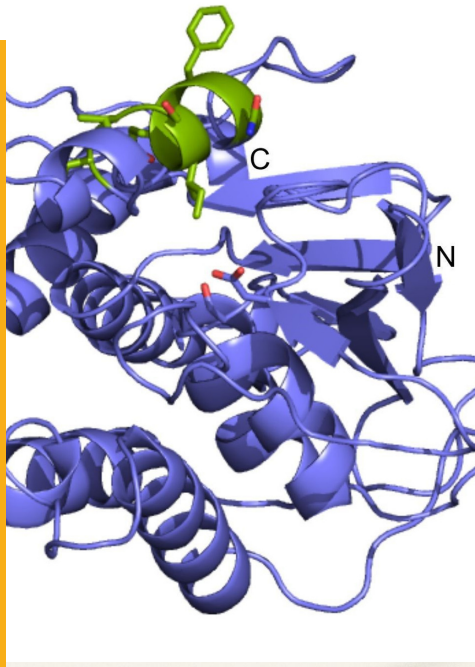
THE 8TH INTERNATIONAL SYMPOSIUM

ON LIPID AND MEMBRANE BIOLOGY

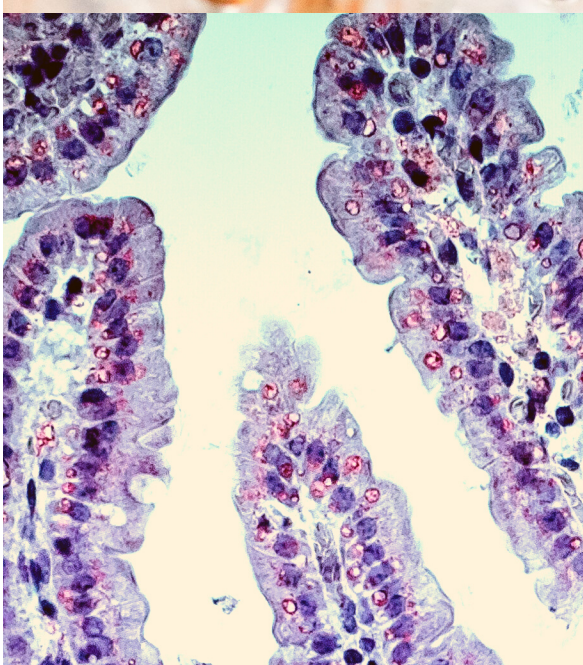


APRIL 21 - 23, 2022

MEDICAL UNIVERSITY OF GRAZ

LECTURES, SHORT TALKS & POSTERS

ABSTRACT BOOK



SFB Lipid Hydrolysis

8TH INTERNATIONAL GRAZ SYMPOSIUM ON LIPID AND MEMBRANE BIOLOGY

APRIL 21-23, 2022 GRAZ, AUSTRIA

PROGRAM

Conference Venue: Medical University of Graz, Aula, Neue Stiftingtalstrasse 6, 8010 Graz

THURSDAY, April 21, 2022

12^{15} - 12^{30}	Registration and Mounting of Posters	
12^{30} - 12^{45}	**Opening of the Scientific Symposium**	
Session 1		Chair: Dagmar Kratky
12^{45} - 13^{30}	Plenary Lecture 1	**Richard LEHNER**, Edmonton, Canada Deletion of arylacetamide deacetylase (AADAC) reduces hepatic triacylglycerol and cholesteryl ester turnover and accelerates steatosis?
13^{30} - 14^{15}	Plenary Lecture 2	**Erin KERSHAW**, Pittsburgh, USA A novel transcriptional regulator linked to energy and metabolic homeostasis in humans
14^{15} - 14^{35}	Short Talk 1	**Gabriel CHALHOUB** The role of Carboxylesterase 2 proteins in lipid hydrolysis
14^{35} - 15^{05}	*Coffee Break*	
Session 2		Chair: Guenter Haemmerle
15^{05} - 15^{50}	Plenary Lecture 3	**Rosalind COLEMAN**, Chapel Hill, USA Lipid Droplets and the Compartmentalization of Lipid Metabolism
15^{50} - 16^{10}	Short Talk 2	**Peter HOFER** Towards a mathematical model of intracellular, neutral lipolysis
16^{10} - 16^{30}	Short Talk 3	**Margarita SCHRATTER** PNPLA1 is a triacylglycerol estolide hydrolase/transacylase involved in omega-O-acylceramide biosynthesis
16^{30} - 17^{00}	*Coffee Break*	
Session 3		Chair: Rudolf Zechner
17^{00} – 18^{00}	**Keynote Lecture 2022** BioTechMed GRAZ	**Jeffrey FRIEDMAN**, New York, USA Leptin and the regulation of food intake and the body weight

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APRIL 21-23, 2022 GRAZ, AUSTRIA

FRIDAY, APRIL 22, 2022

Session 4		Chair: Ruth Birner-Gruenberger and Michael Trauner
09^{00} - 09^{45}	Plenary Lecture 4	**Stefano ROMEO**, Gothenburg, Sweden PSD3 as a promising therapeutic target to treat fatty liver disease
09^{45} - 10^{30}	Plenary Lecture 5	**Judith STORCH**, New Brunswick, USA Intracellular cholesterol trafficking by NPC proteins
10^{30} - 11^{00}	*Coffee Break*	
11^{00} - 11^{45} Ph.D. training MOLECULAR METABOLISM - MOBILES	Plenary Lecture 6	**Martin KLINGENSPOR**, Munich, Germany Prandial secretin-mediated thermogenesis in brown fat controls food intake
11^{45} - 12^{05}	Short Talk 4	**Isabella POTOTSCHNIG** Mechanisms of adipose tissue atrophy in cancer cachexia
12^{05} - 12^{25}	Short Talk 5	**Isabel REINISCH** P53 shapes adipose tissue immune cell infiltration upon intermittent fasting in obese mice
12^{25} - 14^{20}	*Lunch*	Poster Session
Session 5		Chair: Ulrike Taschler and Robert Zimmermann
14^{20} - 15^{05}	Plenary Lecture 7	**Grant Mitchell**, Montreal, Canada Lipolysis and biochemical genetics; a clinical perspective
15^{05} - 15^{25}	Short Talk 6	**Dominik BULFON** Bis(monoacylglycerol)phosphate protects from cationic amphiphilic drug - induced cytotoxicity
15^{25} - 15^{45}	Short Talk 7	**Manu Manjunath KANTI** Adipose triglyceride lipase mediated lipid catabolism is essential for bronchiolar regeneration
15^{45} - 16^{15}	*Coffee Break*	
16^{15} - 16^{35}	Short Talk 8	**Anne GEMMINK** Functional ATGL is important for maintaining mitochondrial network integrity and respiration in human primary myotubes
16^{35} - 17^{20} DP-iDP	Plenary Lecture 8	**Dominique LANGIN**, Toulouse, France Hormone-sensitive lipase a central integrator of adipocyte metabolism

17^{20} - 18^{05}	Plenary Lecture 9	**Alison KOHAN,** Pittsburg, USA ApoC-III's inhibition of LDLr: if it's good for the heart, is it good for the gut?
18^{30}	**Conference Dinner**	Departure by bus (meeting point: AULA)

SATURDAY, APRIL 23, 2022

Session 6		Chair: Monika Oberer and Renate Schreiber
09^{00} - 09^{45}	Plenary Lecture 10	**Ulrich KINTSCHER**, Berlin, Germany The role of cytosolic lipolysis in cardiac function and heart failure
09^{45} - 10^{05}	Short Talk 9	**Ivan BRADIĆ** Off-target effects of the lysosomal acid lipase inhibitors Lalistat-1 and Lalistat-2 on neutral lipid hydrolases
10^{05} - 10^{25}	Short Talk 10	**Gernot GRABNER** Small molecule inhibitors targeting lipolysis in human adipocytes
10^{25} - 10^{50}	*Coffee Break*	
10^{50} - 11^{35} PhD Program MOLMED	Plenary Lecture 11	**Sander KERSTEN**, Wageningen, The Netherlands Regulated inhibition of extracellular and intracellular lipolysis
11^{35} - 12^{20} BIOMOL STRUCT	Plenary Lecture 12	**Michael PLOUG,** Copenhagen, Denmark Intrinsically disordered regions and low protein stability drives regulation and compartmentalization of LPL activity
12^{20} - 12^{30}	Best Poster Awards Best Short Talk Award	
	Closing of Symposium	
12^{30} - 13^{30}	*Lunch*	Take down of Posters
Social Program		
afternoon	**Styrian Armoury**	Guided tour from 3 to 4 p.m.

Plenary Lectures

&

Short Talks:

Abstracts

Deletion of arylacetamide deacetylase (AADAC) reduces hepatic triacylglycerol and cholesteryl ester turnover and accelerates steatosis

Richard Lehner

University of Alberta, Department of Cell Biology, *Edmonton*, Alberta, Canada

Nonalcoholic fatty liver disease (NAFLD) is characterized by the accumulation of triacylglycerols (TG) and cholesteryl esters (CE) in the liver. Hepatic TG and CE synthesis, lipolysis and transport are regulated by nutritional status. Fasting increases hepatic TG/CE storage and this storage is normally rapidly depleted during refeeding. We have found that an endoplasmic reticulum-localized lipase arylacetamide deacetylase (AADAC) is required for hepatic TG/CE turnover during refeeding. Hepatic TG/CE depletion was abolished during the refeeding period in AADAC deficient mice resulting in accelerated progression of hepatic steatosis during Western-type diet feeding. Excessive hepatic lipid storage in AADAC deficient mice occurred despite increased abundance of adipose triglyceride lipase (ATGL) and decreased expression of Pnpla3. AADAC deficiency led to impaired whole-body metabolism, aggravated obesity and insulin resistance. These findings indicate that AADAC plays an important role in hepatic TG and CE metabolism and that inactivation of AADAC activity leads to fatty liver and metabolic disorder.

Notes:

A novel transcriptional regulator linked to energy and metabolic homeostasis in humans

Erin Kershaw

Associate Professor of Medicine, Chief, Division of Endocrinology, University of Pittsburgh, Endowed Chair for Obesity and Diabetes Research, University of Pittsburgh, Pittsburgh, PA, USA

Obesity and diabetes are global public health threats. Although multiple factors contribute to these complex diseases, they are well-known to have strong heritable components. Over the last several decades, genome-wide association studies (GWAS) have uncovered multiple loci associated with metabolic traits, and yet these loci collectively explain only a small portion of the phenotypic variance. Most GWAS were performed in majority populations (i.e. Caucasian/European), thereby missing genetic diversity provided by inclusion of underrepresented groups. To overcome this challenge, our collaborative group conducted a GWAS of metabolic traits in Samoans – a population that suffers from the highest prevalence and severity of obesity in the world. These studies identified several novel quantitative metabolic trait loci. One such locus is a missense variant in Creb3 regulatory factor (CREBRF) [rs373863828 (p.Arg457Gln); CREBRFR457Q]. CREBRFR457Q is associated with increased risk of obesity but, unexpectedly, lower risk of diabetes and other obesity-related comorbidities (i.e. dyslipidemia). Very little is known about CREBRF. It was first identified as a "regulatory factor" that inhibits the CREB3-mediated transcriptional response to ER/golgi stress. More recently, the Drosophila ortholog of CREBRF was shown to mediate the majority of the downstream transcriptional response to the well-known cellular nutrient/energy sensor mTOR. These data suggest that CREBRF regulates the transcriptional response to cellular stress, however, the mechanisms by which CREBRF contributes to energy, glucose, and lipid homeostasis remain unknown. To understand the function and physiological relevance of CREBRF and Its CREBRFR457Q variant, we generated mice with global variant knockin (CREBRFR458Q), global knockout (CREBRFKO), and global transgenic overexpression (CREBRFTG) of CREBRF. We performed extensive phenotypic analysis of mice at baseline and in response to acute (fasting/refeeding) and chronic (low- and high-fat diet feeding) nutritional stress. We then performed gene/protein expression analysis to identify mechanisms mediating the resulting phenotypic effects. Additional studies were performed in metabolically-relevant cell types to more specifically determine cell-type-specific and context-specific effects and molecular mechanisms. Results will be discussed in this seminar. Overall, these studies indicate that CREBRF has broad, context-specific molecular, cellular, and physiological effects, and that the CREBRFR458Q variant functions, at least in part, as gain-of-function variant. Future studies will continue to clarify the impact of CREBRF and associated functional variants on disease-relevant phenotypes and to identify causal networks that can be targeted for therapeutic intervention in humans.

