Doctoral Day 2012

Abstractbook

December 7th, 2012

Lecture Hall Center of the Medical University of Graz
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Preface of the Speaker of the Organization Board

On behalf of the organising committee we warmly welcome you to the Doctoral Day of the Medical University of Graz (MUG), which takes place on November 7th 2012 in the MUG Hörsaalzentrum. The meeting gathers together doctoral researchers working on thesis projects in one of our doctoral schools or PhD programs. We want to provide a platform for yearly doctoral and PhD students’ presentations. This we will do in order to strengthen possibilities for interacting and networking of participants in an exciting academic atmosphere. We will try to reach this goal by organizing lectures and poster presentations in scientific sessions according to the multitude of research fields at our university campus. Since statistics show that the majority of scientists move to non-academic positions after pursuing their PhD, we included the already from past years established and by our students highly appreciated Career Options Symposium at the end of this year’s Doctoral Day. This session will aim at illustrating to our fellows which career paths after promotion in principle do exist. Invited speakers will tell about their career choice, explain how they made the jump into another field and will give personal insight into their daily work lives.

We are looking forward to a successful meeting with high quality research presentations, lively discussions and a gain of new perspectives by interaction with fellow students from other fields and academics and professionals from various research areas.

J. Haybaeck
Preface of the Dean

The European Higher Education Area has witnessed a major transition of doctoral curricula within the past decade. The so-called Salzburg Principles of 2005 “emphasize the importance of research and research training and the promotion of interdisciplinarity in maintaining and improving the quality of higher education”. Accordingly, most curricula for doctoral studies now incorporate structured education and training in transferable skills. Above all, the core component of doctoral training is the advancement of knowledge through original research. This entails that doctoral students are embedded in a multidisciplinary faculty, work in a stimulating research environment and have access to sufficient funding.

Under the stewardship of my predecessors, Prof. Günther Gell and Prof. Andrea Olschewski, the Medical University of Graz has established a highly successful line of PhD programmes which stand out as a benchmark of doctoral curricula among the Austrian universities. The curricula and programme structures meet validated quality standards, and this achievement would not have been possible without the contributions of the Study Commission under the guidance of Prof. Andrea Berghold, the programme speakers and the distinguished programme faculties.

The Doctoral Day provides, on the one hand, a unique opportunity for our students to present their research and discuss the results with their fellow students and peers. In so doing, they acquire the skills of presenting, discussing and defending their scientific achievements. On the other hand, the rich programme of the Doctoral Day attests to the broad range of excellent research that is being conducted at our university.

It is with these thoughts that I should like to wish you an enthralling day, full of fascinating research findings and interactive discoveries. May your presentation attract the interest of your fellow students and peers alike and turn out as a building block for your professional career.

Prof. Dr. Peter Holzer
Dean of Doctoral Studies
Medical University of Graz
# Doctoral Day 2012

## Program

Medical University of Graz – Lecture Hall Centre – Auenbruggerplatz 15, A-8036 Graz

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| 9:00 – 9:30   | **Opening by**  
Rector of the Medical University of Graz, Prof. Josef Smolle  
**Words of Welcome by**  
Vice Rector, Prof. Irmgard Lippe  
Dean of Doctoral Studies, Prof. Peter Holzer  
Speaker of the Study Commission, Prof. Andrea Berghold  
Medical Director, Prof. Gernot Brunner  
Head of Organization Committee, Assoc. Prof. Johannes Haybäck |
| 9:30 – 10:30  | **Symposium 1 (talks à 10min plus 5min)**  
Chairperson: Foris Vasile Prietl Barbara  
Speakers: Schwetz Verena Mehta Anita Kuldeep Stancic Angela  
Ragginer Christine  
Responses of Osteocalcin to Oral Glucose Load in Insulin-resistant and Non-insulin-resistant Women  
Steatohepatitis-driven Hepatocellular Carcinoma: a Keratinopathy  
Inhibition of monoacylglycerol lipase (MAGL) improves DSS-induced colitis but not colitis-associated colon cancer  
Thyroid dysfunctions have an impact on oxLDL levels in Sprague Dawley rats |
| 10:30 – 11:15 | **Key Note Lecture**  
Klaus Groschner  
Sense and Sensibility – a molecular tr(i)ip from the sensory system to the heart |
| 11:15 – 11:30 | Break                                                                                                                                   |
| 11:30 – 12:30 | **Symposium 2 (talks à 10min plus 5min)**  
Chairperson: Shanmugam Ganapathy Jäger Kerstin  
Speakers: Cavalieri Margherita Hassan Ahmed  
B vitamins and MRI-detected ischemic brain lesions in patients with recent transient ischemic attack or stroke: The VITATOPS MRI-Substudy  
Psychosocial stress counteracts behavioral changes associated with murine colitis, but not colitis itself |
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<td><strong>Lunch &amp; Poster Session</strong></td>
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<td><strong>Presentation of the Student Representation Doctoral Programmes</strong></td>
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<td>15:30 – 16:10</td>
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<td><strong>Symposium 4 (talks à 5min plus 5min)</strong></td>
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**Chairperson:**
- Anil Paul
- Kober Alexandra
- Martinelli Elisabeth
- Kirsch Alexander
- Heidi Schmitt

