce a plethora of diacylglycerol species varying in chain length, saturation, or linkage to the glycerol backbone. Increasing evidence indicates lipid species specificity of effector proteins, and additionally a dependency on the lipid's subcellular localization. However, the role of individual lipid species in specific signaling pathways is largely understudied mainly due to a lack of methodology to study individual lipid species.

To close this methodological gap and investigate individual lipid species involved in cellular signaling, we synthesize trifunctionalized lipids, which allow for the investigation of individual lipid species in a temporally and spatially defined manner. The lipids are functionalized with a photocage at the headgroup, masking the lipid from effector proteins, driving subcellular localization as well as timely controlled lipid release. The probes further enable the fixation of the lipid to the surrounding proteins to allow for pulse-chase experiments as well as a click handle to attach moieties for visualization or omics-analyses. Using trifunctional diacylglycerols, we would like to elucidate species-specific, time-resolved lipid localization, and metabolic state analysing the lipid's fate after signal initiation. Additionally, we are aiming to identify transporting and metabolizing proteins involved in the post-signaling processes to obtain insights into the role of individual species in signaling.

31 Ziegler: Cyclin-Dependent Kinase 4 regulates mitochondria-ER contacts modulating mitochondrial dynamics, calcium signaling and apoptosis in breast cancer cells

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The shift on energetic demands of proliferating cells during tumorigenesis requires an intense crosstalk between cell cycle and metabolism. Beyond their role in cell proliferation, cell cycle regulators also modulate intracellular metabolism of normal tissues. Nevertheless, in the context of cancer, where CDK4 is upregulated or stabilized, the metabolic role of CDK4 is barely understood. Accounting for 15-20% of breast cancer worldwide, triple-negative breast cancer (TNBC) is characterized by its aggressiveness and remains a challenging disease due to the lack of selective therapies. Using both genetic and pharmacological approaches, we aimed to determine the metabolic role of CDK4 in TNBC cells. Unexpectedly, CRISPR-Cas9-mediated deletion of CDK4 only slightly reduces cell proliferation of TNBC cell line and allows tumour formation. Furthermore, CDK4 deletion deeply affects mitochondrial morphology, leading to hyperfused mitochondria and reduced S616 phosphorylation of pro-fission protein Drp1. Surprisingly, multiple pro-apoptotic stimuli fail to induce proper cell death in CDK4-depleted or long-term CDK4/6 inhibitors-treated TNBC cells. Mechanistically, CDK4 depletion impairs mitochondrial-ER contacts thus reducing calcium fluxes upon pro-apoptotic stimuli. Taken together, these results suggest that CDK4 inhibition leads to cell death resistance limiting mitochondrial apoptotic functions through dampened ER-mitochondrial calcium signalling in breast cancer cells. While CDK4/6 inhibitors constitute future valuable anti-tumoral therapeutic approaches, altered mitochondrial pro-apoptotic function upon CDK4 inhibition could generate cell-death resistant cells, further increasing future risk of cancer relapse in treated patients.