Notes:

The role of carboxylesterase 2 proteins in lipid hydrolysis

Gabriel Chalhoub[1], Helgit Eisner[1], Ulrike Taschler[1], Alina Jamnik[1], Rudolf Schicho[2], Monika Oberer[1], Gabriele Schoiswohl[1], Guenter Haemmerle[1]

[1]Institute of Molecular Biosciences, University of Graz, 8010 Graz, Austria
[2]Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, 8010 Graz, Austria

Human and murine Carboxylesterase 2 (CES2/Ces2) proteins exert a well-established role in hydrolyzing xenobiotics and (pro)drugs. Recently, human CES2 and murine Ces2c have been discovered as triglyceride (TG) hydrolases implicated in the development of obesity and fatty liver disease. In line, we recently showed that intestine-specific Ces2c overexpression counteracts diet-induced obesity and NAFLD in mice. Based on previous studies, Ces2c has been proposed as the murine ortholog of human CES2. Considering that the murine Ces2 family consists of seven homologous proteins it is currently unclear whether other Ces2 members may exert similar roles in lipid metabolism compared to human CES2. The lipid hydrolytic activities of Ces2 protein family members awaits further investigation, which prompted us to examine the enzymatic activities of all mouse Ces2 members toward TGs, diglycerides (DGs), and monoglycerides (MGs) as lipid substrate. Besides CES2/Ces2c, we measured significant TG hydrolytic activities for Ces2a, Ces2b, and Ces2e. Notably, these Ces2 members and CES2 efficiently hydrolyzed DGs and MGs, and their activities even surpassed those measured for TG hydrolysis. Additionally, we observed divergent expression of Ces2 genes in the liver and intestine of mice on a high-fat diet, which could relate to changes in lipid signaling. Finally, reduced CES2 expression in the colon of patients with inflammatory bowel disease and a similar decline in the mRNA expression levels of several Ces2 members in the colon of a murine colitis model implicates a role of CES2/Ces2 members in gut lipid metabolism. Together, these results demonstrate that CES2/Ces2 members are highly efficient DG and MG hydrolases that may play an important role in liver and gut lipid catabolism and signaling.

Notes:

Lipid Droplets and the Compartmentalization of Lipid Metabolism

Rosalind A. Coleman

Professor Emerita of Nutrition and Pediatrics, University of North Carolina at Chapel Hill, Durham, NC 27707, USA

Mammalian lipid droplets (LDs), first described as early as the 1880s, were virtually ignored for more than 100 years. Between 1991 and the early 2000s, however, a series of discoveries and conceptual breakthroughs led to a resurgent interest in obesity as a disease, in the metabolism of intracellular triacylglycerol (TAG), and in the physical locations of LDs as cellular structures with distinct associated proteins. Together with the endoplasmic reticulum, peroxisomes, and the outer mitochondrial membrane, we now recognize LDs as structures that organize the cytosolic compartmentalization of lipid metabolic pathways. In particular, when we consider the physiological changes that control the up- or down-regulation of TAG synthesis from glycerol-3-phosphate and activated fatty acids, we must consider not only alterations in mRNA and protein levels and changes in posttranslational modification, we must think about pathway location. A final critical level of metabolic control lies in the temporal associations of membranes with specific highly organized multi-enzyme assemblies

Notes:

Towards a mathematical model of intracellular, neutral lipolysis

Peter Hofer[1], Jan Elias[2], Yury Sasunov[2], Mariana Colaço-Gaspar[1], Renate Schreiber[1], Klemens Fellner[2], Rudolf Zechner[1,3]

[1]Institute of Molecular Biosciences, University of Graz, Austria.
[2]Institute of Mathematics and Scientific Computing, University of Graz, Austria.
[3]BioTechMed-Graz, Graz, Austria.

Intracellular lipolysis is a central biochemical pathway encompassing the enzymatic degradation of triglycerides (TGs) within cells. Lipolysis-derived products are of fundamental importance since they serve es energy substrates, precursors for a wide range of lipid species, and signaling molecules. From a classical viewpoint, neutral intracellular lipolysis is thought to happen in a linear manner through the consecutive action of three lipases: Adipose triglyceride lipase (ATGL) hydrolyzing TGs, hormone-sensitive lipase (HSL) hydrolyzing diglycerides (DGs) and monoglyceride lipase hydrolyzing monoglycerides (MGs). However, there is evidence that this simplistic concept of neutral lipolysis needs to be revisited. For example, ATGL was reported to exhibit acylglycerol transacylase as well as DG and phospholipid hydrolase activities in addition to its TG hydrolase activity. HSL is capable of hydrolyzing TGs and MGs in addition to DGs. These findings favor a novel concept of intracellular lipolysis as a process of functional redundancies. Despite literally thousands of studies on the physiological role of lipolysis, a quantitative description of the intricate reaction processes is missing. In an interdisciplinary approach, our main goal is to apply mathematical modeling to quantitatively describe the reactions of neutral lipolysis. A major part of the project is to obtain data from in vitro lipolysis assays with purified ATGL. For the first time, we developed a protocol for the efficient purification of full-length and truncated variants of murine ATGL from a human cell expression system. In addition, we established a reproducible enzymatic assay using phospholipid-emulsified acylglycerol substrates supplemented with radioactive tracers that allow the accurate quantification of reaction products at narrow time intervals. Purified murine ATGL exhibited robust dose- and time-dependent TG hydrolase as well as DG transacylase activities. Phospholipid hydrolase activity could be measured, albeit at comparatively low rates. Our kinetic data from in vitro enzymatic activity assays are currently used to inform a mathematic model which will help us to dissect the relative contribution of ATGL to each step of lipolysis and the physiological role of transacylation.

Notes:

PNPLA1 is a triacylglycerol estolide hydrolase/transacylase involved in omega-O-acylceramide biosynthesis

Margarita Schratter[1], Patrick Dobrounig[2], Till Schreiner[2], Thomas Züllig[1], Thomas O. Eichmann[3], Rolf Breinbauer[2], Achim Lass[1], Franz P.W. Radner[1]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria.
[2]Institute of Organic Chemistry, Technical University of Graz, Graz, Austria.
[3]Core Facility Mass Spectrometry, ZMF, Medical University Graz, Graz, Austria.

Patatin-like phospholipase domain-containing 1 (PNPLA1) catalyzes the final step of omega-O-acylceramide (acylCer) biosynthesis essential for skin permeability barrier formation. This transacylation reaction requires the co-activator alpha/beta-hydrolase domain-containing 5 (ABHD5) for efficient esterification of omega-OH-ceramides with linoleic acid (LA). Triacylglycerol (TAG) has been suggested as LA donor for this reaction. However, our analyses of epidermal lipids from PNPLA1- and ABHD5-deficient mice revealed that LA does not specifically accumulate in TAG, but instead in TAG estolides. This endogenous lipid class represents a major storage form of bioactive estolides in adipose tissue, which exhibit anti-inflammatory and anti-diabetic effects in mice and humans. To date, no published data exist on the role of TAG estolides in the formation of the skin permeability barrier. Based on our findings, we hypothesize that-TAG estolides may act as LA donor for PNPLA1-mediated acylCer biosynthesis. We therefore performed in vitro transacylase assays using a cell-free protein expression system. In this assay, we directly expressed PNPLA1 into the lipid membrane of liposomes containing the putative LA donor and omega-OH-ceramides as LA acceptor. Mass spectrometry analysis then revealed that acylCer formation was increased in the presence of TAG estolides compared to TAG substrate. Taken together, in this study we identified PNPLA1 as a hydrolase/transacylase of TAG estolides involved in acylCer formation. The regulation of TAG estolide metabolism in the epidermis might provide a potential novel target for the treatment of rare cornification disorders.

Notes:

Keynote Lecture 2022

Leptin and the Regulation of Food Intake and Body Weight

Jeffrey M. Friedman

Marilyn M. Simpson Professor, Investigator, Howard Hughes Medical Institute, The Rockefeller University, New York, USA

Leptin is an adipose tissue hormone that maintains homeostatic control of adipose tissue mass. This endocrine system thus serves a critical evolutionary function by protecting individuals from the risks associated with being too thin (starvation) or too obese (predation). Mutations in leptin or its receptor cause massive obesity in mice and humans, and leptin can effectively treat obesity in leptin deficient patients. The identification of leptin has thus provided a framework for studying the regulation of feeding behavior and the pathogenesis of obesity.
While most obese patients have high endogenous levels of leptin indicating that they are leptin resistant , obese patients with low endogenous levels show robust weight loss with leptin treatment. Leptin also links changes in nutrition to adaptive responses in other physiologic systems with effects on insulin sensitivity, fertility, immune function and neuroendocrine function (among others). Leptin is an approved treatment for generalized lipodystrophy, a condition associated with severe diabetes, and has also shown promise for the treatment of other types of diabetes and for hypothalamic amenorrhea, an infertility syndrome in females. Studies of leptin gene regulation also suggest that leptin should be an effective treatment for the subset of obese patients with low endogenous levels of the hormone.
The identification of leptin has also advanced our understanding of the neural mechanisms that control feeding. Current research focuses on the function of specific neural populations in the hypothalamus and other brain regions to control feeding behavior and energy balance. Recent studies have linked arcuate neurons that respond to leptin and a brainstem motor output that coordinates motor programs associated with food consumption. These findings identify the components of a simple subcortical circuit that regulates feeding thus providing a possible framework for elucidating how complex information is processed to generate specific behaviours.In other studies , we are studying the transcriptional mechanisms that regulate leptin gene expression as well as the leptin regulated neural circuits that regulate metabolism.

Notes:

PSD3 as a promising therapeutic target to treat fatty liver disease

Stefano Romeo

Professor/ Specialized Physician, Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden

Fatty liver disease (FLD) is a growing health issue with burdening unmet clinical needs. FLD has a genetic component but, despite the common variants already identified, there is still a missing heritability component. Using a candidate gene approach, we identify a locus (rs71519934) at the Pleckstrin and Sec7 domain-containing 3 (PSD3) gene resulting in a leucine to threonine substitution at position 186 of the protein (L186T) that reduces susceptibility to the entire spectrum of FLD in individuals at risk. PSD3 downregulation by short interfering RNA reduces intracellular lipid content in primary human hepatocytes cultured in two and three dimensions, and in human and rodent hepatoma cells. Consistent with this, Psd3 downregulation by antisense oligonucleotides in vivo protects against FLD in mice fed a non-alcoholic steatohepatitis-inducing diet. Thus, translating these results to humans, PSD3 downregulation might be a future therapeutic option for treating FLD.

Notes:

Intracellular cholesterol trafficking by NPC proteins

Judith Storch

Distinguished Professor of Nutritional Sciences, Rutgers, The State University of New Jersey, Department of Nutritional Sciences, School of Environmental and Biological Sciences, New Jersey, USA

In Niemann-Pick type C (NPC) disease, cholesterol accumulates in the late endosomal/lysosomal compartment (LE/LY) of cells secondary to mutations in the genes encoding the NPC1 or NPC2 proteins. This aberrant sterol accumulation can lead to neuronal dysfunction and ultimately death, thus strategies to prevent or reduce intracellular sterol accumulation in NPC disease are urgently needed. By examining the fundamental mechanisms by which the NPC proteins normally traffic cholesterol, we hope to provide mechanism-based strategies to treat NPC disease. We used in vitro kinetic studies to demonstrate that NPC2, a 16kDa glycosylated protein, could function as a cholesterol transporter within the LE/LY. Notably, the rate of cholesterol transfer by NPC2 was dramatically stimulated by the atypical phospholipid lysobisphosphatidic acid (LBPA, also known as bismonoacylglyerophosphate, BMP). Although a minor component of total cell phospholipid, LBPA is resident only in the LE/LY compartment where it can comprise almost 20% of membrane phospholipids. We found that NPC2 interacts directly with LBPA and identified the “hydrophobic knob” on NPC2 as the LBPA sensitive domain. It has been shown that enriching NPC1-deficient cells with LBPA led to cholesterol egress, but we found that similar LBPA enrichment did not work at all in NPC2-deficient cells, underscoring an obligate interaction of NPC2 and LBPA for normal cholesterol trafficking. Since increasing LBPA levels, either directly or via incubation with its presumed precursor phosphatidylglycerol (PG) can lead to cholesterol clearance in NPC1 deficient cells, we are seeking to understand the mechanisms underlying this apparent rescue of the sterol accumulation phenotype by LBPA. Using multiple NPC1-deficient cell types including iPSC neurons and primary neurons, our results indicate that LBPA enrichment is working by increasing exosome biogenesis and secretion, and by stimulating autophagic flux. Manipulation of LE/LY levels of LBPA, either by stimulating synthesis or blocking degradation, could thus be a new strategy to treat NPC1 disease, which accounts for 95% of cases. The biosynthetic and degradative pathways of LBPA are not known, however, thus current studies are aimed at identifying the enzymes of LBPA metabolism so as to develop compounds that will specifically raise the levels of LBPA in NPC1 disease cells, thereby effecting cholesterol clearance.

All organisms face fluctuations in the availability and need for metabolic energy. To buffer these fluctuations, cells use neutral lipids, such as triglycerides, as energy stores. We study how lipids are stored as neutral lipids in cytosolic lipid droplet organelles. Specifically, we investigate the molecular processes that govern the synthesis of energy storage lipids as well as their storage in and mobilization from lipid droplets. In modern societies, individuals often face metabolic energy excess, leading to metabolic diseases. We will present our studies on the causal link between energy excess and metabolic disease.

Notes:

Prandial secretin-mediated thermogenesis in brown fat controls food intake

Martin Klingenspor

Chair of Molecular Nutritional Medicine, Department of Molecular Life Sciences, Technical University of Munich, TUM School of Life Sciences, Freising, Germany

In the defense of cold, uncoupling protein 1 (UCP1) in brown and beige fat dissipates the chemical energy of fatty acids and glucose for nonshivering thermogenesis. In several mouse models, the recruitment and activation of brown fat thermogenesis alleviates diet-induced obesity, dyslipidemia, and diabetes. In humans, cold-inducible brown fat activity is positively associated with a lower risk for cardiometabolic diseases and declines in the elderly as well as in people with obesity and diabetes. These beneficial metabolic effects are mostly accredited to increased energy expenditure, though in humans the thermogenic capacity of brown fat is rather limited. Earlier studies in rodents and recent studies in humans demonstrated meal-induced brown fat activity linked to meal termination (satiation). Thus, brown fat may be more than a mere heater organ and play an additional role in the control of energy intake. So far, increased sympathetic nerve activity in brown fat and beta3-adrenergic receptor signalling in brown adipocytes was considered as the canonical pathway mediating cold-induced as well as meal-induced brown fat activity. In contrast, our key observations identified the gut hormone secretin, activating secretin receptor signalling in brown adipocytes, as the physiological mediator of meal-induced brown fat and satiation. Secretin activated brown fat operates as a relay station for gut-brain communication in the control of food intake. The beneficial metabolic effects of brown fat may be partly attributable to this gut-brown fat-brain axis.

Notes:

Mechanisms of adipose tissue atrophy in cancer cachexia

Isabella Pototschnig[1], Ursula Feiler[1], Rudolf Zechner[1,2,3], Martina Schweiger[1,2,3]

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[3]BioTechMed-Graz, Graz, Austria.

Cancer-associated cachexia (CAC) is a devastating multifactorial syndrome affecting up to 80% of cancer patients. It is characterized by massive bodyweight loss due to adipose tissue (AT) and muscle atrophy. Surprisingly, survival rates of patients with solely AT atrophy are as poor as of patients with AT and muscle atrophy. Therefore, the aim of my study focused on the mechanisms of AT loss in CAC. As a preclinical CAC model, we use CHX207 fibrosarcoma cells, which are syngeneic to C57BL/6J mice and robustly induce cachexia. We found that CHX207-tumor bearing mice show drastically reduced AT mass already 13 days after cancer cell injection. Moreover, we discovered that AT atrophy in CHX207-tumor bearing mice results from a combination of decreased adipolipogenesis, reduced lipid uptake, and increased lipolysis in white AT. In contrast to other cachexia models, elevation of lipolysis in AT of CHX207-tumor bearing mice does not result from Protein kinase A-mediated phosphorylation of Hormone sensitive lipase, but rather from increased Adipose triglyceride lipase (ATGL) expression and activity. In ex-vivo lipolysis assays using fat explants of control- and CHX207-tumor bearing mice we observed unchanged basal lipolysis, but a strong increase in glycerol release upon β-adrenergic stimulation of fat explants isolated from CHX207- tumor bearing mice compared to control mice. Moreover, we found highly increased expression of the ATGL co-activator Comparative gene identification-58 (CGI-58) in white AT of cachectic CHX207-tumor bearing mice. These data suggest, that AT atrophy in cachectic CHX207-tumor bearing mice results from elevated lipolysis due to augmented activation of ATGL by CGI-58 upon β-adrenergic stimulation.

Notes:

P53 shapes adipose tissue immune cell infiltration upon intermittent fasting in obese mice

Isabel Reinisch[1], Helene Michenthaler[1], Jelena Krstic[1], Markus Galhuber[1], Moritz Oster[2], Georgia Lenihan-Geels[3], Tongtong Wang[5], Nemanja Vujic[1], Tobias Madl[1,4], Dagmar Kratky[1,4], Renate Schreiber[6], Maria Rohm[7], Tim J. Schulz[3], Michael Schupp[2], Albert Heck[8], Anastasia Georgiadi[7], Stefan Herzig[7], Christian Wolfrum[5], Andreas Prokesch[1,4]

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[2]Charite Berlin, Institute of Pharmacology, Berlin, Germany.
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Historically viewed as a passive site for energy storage, it is now well accepted that limitations in the degree of adipose tissue (AT) plasticity drive the progression of obesity-associated sequelae. As effective interventions against the metabolic syndrome, fasting regimens like intermittent fasting (IF) confer health benefits beyond weight reduction. Tissue-specific metabolic adaptions to fasting include marked changes in AT morphology, physiology, and cellular composition. In this context, recent studies emphasized an involvement of nutrient-responsive transcription factor p53 in regulating metabolism and tissue homeostasis in noncancerous settings, but the distinct function of p53 in the fasting response of adipocytes remains poorly characterized. Therefore, we generated an inducible, adipocyte-specific p53 knock-out (p53Ad-/-) mouse model and triggered obesity by high-fat diet feeding. Selective ablation of p53 in adipocytes of obese mice accelerated the catabolic state during IF, as reflected by increased weight loss, decreased adipocyte size, and elevated plasma fatty acid levels. Furthermore, fasted obese p53Ad-/- mice showed improved insulin sensitivity and decreased plasma TNFα levels. Morphologically, we observed an increase in the abundance of crown-like structures in visceral AT (vAT) of IF-obese mice compared to ad libitum fed control, which was largely blunted in IF-p53Ad-/- mice. Performing single-nuclei RNA sequencing of vAT confirmed a marked increase in lipid-associated macrophage (LAM) infiltration upon IF. Strikingly, this fasting-mediated increase in LAMs was completely absent in obese p53Ad-/- mice, in line with a significant reduction in the expression of pro-inflammatory marker genes and abundance of proteins involved in NF-kB signaling. Furthermore, in vitro studies of human and murine, mature adipocytes confirmed the involvement of p53 in the regulation of adipocyte cytokine/chemokine signaling. Taken together, we showed that AT underlies a remarkable heterogeneity, flexibly adapting its composition according to metabolic demands to ensure systemic energy homeostasis. Importantly, we revealed adipocyte p53 as a master regulator of vAT plasticity and innate immune cell composition upon IF in obese mice.

Notes:

Lipolysis and biochemical genetics; a clinical perspective

Grant A. Mitchell

Professor of Medicine, Montreal Diabetes Center, Montréal, Quebec, Canada

Notes:

Bis(monoacylglycerol)phosphate protects from cationic amphiphilic drug - induced cytotoxicity

Dominik Bulfon[1], Nermeen Girgis[1], Gernot Grabner[1], Ulrike Taschler[1,2], Robert Zimmermann[1,2]

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Bis(monoacylglycerol)phosphate (BMP) is a low abundant, negatively charged phospholipid, mainly encountered in late endosomes and lysosomes. It is a structural isomer of phosphatidylglycerol (PG), but features a unique sn-1: sn-1' stereoconfiguration. BMP is compartmentalized in intraluminal vesicles (ILVs) of late endosomes/lysosomes, where it plays an important role in maturation, cholesterol trafficking, and lipid-degradation pathways. Interestingly, BMP is highly increased in drug-induced phospholipidosis (DIPL) and lysosomal storage disorders of genetic origin. DIPL is caused by many clinically relevant cationic amphiphilic drugs (CADs) such as Amiodarone. Here, we investigated whether increased cellular BMP formation protects from Amiodarone-induced phospholipidosis and cytotoxicity. Amiodarone-treatment caused cytotoxic effects and cell death in COS7 and HepG2 cells, which is likely due to mitochondrial damage but not lysosomal membrane permeabilization. BMP synthesis in COS7 and HepG2 cell was promoted by PG supplementation leading to strong increase in cellular BMP formation and an enlargement of the endosomal/lysosomal compartment. Under these conditions, cells were largely protected from the cytotoxic effects of amiodarone. BMP formation improved mitochondrial respiration in COS7 and HepG2 cells when exposed to Amiodarone. Thus, our observations suggest that BMP protects from the cytotoxic effects of Amiodarone. Possibly, increased sequestration of amiodarone in ILVs attenuates mitochondrial damage. Acknowledgment: This work is supported by the SFB "Lipid hydrolysis" (F73) and Austrian Science Fund (FWF) (doc.funds PhD program "Molecular Metabolism"), the University of Graz, the City of Graz and "Land Steiermark".

Notes:

Adipose triglyceride lipase mediated lipid catabolism is essential for bronchiolar regeneration

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The lung airways are constantly exposed to inhaled toxic substances leading to airway epithelial damage, repaired by local expansion of resident club cells. Lung epithelial repair mechanisms are relatively well understood and impaired epithelial repair lays at the core of many prevalent lung diseases including chronic obstructive pulmonary disease (COPD), asthma, lung fibrosis, and lung cancer. Nevertheless, the importance of bronchiolar club cell energy-metabolism in the context of epithelial repair is still unclear. Adipose TriGlyceride Lipase (ATGL) is the rate-limiting enzyme for intracellular lipolysis. ATGL provides energy-rich fatty acids and cognate agonists for Peroxisome Proliferator-Activated Receptor alpha (PPAR-α) activation that are necessary for mitochondrial biogenesis and beta-oxidation of fatty-acids in the mitochondrial matrix. Atgl knockout (Atgl-KO) animals show fatal cardiomyopathy at young age. To study Atgl function in other organs we therefore used Atgl-KO mice with cardiac transgenic Atgl (cTg) expression. In club cells, absence of ATGL induced strong triglyceride accumulation, and decreased mitochondrial numbers and mitochondrial respiration. These defects manifested as bronchiolar epithelial thickening, increased airway resistance, and reduced regenerative potential, following naphthalene-induced epithelial denudation. Here we show that dysfunctional PPAR-α lipid signaling underlies this phenotype, as pharmacological intervention with the PPAR-α agonist WY14643 resulted in full restoration of the airway epithelial regeneration potential. In a time when the metabolic syndrome is spreading and lung health is more important than ever, lung cell metabolism represents a new and promising therapeutic target for pulmonary diseases. Our data emphasize the importance of the cellular energy-metabolism.

Notes:

Functional ATGL is important for maintaining mitochondrial network integrity and respiration in human primary myotubes

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Background: Metabolic diseases and ageing are associated with declined myocellular mitochondrial function, compromised mitochondrial network integrity and altered lipid droplet lipolysis. ATGL-mediated lipolysis derived fatty acids are endogenous ligands for PPAR transcriptional activity, which is key for regulating mitochondrial oxidative capacity. We aimed to examine whether the ATGL-PPAR transcriptional activity axis regulates mitochondrial network integrity and respiration in human primary myotubes.
Methods: Human primary myotubes were cultured from healthy donors (H) and two donors with neutral lipid storage disease with myopathy (NLSDM) caused by a single heterozygous (SH) or a heterozygous compound (HC) mutation in the ATGL gene. Mitochondrial respiration rates, membrane potential, network integrity and dynamics were determined. Subsequently, myotubes were treated with 40 µM NG-497 to inhibit ATGL and/or with the PPARδ agonist GW501516 (1 µM) during differentiation.
Results: ATGL mutations associated with NLSDM reduced basal mitochondrial respiration (H: 3.43±0.26; SH: 1.66; HC: 2.19 pmol/min/µg protein), lowered mitochondrial membrane potential (H: 1.82x107±0.60x107; SH: 1.64x107; HC: 0.95x107 AU), and fragmented the mitochondrial network (H: 1.04±0.16; SH: 1.92; HC: 3.00). Protein content of dynaminrelated protein 1 (H: 0.024±0.003; SH: 0.008; HC: 0.010 AU) and mitofusin 1 (H: 0.20±0.05; SH: 0.10; HC: 0.11 AU) was lower in myotubes of NLSDM patients. ATGL inhibition in myotubes from healthy donors reduced basal mitochondrial respiration (0.71±0.09 fold change), lowered mitochondrial membrane potential (0.77±0.14 fold change) and fragmented the mitochondrial network (1.57±0.77 fold change). PPARδ agonist treatment increased basal mitochondrial respiration (H: 1.62±0.30; SH: 1.31; HC: 1.06; H+NG-497: 1.14±0.05 fold change) regardless of changes in mitochondrial membrane potential and network integrity.
Conclusion: ATGL activity is important in regulating mitochondrial network quality and respiration. PPARδ agonist treatment was more effective on mitochondrial respiration in ATGL proficient than ATGL deficient primary myotubes, indicating that ATGL activity increases the positive effects of PPARδ agonism on mitochondrial respiration.

Notes:

Hormone-sensitive lipase, a central integrator of adipocyte metabolism

Dominique Langin

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Hormone-sensitive lipase (HSL) has initially been characterized as the hormonally regulated neutral lipase responsible for the breakdown of adipose tissue triacylglycerols into fatty acids. Compared to other neutral lipases, HSL has a broad substrate specificity. HSL singularity is also provided by a regulatory domain containing phosphorylation sites embedded in the catalytic domain. Besides the classical role in lipolysis, we recently showed that HSL interacts in the cytosol with the glucose-responsive transcription factor ChREBP to control fatty acid synthesis from glucose carbons. In adipocyte nuclei, HSL exerts transcriptional repression of mitochondrial oxidative phosphorylation genes. Shuttling of adipocyte HSL between the nucleus, the cytosol and lipid droplets allows coordinated control of three major triacylglycerol metabolic pathways, lipolysis, de novo lipogenesis and fatty acid oxidation. HSL emerges as a central integrator of adipocyte metabolic control and adaptation.

Notes:

ApoC-III's inhibition of LDLr: if it's good for the heart, is it good for the gut?

Alison Kohan

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Objective: Cellular metabolism is a key regulator of $CD4^{+}Foxp3^{+}$ regulatory T cell (Treg) homeostasis, but the foundational studies in this area use free fatty acid treatment as a proxy for plasma triglycerides. In vivo, plasma triglyceride is the main source of fatty acids for cells, not free fatty acids.
Design/Results: Using apolipoprotein C-III transgenic and $LDLr^{-/-}$ mice, we report that the loss of lipoprotein triglycerides transport in these models results in protection from DSS-colitis and accumulation of intestinal Tregs and plasma IL-10. Total loss of apoC-III increases colitis severity. Tregs exposed to apoC-III increase lipolysis and fatty acid oxidation and apoC-III inhibits Bodipy- triglyceride uptake. Therapeutic treatment of WT mice with apoC-III-containing lipoproteins protects mice from colitis.
Conclusion: Our data suggest that therapies that reduce apoC-III could have negative effects in patients who are at risk of IBD, and conversely, that apoC-III could be a new therapeutic target to stimulate intestinal Tregs and IL-10 for the management of IBD. These data identify apoC-III and lipoprotein metabolism as a novel regulator of tolerance in the intestine.

Notes:

The role of cytosolic lipolysis in cardiac function and heart failure

Ulrich Kintscher

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Heart failure is one of the leading causes of death worldwide. New therapeutic concepts are urgently required to lower the burden of both major forms of heart failure: with reduced or preserved ejection fraction (HFrEF/ HFpEF). Lipolytic processes are induced during the development of heart failure through an increased sympathetic tone, and occur in multiple organs such as the heart, and adipose tissue. Increasing evidence suggests that cellular lipolysis, in particular adipose triglyceride lipase (ATGL) activity, has an important function in cardiac (patho)physiology. This presentation will summarize the crucial role of cellular lipolysis, in particular cytosolic and ATGL-mediated lipolysis for normal cardiac function and for the development of HFrEF and HFpEF. The most relevant pre-clinical studies including the work form our lab in flies and mice will be presented. Potential underlying molecular mechanisms how lipolytic processes in the heart and other tissues may affect cardiac functions will be outlined. Finally, recent data on the therapeutic importance of pharmacological ATGL inhibition as a potential treatment option for HFrEF and/ or HFpEF in the future will be presented.

Notes:

Off-target effects of the lysosomal acid lipase inhibitors Lalistat-1 and Lalistat-2 on neutral lipid hydrolases

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Lysosomal acid lipase (LAL) is the key enzyme of lysosomal lipid hydrolysis responsible for the degradation of cholesteryl esters and triacylglycerols at an acidic pH. LAL deficiency is a rare lipid storage disorder associated with massive lipid accumulation, inflammation, and premature mortality. The role of LAL in various cellular processes has mostly been studied in LAL-deficient (Lal-/-) mice, which share phenotypical characteristics with humans suffering from LAL deficiency. Additionally, LAL inhibitors Lalistat-1 (L1) and Lalistat-2 (L2) are commonly used to investigate how the inhibition of LAL activity impacts various cellular processes in different cell types. Since LAL degrades the same substrates as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) in the cytosol at neutral pH, L1 and L2 must be highly specific to LAL. We aimed to investigate whether genetic LAL deficiency and pharmacological inhibition of LAL activity resulted in comparable cell dysfunction. We observed impaired isoproterenol-stimulated lipolysis in L2-treated adipocytes but not in Lal-/- adipocytes. Further detailed analyses revealed that both L1 and L2 impair the activity of neutral lipid hydrolases. We confirmed these results by performing activity assays using mouse tissues lacking HSL or ATGL and cells with overexpression of mouse or human ATGL or HSL. By performing serine hydrolase-specific activity-based labeling in combination with quantitative proteomics, we identified additional enzymes that were inhibited by L1 and L2. Our findings are critically important since they demonstrate that commonly used concentrations of L1 and L2 are not suitable to investigate the role of LAL-specific lipolysis in lysosomal function, signaling pathways, and autophagy. The interpretation of their effects on lipid metabolism should be taken with caution and the applied inhibitor concentrations in cell culture studies must be less than 1 µM.

Notes:

Small Molecule Inhibitors Targeting Lipolysis in Human Adipocytes

Gernot F. Grabner[1], Nikolaus Guttenberger[2], Nicole Mayer[2], Anna K. Migglautsch-Sulzer[2], Christian Lembacher Fadum[2], Nermeen Fawzy[1], Dominik Bulfon[1], Peter Hofer[1], Thomas Züllig[1], Lennart Hartig[1], Natalia Kulminskaya[1], Gabriel Chalhoub[1], Margarita Schratter[1], Franz P.W. Radner[1], Karina Preiss-Landl[1], Sarah Masser[1], Achim Lass[1,3], Rudolf Zechner[1,3,4], Karl Gruber[1,3,4], Monika Oberer[1,3,4], Rolf Breinbauer[2,3], Robert Zimmermann[1,3,4]

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Chronically elevated circulating fatty acids levels promote lipid accumulation in non-adipose tissues and cause lipotoxicity. Adipose triglyceride lipase (ATGL) critically determines the release of fatty acids from white adipose tissue, and accumulating evidence suggests that inactivation of ATGL has beneficial effects on lipotoxicity-driven disorders including insulin resistance, steatohepatitis, and heart disease, classifying ATGL as a promising drug target. Here, we report on the development and biological characterization of the first small molecule inhibitor of human ATGL. This inhibitor, designated NG-497, selectively inactivates human and non-human primate ATGL but not structurally and functionally related lipid hydrolases. We demonstrate that NG-497 abolishes lipolysis in human adipocytes in a dose-dependent and reversible manner. The combined analysis of mouse- and human-selective inhibitors, chimeric ATGL proteins, and homology models revealed detailed insights into enzyme-inhibitor interactions. NG-497 binds ATGL within a hydrophobic cavity near the active site. Therein, three amino acid residues determine inhibitor efficacy and species selectivity and thus provide the molecular scaffold for selective inhibition.

Notes:

PhD Program

MOLMED

Regulated inhibition of extracellular and intracellular lipolysis

Sander Kersten

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Extracellular lipolysis is an essential step in the uptake of triglycerides by adipose tissue and is catalyzed by the enzyme lipoprotein lipase (LPL). Intracellular lipolysis is essential for mobilizing fatty acids, allowing them to be used internally or be exported to other tissues. The key step in intracellular lipolysis is catalyzed by adipose triglyceride lipase (ATGL). The activities of LPL and ATGL are carefully regulated mainly through post-translational mechanisms.

During fasting, LPL is inhibited in adipose tissue to prioritize the uptake of plasma triglycerides by other tissues. The inhibition of LPL during fasting is triggered by the protein ANGPTL4, which promotes the unfolding, cleavage, and subsequent degradation of LPL in adipocytes. Human genetic studies indicate that inactivating variants of ANGPTL4 are associated with reduced serum triglyceride levels and a reduced risk of atherosclerotic cardiovascular disease, suggesting that ANGPTL4 may be pharmacologically targeted to reduce the risk of atherosclerotic cardiovascular disease. We and others have found that silencing of ANGPTL4 via antisense oligonucleotides effectively reduces plasma triglycerides and glucose in mice without raising major safety concerns. The results pave the way for the first clinical trials with ANGPTL4 antisense oligonucleotides in humans.

Similar to LPL, ATGL is subject to extensive regulation, which includes via phosphorylation as well as the physical interaction with (in)activating proteins such as CGI-58 and G0S2. In addition, ATGL interacts with and is inhibited by the protein HILPDA. We found that HILPDA mediates the induction of lipid droplet accumulation by LPS treatment in macrophages. Besides inhibiting ATGL, our cell-based studies suggest that HILPDA also reduces ATGL protein content, possibly by promoting ATGL degradation. Overall, we provide evidence that LPS-mediated activation of macrophages is accompanied by suppression of lipolysis via induction of HILPDA, thereby reducing the availability of pro-inflammatory lipid precursors and downregulating the production of PGE2 and IL-6.

Notes:

Intrinsically disordered regions and low protein stability drives regulation and compartmentalization of LPL activity

Michael Ploug

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Hydrolysis of triglyceride-rich lipoproteins (TRLs) by lipoprotein lipase (LPL) fuels intravascular lipid metabolism—a process suppling energy to vital organs such as heart and skeletal muscles. Tissue-specific partitioning of TRLs and their intravascular processing by LPL is regulated by a highly complex interplay between endogenous activators and inhibitors. Unprecedentedly, angiopoietin-like protein 4 (ANGPTL4) inhibits LPL by lowering its melting temperature (T_m) from 35°C to < 15°C and thus catalyzes the irreversible unfolding of LPL's α/β-hydrolase domain. Binding to LPL's endothelial transporter (GPIHBP1) mitigates this process and increases T_m to 58°C. With hydrogen–deuterium exchange mass spectrometry (HDX-MS), we mapped the ANGPTL4 binding site on LPL and outlined the downstream unfolding trajectory leading to irreversible LPL inactivation. In contrast, the binding site for apolipoprotein C2 (APOC2) and the molecular mechanisms by which this cofactor activates LPL remains unclear and controversial. Here, we show by HDX-MS that the C-terminal α-helix in APOC2 (residues 75–82) binds to regions proximal to the catalytic pocket in LPL (residues 49–60, 87–99, and 218–236). Notwithstanding these sites are identical to those used to bind ANGPTL4, APOC2 binding activates LPL and increases its thermostability (T_m) to 50°C rather than destabilizing it—as ANGPTL4 does. Corroborating this unexpected observation, we find that APOC2- and ANGPTL4-binding have opposite impacts on regions proximal to LPL's catalytic pocket (residues 178–217 and 239–249), which are stabilized by APOC2 and destabilized by ANGPTL4. Our current study provides new molecular insight into the mechanism by which APOC2 binds and regulates LPL.

Notes:

Posters:

Abstracts

Poster 1 – Group A

Consequences of (intestinal) LAL deficiency on whole body lipid metabolism

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Lysosomal acid lipase (LAL) is the only enzyme responsible for the degradation of cholesteryl esters and triglycerides in the lysosome at an acidic pH. Mutations in its gene cause LAL deficiency, a rare autosomal recessive disease. One of the most common symptoms of LAL deficiency is lipid malabsorption in the small intestine, accompanied by macrophage infiltration. We aim to investigate the consequences of whole-body and intestinal LAL deficiency on lipid metabolism and absorption and the impact of infiltrating macrophages in this process. We collected three parts of the small intestine (duodenum, jejunum, ileum) from mice with a global (LAL KO) or intestine-specific deletion of LAL (iLAL KO) and corresponding controls, either fed chow or high fat/high cholesterol diet. We found massive lipid accumulation in the small intestine of LAL KO mice, particularly in macrophages in the lamina propria. We were able to demonstrate that these mice absorb less dietary lipids and secrete fewer chylomicrons, but have increased fecal lipid loss. However, we proved that the lipids accumulated in the small intestine of LAL KO mice originate mainly from the circulation. We also observed an increased expression of inflammatory markers and lipid metabolism related genes in the jejunum of LAL KO mice, which increased with age. Despite the drastically reduced LAL activity in iLAL KO enterocytes, villus morphology, lipid concentrations, expression of lipid transporters and inflammatory genes as well as lipoprotein secretion were unaltered in the small intestine of these mice. We conclude that enterocyte-specific deletion of LAL does not phenocopy the intestinal phenotype of global LAL KO mice. Therefore, loss of LAL in enterocytes alone is not sufficient to cause lipid deposition in the small intestine, implying that infiltrating macrophages play a crucial role in this process.

Notes:

Poster 2 – Group A

The role of platelets in ER stress-induced hepatic steatosis

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Background Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and characterized by excessive accumulation of hepatic triglycerides. It can further progress into non-alcoholic steatohepatitis (NASH) and liver fibrosis/cirrhosis, which potentially reduce life expectancy and life quality. Platelets accumulate in steatotic livers of NAFLD patients and potentially contribute to hepatic inflammation and endoplasmic reticulum (ER) stress, which fuels disease progression. By interacting with circulatory and liver-resident (immune) cells such as hepatocytes, Kupffer cells, monocytes, and stellate cells, platelets finetune liver homeostasis, and selective platelet inhibition might comprise a target for future therapies. Aims The central aim of this project is to elucidate the role of platelets in the development of hepatic ER stress and associated lipid accumulation and inflammation. Methods Mice were treated with tunicamycin to induce hepatic ER stress and depleted of platelets using an anti-GPIb-antibody. Hepatic tissue was analyzed on mRNA, protein, and lipid levels. Furthermore, immune cell influx was determined by flow cytometry. Additionally, in vitro studies were performed by coincubation of murine hepatocytes with platelets. Results Tunicamycin led to a significant increase of hepatic triglycerides, which was associated with elevated hepatic expression of very-low-density lipoprotein receptor (VLDLR) and decreased ApoB100 expression. Depletion of platelets alleviated hepatic steatosis and lowered plasma levels of alanine transaminase (ALT) and aspartate transaminase (AST). In addition, the absence of platelets significantly reduced inflammasome activation, indicated by decreased mRNA levels of interleukin-1b and NLR family pyrin domain containing 3 (NLRP3). In contrast to the in vivo data, in vitro co-incubation of platelets with hepatocytes did not alter ER stress and only mildly affected lipid accumulation, indicating the need for additional intercellular crosstalk. Conclusion Platelets exert detrimental effects on ER stress-induced hepatic steatosis, which is potentially mediated by interacting with (liver-resident) immune cells and by modulating inflammasome activity.

Notes:

Poster 3 – Group A

Chemical suppression of adipose triglyceride lipase improves nonalcoholic steatohepatitis in a diabetic and hyperlipidemic mouse model

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Background and Aims: Metabolic comorbidity such as diabetes, obesity and metabolic syndrome plays a key role in the pathogenesis and progression of non-alcoholic fatty liver disease (NAFLD). Increased adipocyte triglyceride lipase (ATGL/PNPLA2) activity, because of insulin resistance, may result in enhanced release of fatty acids from white adipose tissue to the liver, where it precipitates the development and progression of NAFLD. Therefore, ATGL is an attractive target to avert metabolic liver injury. We tested whether the pharmacological ATGL inhibitor Atglistatin hinders metabolic syndrome and improves liver injury in STAM mice as a model of on-alcoholic steatohepatitis (NASH).
Method: Two-days-old C57BL/6j male mice received a single subcutaneous streptozotocin (STZ) injection (100mg/kg body weight). HFD intervention in the STZ-injected mice representing STAM (diabetic and hyperlipidemia condition) commenced at the age of 4 weeks and continued for four weeks. NASH developed within 8 weeks. For the pharmacological inhibition of ATGL, STZ-injected mice were fed an HFD supplemented with 2 mmol/kg Atglistatin for four weeks.
Results: Atglistatin reduced body weight (-2.54 g), despite a comparable consumption of food between the STAM and Atglistatin groups. The bodyweight reduction was due to a decrease in fat (-40%) and liver mass (-23%). Atglistatin treatment significantly improved fasting blood sugar (-12%). Interestingly, the intestinal length was longer (+50%) in the Atglistatin mice in comparison to the STAM mice. In line with a trend for reduced ALT, there was a significant improvement in histological liver injury assessed by H&E staining and NAFLD score (-45%). The NAFLD score revealed improvement of steatosis (-60%) and inflammation (-35%) but not ballooning in the Atglistatin group. Immunochemistry showed that Atglistatin treatment significantly reduces Mac2 positive cells in the liver (-50%) and gonadal white adipose tissues (gWAT) (-60%) compared to the untreated STAM group, indicating a reduced infiltration of immune cells into the liver and gWAT.
Conclusion: Atglistatin improved metabolic features and liver injury in the STAM mice by reducing inflammation and fat accumulation in key metabolic tissues such as the liver and adipose tissue.

Notes:

Poster 4 – Group A

The Co-enzyme A Rejection Mechanism of Fatty-acyl AMP Ligases

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Fatty acyl AMP ligases (FAALs) are structurally highly similar to the fatty acyl CoAligases(FACLs). Both enzymes perform their function in a two-step reaction, wherein in the first step the fatty acids are activated (adenylation), and in the second step thioesterification of these activated fatty acids occurs. The second step is the decisive factor for the fate of thefatty acid. The thioesterification by FAALs leads to transfer onto Acyl carrier proteins (ACP)which leads to complex lipid synthesis (anabolic processes). FACLs on the other handtransfer the activated fatty acid to CoenzymeA , the highly abundant and diffusible molecule in the cell, thereby leading to catabolic processes. In this work, we carried out mutation in the canonical and novel ACP binging pocket based on structural features of FACLs and FAALs. If the novel pocket was blocked by an exchanged amino acid, the FAALs transfer was not meditated. Summing up, we identified the CoA rejection mechanism by FAALs combining biochemical and structural data.

Notes:

Poster 5 – Group A

Mapping the structural landscape of carboxylesterase 2c with X-ray crystallography

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Carboxylesterases (CES) are serine hydrolases of the alpha beta hydrolase fold family which hydrolyze a wide range of endogenous and xenobiotic amides, thioesters and esters including lipids. Some carboxylesterases show important physiological functions in lipid metabolism and human diseases. However, the physiological role of the different isoforms of Ces2 protein family members is not completely understood. Therefore, structural characterization of the carboxylesterase 2 family is an indispensable part to get a better insight into the metabolic roles of this enzymes. Here, we present first crystal structure of the murine carboxylesterase 2c (mCes2c) to 2.1Å resolution in its native form. The overall structure of mCes2c resembles that of the human CES1. The core domain adopts the α/β hydrolase-fold containing a catalytic triad (Ser-230, Glu-347, His-459) and a “lid” domain, which might regulate the substrate entry to the catalytic site. Similarities and differences to other carboxylesterase will be discussed in this contribution.

Notes:

Poster 6 – Group A

Towards a mathematical model of intracellular lipolysis

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Fatty acids (FAs) can be stored in the form of triglycerides (TGs) in cytosolic lipid droplets. In a process known as intracellular lipolysis, FAs are liberated from LDs by consecutive enzymatic hydrolysis of TGs supplying the organism with energy substrates during deprivation. Adipose triglyceride lipase (ATGL) initiates intracellular lipolysis due to its robust TG hydrolase activity. The second step of lipolysis is mainly catalyzed by hormone-sensitive lipase (HSL) which hydrolyzes diglycerides (DGs) producing monoglycerides (MGs) and FAs. However, HSL was also reported to exhibit TG and MG hydrolase activities indicating functional redundancies between the enzymes. In contrast to the other enzymes involved in lipolysis (ATGL and HSL), monoglyceride lipase (MGL) can only hydrolyze MGs having no activity towards TGs and DGs. Even though lipolytic enzymes have been functionally characterized in great detail, there is still a lack in understanding their kinetic properties and relative contributions to each particular step of lipolysis. Hence, our general aim is to develop a mathematical model of intracellular lipolysis based on kinetic data obtained from in vitro lipolysis assays with purified enzymes. In this particular study, we focused on HSL- and MGL-mediated lipolysis. Recombinant murine His6-tagged MGL was expressed in Expi293F cells. Automated affinity chromatography through a Ni2+ column yielded isolated MGL with minor impurities. Discontinuous enzymatic assays with thin layer chromatographybased separation of reaction products proved that purified MGL exhibits strong and time-dependent activity towards a substrate containing monoolein (MO) and phospholipids. MGL hydrolyzed MO in a fully saturated regime. Experimental data and mathematical modelling suggest that in vitro MO hydrolysis is exclusively determined by MGL deactivation. His6-tagged HSL was enriched by automated affinity chromatography and showed strong activity towards phospholipidemulsified diolein (DO) and MO substrates, which could be increased even further upon in vitro phosphorylation. HSL activity towards TO could be measured, albeit at very low levels. These data are currently used to inform the mathematical model which will provide deeper insights into HSL-mediated lipolysis.

Notes:

Poster 7 – Group A

Hepatic response to fatty acid treatment: A study of lipotoxicity, rescue and trans fatty acids

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Canonically the liver responds to high fatty acid load in the plasma (caused either by food intake or starvation induced release from adipose tissue) with accumulation of triglycerides in intracellular lipid droplets, thus acting as a short-term buffer. Fatty acids can be remobilized for lipoprotein synthesis, burned in mitochondria as energy substrates, used for cellular membrane biosynthesis or act as signaling molecules. In surplus amounts fatty acids can be lipotoxic. Our aim was to improve our understanding of the molecular mechanisms that cause lipid induced hepatocyte death. We incubated human hepatoma (HepG2) cells with fatty acid bovine serum albumin conjugates of five different long chained fatty acids in different concentrations and combinations to assess changes in the lipidome and proteome as well as to morphology. Initial treatments with varying fatty acids unveiled that all tested saturated fatty acids in contrast to the unsaturated oleic acid led to dose dependent decline in cellular growth rates. Moreover, saturated fatty acids caused increased rates of mitochondrial fragmentation preceding cell death, while less lipid accumulation was observed compared to oleic acid. All effects could be abolished after oleic acid was administered together with any saturated fatty acid in a ratio of at least 1:2. Elaidic acid, the trans variant of oleic acid, on the other hand, did not increase lipid droplet volume at higher concentrations but still abolished the negative impact of saturated fatty acids. Data from the proteomics analysis reviled a small set of proteins, modulators of transcription and the immune system, altered in conditions that could be considered lipotoxic. Lipidomic analyses, to our surprise, showed a dose dependent increase of triglycerides for both, unsaturated as well as saturated fatty acids, suggesting that efficient staining of lipid droplets was affected by their fatty acid composition. Exclusive to the treatment with saturated fatty acids, an increase of ceramides and phospholipids was observed, which was rescued by addition of either oleic or elaidic acid. Our data do not support the previously proposed hypothesis that survival is linked to the cells´ capacity to store fatty acids as triglycerides but rather suggest that unsaturated fatty acids inhibit metabolic pathways of saturated fatty acids, thus preventing lipotoxic effects.

Notes:

Poster 8 – Group A

Transcriptome and enhancer dynamics in the fasted liver revealed by PRO-seq

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Periodic fasting has numerous health benefits but the underlying tissue-specific, regulatory mechanisms are still illdefined. The liver is strongly responsive to changes in nutrient intake such as fasting. Here, we aimed to reveal dynamic changes in enhancer/gene activation and the underlying transcription factor (TF) networks. Therefore, we generated qPRO-seq (quick precision run-on followed by sequencing) data in mouse liver sampled 1, 3, 6, 12, and 24 hours after food withdrawal. Livers from ad-libitum fed mice served as circadian-matched control. qPRO-seq enables detection of activated enhancers, through enhancer RNA (eRNA) detection, and of direct RNA synthesis on gene bodies, providing much deeper regulatory insights than conventional RNA-seq data. In our hands, qPRO-seq is highly reproducible between independent experiments and superior to the established GRO-seq method with regard to sequence coverage and sensitivity. Genome track visualizations of known fasting-responsive genes (e.g., Pck1 and Fasn) served as a proof of principle. Supervised clustering of fasting-activated gene bodies revealed nine distinct gene expression profiles across the time course. Gene ontology mapping showed progressive enrichment of processes involved in lipid, protein, and steroid metabolism. Interestingly, immediately early (1 and 3 hours of fasting) activated genes yielded enrichment in terms like “chromatin remodeling” and “RNA splicing”, regulatory mechanisms that will be further investigated. eRNA analysis revealed over 15,000 fasting-specific enhancers. Motif finding within these eRNA regions uncovered known TF motifs, like glucocorticoid receptor, PPARα, and forkhead TFs (e.g. FOXO1 and FOXO3). Early, transiently detected TF motifs included GATA factors, AP-2 TFs, and orphan nuclear receptors, all previously not described in the fasting context. In sum, first analyses of our qPRO-seq time-series data set of fasted liver support the notion that fasting greatly reorganizes the enhancer and transcriptome landscape, revealing novel pioneer TFs involved in immediate early changes. Further analyses will be directed towards identification of fasting-selective super enhancers clusters and Pol2 pause/release mechanisms on transcription start sites to provide in-depth insight into the fasting-mediated regulatory landscape.

Notes:

Poster 9 – Group A

The role of ATGL in intestinal and systemic lipid homeostasis

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The rising incidence of metabolic and cardiovascular diseases in the last decades is mainly linked to an increased intake of dietary lipids. Enterocytes of the small intestine (SI) play a crucial role in maintaining whole body lipid homeostasis by mediating dietary lipid uptake as well as ensuring the supply of fatty acids to other organs. Excessive triglycerides (TGs), originating from apical (diet) or basolateral (circulation) lipid uptake, are transiently stored in form of cytosolic lipid droplets (cLDs) and can be mobilized upon need. Hydrolysis of cLDs in interprandial periods is believed to maintain whole-body lipid homeostasis, but the underlying mechanism(s) are still elusive. Therefore, we aimed to elucidate whether the major neutral TG hydrolase ATGL is involved in the degradation of apically and/or basolaterally derived cLDs using mutant mouse models either overexpressing ATGL (Atgl iTg) or lacking ATGL and its coactivator CGI-58 (Atgl/Cgi-58 iDKO) exclusively in the SI. Independent of the diet, Atgl/Cgi-58 iDKO mice displayed massive cLD accumulation within enterocytes, while overexpression of ATGL only had beneficial effects after triggering intestinal steatosis by acute or chronic lipid exposure. Of note, alimentary lipids failed to accumulate in the SI of iDKO mice in the early phase of absorption, but got incorporated into cLDs 2 h post gavage. These findings together with persistent cLD abundance after restriction to endogenous lipids and accumulation of intravenously applied fatty acids indicated the existence of a secretion/reuptake cycle in enterocytes. Increased deposition of basolaterally-derived lipids in mice lacking either only CGI-58 or ATGL indicated a significant role of CGI-58 in this process. In agreement with this, mice overexpressing solely ATGL showed no detectable changes in the accumulation of basolaterally derived lipids, in part because physiological cLD formation from basolateral-derived lipids is quite low in wildtype-mice. Unexpectedly, overexpression of ATGL affected whole-body cholesterol homeostasis, which was in line with data obtained from Atgl iKO, but not Cgi-58 iKO or Atgl/Cgi-58 iDKO mice. Furthermore, changes in cholesterol metabolism in Atgl iTg mice was strongly dependent on the nutritional status. Our data provide evidence that ATGL together with its coactivator CGI-58 plays an important role in the mobilization of basolaterally derived cLDs in the SI. Dietary TG absorption and secretion of dietary lipids within chylomicrons remained comparable in all genotypes studied. Unexpectedly, overexpression of only ATGL strongly affects intestinal and systemic cholesterol metabolism, independent of CGI-58 availability.

Notes:

Poster 10 – Group A

Double-targeting energy metabolism in hepatocellular carcinoma

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A common mechanism of drug resistance is the metabolic rewiring of cancer cells, calling for novel metabolic combination therapies. Here, we explored whether simultaneously targeting the main energy-producing pathways, aerobic glycolysis and oxidative phosphorylation (OxPhos), can be utilized as treatment for hepatocellular carcinoma (HCC). To target aerobic glycolysis, we reduced glucose availability both in vitro (by using glucose deprived media), and in HCC mouse models (by periodically fasting mice). To target OxPhos, we used sorafenib, a standard-of-care drug for HCC, and other OxPhos inhibitors (e.g., IM156, Lonidamine, Atovaquone) which have been approved for in vivo use or are currently tested in clinical trials. We show that periodic fasting can sensitize resistant HCC to sorafenib in an HCC cell line, in xenografts, and in patient-derived organoids. Metabolic characterization shows that sorafenib acts as a potent inhibitor of mitochondrial respiration, causing resistant cells to switch to glycolysis for survival. Synergistically reducing nutrients prevents this Warburg shift and leads to sensitization to sorafenib in vitro and in vivo. Functional experiments show that glucose is the limiting nutrient crucial for curtailing this metabolic flexibility. Furthermore, we show in several HCC cell lines and patient-derived HCC organoids that combining nutrient restriction with other OxPhos inhibitors increases their cytotoxicity, i.e. reduces the IC50 values of the therapeutics. The assessment of the cells' metabolic function showed significant inhibition of both mitochondrial respiration and aerobic glycolysis upon combination treatment. Together, our data suggest simultaneous targeting of both main energy-producing pathways as a potential strategy to overcome resistance and improve the therapeutic efficacy in HCC. Thus, we identify the combination of OxPhos inhibitors with dietary restriction regimens as valuable therapeutic approach in HCC therapy that warrants further preclinical investigation.

Notes:

Poster 11 – Group A

Domain scouting and variation of expression hosts pave the way for expression and purification of highly active ATGL protein

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Adipose triglyceride lipase (ATGL) is an essential lipase involved in intracellular fat degradation in mammals. It hydrolyzes triacylglycerols (TGs) to diacylglycerols (DGs) and FAs, yet also carries out a transacylation reaction. Malfunction of lipid hydrolysis leads to metabolic diseases such as obesity, type 2 diabetes, non-alcoholic fatty liver disease/steatohepatitis, and cancer. The physiological importance of ATGL, is also emphasized by multiple mechanisms regulating its enzymatic activity including the ATGL co-activator protein CGI-58, and the inhibitory protein G0S2. To unveil the underlying mechanisms of ATGL function and regulation on a molecular level, it is of a great importance to get the ATGL in its pure and enzymatically active form. Therefore, this scientific report focusses on the optimization of protocols that allow expression and purification of active mouse ATGL. In a large-scale effort, 42 different constructs were tested for expression and solubility in different expression hosts including E. coli, mammalian cells HEK(293T) and insect cells (Sf9). We identified suitable vector systems, fusion proteins, and expression organisms for our target. We also optimized construct size resulting in mouse ATGL M1-D288 which was eventually expressed and purified from the E. coli strain ArcticExpress (DE3). Activities of this ATGL-variant were characterized in vitro using enzymatic assays and thin layer chromatography.The assays reconfirmed the hydrolytic and transacylation activities of ATGL which implies key intrinsic regulation mechanisms of intracellular lipolysis.

Notes:

Poster 12 – Group A

Living in the cold without brown adipose tissue lipolysis

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Brown adipose tissue (BAT) burns energy to produce heat in a process termed non-shivering thermogenesis (NST), mediated by uncoupling protein 1 (UCP1). Fatty acids (FAs) activate UCP1 and are important energy substrates. These FAs are mobilized from intracellular triglycerides (TG) stored in cytoplasmic lipid droplets by adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) in a process called lipolysis. Intracellular lipolysis has been considered essential for NST but this dogma has been challenged when BAT-specific ATGL knockout mice showed normal NST. Next to ATGL, HSL exhibits minor TG hydrolase activity. Thus, HSL-mediated FA release might be sufficient to allow NST. To address this question, we studied tamoxifen-inducible BAT-specific ATGL and HSL double knockout mice (BAT-iDAKO). BAT-iDAKO mice showed markedly reduced ATGL and HSL protein expression in BAT resulting in ≥60% lower TG hydrolase activities. Subsequently, BAT mass was ~7-fold higher in BAT-iDAKO than in control mice showing a "classical white" unilocular lipid droplet histology. BAT content of UCP1 and other mitochondrial proteins upon cold adaptation (CA) was markedly lower in BAT-iDAKO than in control mice. Consistently, mitochondrial respiration upon CA was 50% lower in BAT homogenates of BAT-iDAKO than of control mice. Nevertheless, upon CA at 5°C for 3 weeks, body temperature was 0.8 °C higher in BAT-iDAKO than in control mice. Inline, β3-adrenoreceptor activation using CL316,243 revealed no differences in whole-body oxygen consumption or plasma FA and glycerol levels between BAT-iDAKO and control mice indicating that NST was intact. Food intake was 25% higher during light and slightly lower during dark in BAT-iDAKO than in control mice that was associated with reduced metabolic flexibility. Indirect calorimetry upon fasting during CA showed similar energy expenditure between BAT-iDAKO and control mice. Yet, fasting plasma FA and TG levels were 36% and 180% higher, respectively, in BAT-iDAKO mice suggesting that increased white adipose tissue lipolysis fuels BAT. Together, our data suggest that ATGL- and HSL-mediated lipolysis in BAT is not mandatory for NST.

Notes:

Poster 13 – Group B

p53 regulates a miRNA-fructose transporter axis in brown adipose tissue under fasting

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Active thermogenic adipocytes voraciously consume energy substrates like fatty acids and glucose to maintain body temperature upon cold exposure. Despite strong evidence for the involvement of brown adipose tissue (BAT) in controlling systemic energy homeostasis upon nutrient excess, it is unclear how the activity of brown adipocytes is regulated in times of nutrient scarcity. Therefore, this study aimed to scrutinize factors that modulate BAT activity to balance thermogenic and energetic needs upon simultaneous fasting and cold-exposure. For an unbiased view we performed transcriptomic and miRNA sequencing analysis of BAT from acutely fasted (24 hours) mice under mild cold exposure. Combining these data with in-depth bioinformatic analyses and in vitro experiments, we defined a previously undescribed axis of p53 and miR92a-1-5p that is highly upregulated by fasting in thermogenic adipocytes. p53, a fasting-responsive transcription factor, was previously shown to control genes involved in the thermogenic program and miR-92a-1-5p was found to negatively correlate with human BAT activity. Here, we identified fructose transporter Slc2a5 as one direct downstream target of this axis and show that fructose can be taken up by and metabolized in brown adipocytes. In sum, this study delineates a fasting-induced pathway involving p53 and miR-92a-1-5p impinging on Slc2a5 and suggests a contribution of fructose as an energy substrate in thermogenic adipocytes.

Notes:

Poster 14 – Group B

Adipocyte-HSL deficiency leads to brown adipose tissue inflammation but does not affect its thermogenic ability

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The increasing understanding of adipose tissue as a central regulator of systemic energy metabolism and metabolic health has directed the attention to distinct fat depots and their implication in the pathophysiology of metabolic diseases. Obesity is associated with adverse alterations in white adipose tissue (WAT) biology and health including increased immune cell infiltration and activation of inflammatory pathways. Since the discovery of brown adipose tissue (BAT) in adult humans, this thermogenic adipose tissue specialized in dissipating energy such as fatty acids (FA) generated by lipolysis, has been attracted considerable interest as an important organ to target obesity and associated metabolic alterations. In contrast to WAT, relatively little is known about the impact of BAT dysfunction and inflammation during obesity and pathophysiology on metabolic health. In this study, we investigated the impact of impaired adipocyte lipolysis on BAT health and function using adipocyte-specific HSL knockout (AHKO) mice. We showed that AHKO mice developed BAT expansion accompanied by a more “WAT-like” appearance and increased immune cell infiltration and activation of inflammatory pathways. In accordance, insulin mediated phosphorylation of AKT was significantly decreased in AHKO mice. BAT hypertrophy, mainly caused by reduced lipolytic activity, was partially compensated by decreased FA uptake and impaired expression of genes involved in FA and triglyceride synthesis. Notably, impaired BAT lipolysis did not affect the thermogenic ability of AHKO mice during acute cold exposure. Thus, HSL is critical for BAT metabolic health, but does not regulate its thermogenic function.

Notes:

Poster 15 – Group B

Adipose triglyceride lipase is needed for homeostatic control of sterol element-binding protein-1c driven hepatic lipogenesis

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Sterol Regulatory Element-Binding Proteins (SREBPs) are transcription factors that regulate cholesterol and fatty acid (FA) metabolism. SREBPs are translated as inactive precursors (P)-SREBP into the Endoplasmic Reticulum (ER) membrane. In the ER, SREBPs form complexes with SCAP and Insulin-Induced Gene-1 protein (INSIG-1). When lipids are scarce, proteasomal degradation of INSIG liberates the SREBP-SCAP complex that subsequently moves to the GolgiApparatus (Golgi), where it is proteolytically processed by membrane proteases S1P and S2P. The emerging nuclear (N)- SREBP-1c migrates to the nucleus where it activates genes involved in FA biosynthesis. FA may be derived from nutrition, de-novo lipogenesis, or enzymatic triglyceride (TG) catabolism (lipolysis). During fasting, however, FA are almost exclusively supplied by hydrolysis of TGs from adipose tissue stores. Adipose TriGlyceride Lipase (ATGL) is the rate-limiting enzyme for lipolysis, and it preferentially hydrolyzes Unsaturated FA (uFA). uFA stabilize SREBP-1c in the ER, but when they are scarce, SREBP-1c is proteolytically activated, and drives expression of lipogenic genes. Therefore, we hypothesized that ATGL-derived uFA regulate SREBP-1c proteolytic activation in the liver. Aim: To understand if FA derived from ATGL lipolysis contribute to the regulation of SREBP-1c in the liver. We analyze the interplay between the master regulator of FA biosynthesis, SREBP-1c, and ATGL in vitro and in vivo, in Atgl-knockout (KO) mice. Results: uFA liberated by ATGL suppress P SREBP 1c activation. SREBP-1c was hyper-activated in livers of global- and adipose-tissue specific Atgl-KO mice, which also showed reduced uFA levels in plasma and liver. Pharmacological inhibition of ATGL by Atglistatin selectively activated SREBP1c in primary hepatocytes. Conclusion: ATGL lipolysis liberates uFA from adipose tissue and suppresses lipogenic gene-expression in the liver. ATGL derived uFA block P-SREBP1c/SCAP export from the ER to the Golgi, which prevents the proteolytic activation of SREBP1c. Our findings highlight an ATGL/SREBP-1c axis, instrumental to coordinate lipogenesis and lipolysis, whose homeostatic regulation is crucial to avoid severe diseases including diabetes, cardiomyopathy, and cancer.

Notes:

Poster 16 – Group B

PPAR-α signaling triggers a metabolic switch essential for bronchiolar regeneration

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Dysregulation of the energy homeostasis often leads to development of metabolic syndrome, associated with cardiovascular disease, diabetes, liver steatosis, and cancer. The influence on the lung, however, is rather poorly characterized. Nevertheless, central obesity, type-2 diabetes, hypertension, and enhanced blood triglyceride levels are linked to reduced lung function in several epidemiological studies. Our data show that Peroxisomal Proliferator Activated Receptor-α (PPAR-α) signaling is essential for lung regeneration (Kanti et al., 2022). PPAR-α is a nuclear receptor that drives the transcriptional program for fatty acid β-oxidation and mitochondrial biosynthesis upon binding of fatty acids and cognate ligands. We challenged mice with naphthalene to induce airway epithelial injury. 1 to 2 days post naphthalene exposure, nearly complete bronchiolar epithelial denudation was apparent. In the following week, the bronchiolar epithelium fully recovered. Using RNAscope in situ hybridization, we found that PPAR-α target genes were selectively upregulated in the bronchiolar epithelium during repair. Pyruvate Dehydrogenase Kinase 4 (Pdk4) mRNA showed the strongest induction in bronchioles three days post naphthalene administration. Intriguingly, PDK-4 inhibits Pyruvate Dehydrogenase activity, and, thereby, induces a metabolic shift from sugar to fatty acid catabolism. Pharmacological activation of PPAR-α signaling enhanced the bronchiolar epithelial regeneration potential in mice (Kanti et al., 2022). Therefore, we hypothesize that PPAR-α signaling triggers a metabolic switch during bronchiolar regeneration. Adopting a powerful and novel combination of RNAscope in situ hybridization and immunofluorescence, we aim to understand which cell type(s) of the airway epithelium undergo metabolic shift for injury-repair. Mechanistical analyses of these cells should clarify the metabolic requirements during bronchiolar regeneration.

Notes:

Poster 17 – Group B

p53 coordinates immune infiltration determining the response to hepatocellular carcinoma combination therapy

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Hepatocellular carcinoma (HCC) is a common cancer with high mortality rate and limited therapeutic possibilities if diagnosed mid-to-late stage. While recently developed immune checkpoint therapies provide the best survival improvement, they are expensive and only effective in a subset of patients. Conventionally used systemic therapies are tyrosine kinase inhibitors such as lenvatinib or sorafenib, which are plagued by rapid development of resistance, followed by tumor progression. We recently showed that nutrient withdrawal (fasting in vivo and glucose starvation in vitro) can improve the therapeutic response to sorafenib in HCC cell lines, mouse models, and in patient-derived HCC organoids. Intriguingly, we found that the synergism between fasting and sorafenib depends on functional p53, a tumor suppressor mutated in a third of HCC pathologies. Here, we used our model of hepatocyte-specific, inducible p53 knock out (p53KO) in orthotopic HCC to investigate p53's impact on tumor immune infiltration upon combination treatment. Performing single cell RNA sequencing of HCC nodule-infiltrating immune cells showed a remodelling of the immune landscape towards an increased neutrophilto-lymphocyte ratio in p53KO nodules. This immunosuppressive signature comprises a biomarker for poor prognosis in many cancers and we confirm the increased presence of neutrophils with Ly6G immunohistochemistry. RNA-seq data from HepG2 cells with wild-type or CRISPR-knocked out p53 revealed an increase in expression of chemokine ligands (CXCLs) in starved cells without p53. Moreover, in vitro assays using supernatants from starved p53 wild-type and p53KO cells confirmed higher migration of neutrophils from three human donors in p53KO conditions. Together, our data suggest that p53 is coordinating neutrophil infiltration into the tumor microenvironment in response to fasting/sorafenib combination treatment. These observations harbour therapeutic potential in the light of increasing evidence that suggests targeting of tumor-promoting neutrophils.

Notes:

Poster 18 – Group B

Whole mount tissue imaging and quantitative assessment of sympathetic innervation in mouse white adipose tissue

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Intracellular lipolysis is a tightly regulated process essential for adipose tissue (AT) function and systemic energy homeostasis. One level of regulation is the post translational activation of lipases upon beta adrenergic stimulation. The catecholamine norepinephrine is released from local sympathetic neurons and represents the main ligand for beta adrenergic receptors of adipocytes. Thus, the abundance of the enzyme tyrosine hydroxylase which is rate-limiting for sympathetic norepinephrine synthesis, largely defines the lipolytic response in AT. Recent advances in whole mount tissue imaging unveiled that sympathetic innervation is highly heterogeneous within AT depots, questioning the informative value of expression analyses using tissue homogenates. 3D imaging of immunolabeled whole tissue samples typically requires specialized microscopy techniques and generates a large quantity of data. Quantitative analysis of these data is only applicable using advanced, commercial software that requires potent work station class computers. We currently establish a workflow utilizing confocal laser scanning microscopy and a freeware pipeline for quantitative analysis of nerve fiber density. This workflow is highly automated, can be performed on consumer grade computers and comprises validation on a rolling basis. Using this approach, we study the heterogeneity of sympathetic neuron density in adipose tissue in health and disease.

Notes:

Poster 19 – Group B

Mutation studies shed light on the protein-protein interaction interface and regulation of ATGL by G0S2

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The protein encoded by the G0/G1 switch gene 2 (G0S2) is a potent inhibitor of adipose triglyceride lipase (ATGL) and thus an important regulator of intracellular lipolysis. Since dysfunction of lipolysis is associated with metabolic diseases including diabetes and obesity, inhibition of ATGL is considered a therapeutic strategy. G0S2 interacts with ATGL's patatindomain to mediate non-competitive inhibition, however atomic details of the inhibition mechanism are incompletely understood. Sequences of G0S2 from higher organisms show a highly conserved N-terminal part, including a hydrophobic region covering amino acids 27 to 42. We show that predicted G0S2 orthologs from platypus, chicken and Japanese ricefish are able to inhibit human and mouse ATGL, emphasizing the contribution of conserved amino acid to ATGL inhibition. Our site directed mutagenesis and truncation studies give insights in the protein-protein interaction on a per-residue level. We determine that the minimal sequence required for ATGL inhibition ranges from amino acids 20 to 44. Residues Y27, V28, G30, A34 G37, V39 or L42 within this sequence play a substantial role in ATGL inhibition. Furthermore, we show that unspecific interactions of the N-terminal part (amino acids 20-27) of the minimal sequence facilitate the interaction to ATGL. Our studies also demonstrate that full-length G0S2 shows higher tolerance to specific single amino acid exchanges in the hydrophobic region due to the stronger contributions of unspecific interactions. However, exchanges of more than one amino-acid in the hydrophobic region also result in the loss of function as ATGL inhibitor even in the full-length protein.

Notes:

Poster 20 – Group B

Purification and biophysical characterization of the lipid-droplet associated G0/G1 switch protein (G0S2)

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Most organisms store excess energy in form of triacylglycerols (TGs) in lipid droplets (LDs). These LDs have a unique ultrastructure consisting of a core of neutral lipids that is surrounded by a phospholipid monolayer. Numerous different proteins are associated to the LDs. These energy stores can be mobilized in a process termed intracellular lipolysis, which generates glycerol and fatty acids. Enzymatic mobilization of TGs occurs via consecutive actions of three lipases, namely adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoacylglycerol lipase (MGL). The activity of ATGL is regulated by direct interaction with the LD-proteins, especially the co-activator CGI-58 and the inhibitory protein G0/G1 switch protein 2 (G0S2). Determining the structures of proteins involved in lipolysis is of great interest to understand their mechanism of action and interactions. Our studies focus on G0S2 using an integrated structural biology approach. Due to highly hydrophobic patches G0S2 tends to aggregate in aqueous buffer. We embed G0S2 in circular nanodiscs and DPC micelles, which serve as a LDmimicking system. We outline the protocol for purification and first steps towards biophysical characterization using small angle X-ray scattering (SAXS), circular dichroism spectroscopy (CD-spectroscopy), and dynamic light scattering (DLS).

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Poster 21 – Group B

Obstructing lipolysis in liver cells decreases activation of neighboring hepatic stellate cells

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Introduction: The human liver has suffered immensely due to the recent obesity epidemic. Non-alcoholic fatty liver disease (NAFLD) is one of the many consequences of excess caloric intake. Progression of a healthy liver to an irreversibly damaged cirrhotic liver is attributed to a chronically inflamed liver environment. This condition is propagated by large accumulations of lipid droplets (LDs) in hepatocytes. However, LDs themselves are not cytotoxic, but in large numbers and volumes, they tend to create an inflammatory environment in the liver which can trigger cell damage and prolonged immune responses. Fibrosis is the deposition of collagen in the liver and the consequence of this persistent, non-resolved liver inflammation. Fibrosis is carried out by otherwise resting hepatic stellate cells (HSC). These specialized cells are activated by a wide variety of factors, including certain fatty acids, metabolites and signalling proteins. In this work, we show that a decrease of the lipolysis activator CGI-58 (ABHD5) in hepatocytes leads to a decrease in the activation of neighbouring HSCs. This phenomenon is the focus of our current investigation. Furthermore, we investigate a mutation (I148M) of a lipase (PNPLA3), which plays an important role in the development of liver cancer.
Methods: We use lentivirally induced CGI-58 knock-down (KD) and PNPLA3 overexpression (OE) in hepatocytes (Hep3B) as well as HSC (LX-2). Here, we employ proteomic analysis on both cell types to gain deeper insight into the function of CGI-58 and the PNPLA3 I148M mutation in the liver. Furthermore, we validate findings suggested from proteomic analysis, e.g. proliferation, expression of certain proteins, accumulation and loss of lipid droplets, by employing techniques like wide field and confocal microscopy, western blotting and analysis of lipids via FAMES GC-MS/MS, as well as metabolomic analysis.
Results and Discussion: A constantly inflamed liver environment is often a trigger for the transformation of quiescent HSC into an activated, fibrotic phenotype. Via proteomic and phenotype analysis, we show that a KD of CGI-58, the major regulator of TG hydrolysis, in hepatocytes leads to a decrease in the activation of neighbouring HSCs. Here, we identified several characteristics of HSC activation, e.g. increased growth, loss of LDs and excessive production of key activated HSC proteins. Right now, we are in the process of identifying important players which could be responsible for this phenomenon. This study provides new insight in the complex relationship between liver lipid storage, lipolysis and fibrosis.

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Poster 22 – Group B

The role of polyamines in lipid metabolism

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Polyamines are an evolutionarily conserved class of metabolites and are involved in many cellular processes. Our lab has previously shown that dietary supplementation of the polyamine spermidine extends the health and lifespan of various species, including Drosophila. In flies, spermidine supplementation impinges on phospholipid profiles and triglyceride levels (PMID: 25010732), indicating that spermidine (or polyamines in general) might regulate lipid metabolism. In addition, genetic inhibition of polyamine pathway generated flies with elevated triglyceride stores (PMID: 34063217; 31870547). On the molecular level, spermidine acts through different routes, for instance in protein synthesis through a unique posttranslational modification of the eukaryotic translation factor eIF5A, termed “hypusination”. Here, we want to explore the relationship between polyamines, hypusination, and lipid metabolism with the help of genetic fly mutants of polyamine/hypusination pathways. We will test the hypothesis that the increase in spermidine levels leads to eIF5A hypusination-associated translation of proteins involved in lipid metabolism. This study will provide new insight into the crosstalk of two central metabolic pathways under fasting conditions.

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Poster 23 – Group B

Loss of ANGPTL4 locks the lipofibroblast phenotype and attenuates lung fibrosis.

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Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by the extensive and irreversible lung tissue scarring and high extracellular matrix (ECM) deposition. The mechanism of IPF development is not well understood. In healthy lung, lipofibroblasts, the ADRP positive cells, are responsible for the lipid homoeostasis, by transferring the surfactant components. In IPF, homeostasis of surfactant production is compromised, leading to the progression of the disease. One of the lipid metabolism regulator is Angiopoietin Like 4 (ANGPTL4). ANGPTL4 control free fatty acids circulation, which could change lipofibroblasts physiology. We hypothesized that the high levels of ANGPTL4, negatively influence the fatty acids levels contribute to the profibrotic development.

After the bleomycin challenge, in ANGPTL4 KO mice, levels of AMPK were higher compared to the WT counterpart. Collagen deposition in ANGPTL4 KO mice was lower, which suggest inhibition of ANGPLT4 is connected to lower lung fibrosis. Staining of ADRP demonstrated higher numbers of ADRP positive cells in ANGPTL4 KO BLM treated mice, compared to controls. This indicates a higher number of lipofibroblasts in KO animals. Metformin and Rosiglitazone, drugs used in the diabetes treatment, can improve the lung fibrosis by stimulating the lipogenesis and therefore, lipofibroblasts homeostasis. They are able to induce AMPK pathway, therefore inhibit collagen deposition. After rosiglitazone and metformin treatment we observed decrease of the bleomycin induced-fibrosis and increased ADRP expression. Similarly, rosiglitazone and metformin increased AMPK expression, both in vivo and in vitro on parenchymal lung fibroblasts (hPF) isolated from IPF patients.

The ANGPLTL4 KO showed similar lowering of the collagen deposition and AMPK elevation, like the rosiglitazone and metformin treatment. Therefore, ANGPTL4 could be a potential target for the treatment of lung fibrosis.

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