**Speakers:**
- Tomazic Peter
- Durchschein Franziska
- Kober Alexandra
- Anil Paul
- Kober Alexandra
- Martinelli Elisabeth
- Kirsch Alexander
- Heidi Schmitt
- Andrea Olschewski
- Jaap Wieling
- Siegling Angela
- Christian Rosker
- Heribert Häusler
- Berghold Veronika
- Binder Corinna
- Curcic Sanja
- Heidary Maryam
- Krones Elisabeth
- Martínez Vera Naira Pilar
- Mohan Sumitra
- Pichler Karin
- Andrea Olschewski
- Medical University of Graz / LBI for Lung Vascular Research
- QPS Austria
- Austria Wirtschaftsservice
- Novartis Pharma GmbH
- University of Mainz / Böhringer Ingelheim

**Abstracts:**
- Seasonal differences in nasal mucus proteome between allergic rhinitis patients and healthy controls
- Genetic Loss of Muscarine-3 Receptor Decreases Biliary Bicarbonate Secretion and Aggravates Cholestatic Liver Injury in Mice
- Phospholipid scramblase 1 is not involved in human trophoblast fusion
- Influence of an open ductus arteriosus on cerebral tissue oxygenation during the first day of life in preterm and term neonates
- Uremia alters HDL composition and anti-inflammatory properties
- Characterization of CTCs with Stem Cell Features from Patients with Breast Cancer
- norUDCA protects Common Bile Duct Ligated Mice from Collecting Duct Tubular Epithelial Lesions
- Assessment of the uptake of magnetite labeled nanoparticles in the rat brain using MRI
- Analysis of Circulating Cell Free Tumor DNA in Plasma in Colon Cancer Patients
- Matrix metalloproteinases’ expression in human growth plate chondrocytes is enhanced at high levels of mechanical loading – a possible explanation for overuse injuries in children
## Poster Presentations

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Key Note Lecture

SENSE AND SENSIBILITY – A MOLECULAR TR(I)P FROM THE SENSORY SYSTEM TO THE HEART

Klaus Groschner

Institute of Biophysics, MUG

During the past two decades, a number of apparently “last white spots” on our scientific map of cellular ion transport systems, specifically of plasma membrane ion conductances, have been eliminated, and a fairly complete picture of the ion channel repertoire in excitable and non-excitable cells has emerged. These advances enabled impressive insights into the molecular basis of cellular perception, processing and interpretation of environmental stimuli. Recent progress in ion channel physiology has substantially improved our understanding of the human sensory system and the molecular principles underlying sensory organ function. The transient receptor potential (TRP) family of cation channels is a large family of signal transduction molecules, characterized by a six-transmembrane domain topology and the ability to form tetrameric channel complexes. TRP and related cation channels have been identified in almost every tissue of the body and recognize/sense a wide variety environmental stimuli as well as intracellular messengers, thereby serving as common and essential elements of cellular signal integration. Importantly, their (patho)physiological role is not restricted to sensory organs and the sensory nervous system, but these signaling molecules determine also the sensitivity of many tissues to pathologically relevant stress situations, including susceptibility of the heart to hypertrophic and arrhythmogenic stimuli. Recent progress in identifying structure-function relations and in translating this molecular knowledge into novel therapeutic strategies will be outlined and discussed.
# Curriculum Vitae

**Prof. Dr. Andrea Olschewski**

<table>
<thead>
<tr>
<th>Current position</th>
<th>Managing Director</th>
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<tr>
<td>Organisation</td>
<td>Ludwig-Boltzmann Institute for Lung Vascular Research, Graz</td>
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<tr>
<td>since</td>
<td>2010</td>
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<td>Professional career</td>
<td>2009</td>
</tr>
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<td></td>
<td>Chair of the Research Promotion Commission of the MUG</td>
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<td>2006-present</td>
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<td>Full Professor of Anesthesiology, Medical University of Graz, Austria</td>
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<td></td>
<td>2002-2005</td>
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<td></td>
<td>Deputy Director for academic affairs of the Department of Anesthesiology, Justus-Liebig-University Giessen, Germany</td>
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<td>2002-2005</td>
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<tr>
<td></td>
<td>Associate Professor, Department of Anesthesiology, Justus-Liebig-University Giessen, Germany</td>
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<tr>
<td></td>
<td>2002</td>
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<td></td>
<td>Habilitation in Anesthesiology</td>
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<td>2000-2002</td>
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<td></td>
<td>Postdoctoral Fellow of the German Research Foundation (DFG) at the University of Minnesota, Minneapolis, MN, USA</td>
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<td>1999-2005</td>
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<td></td>
<td>Senior physician, Department of Anesthesiology, Justus-Liebig-University Giessen; Germany</td>
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<td></td>
<td>1993-1999</td>
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<td></td>
<td>Resident, Department of Anesthesiology, Justus-Liebig-University Giessen, Germany</td>
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| Education               | 2005              |
|                        | Board certification in Pain Therapy |
|                        | 1999              |
|                        | Board certification in Anesthesiology |
|                        | 1999              |
|                        | PhD thesis in Neurophysiology, Justus-Liebig-University Giessen, Germany |
|                        | 1986-1992         |
|                        | Study of Medicine, MD, University of Debrecen, Hungary |
**Curriculum Vitae**

**Dr. Christian Rosker**

Current position  
MSL Respiratory

Organisation  
Novartis Pharma (Austria)

since  
08.2011

Professional career  
09.2008-07.2011  
University Assistant at the Physiology Department of the University of Bern, Switzerland (focus on cardiac remodelling/arrhythmia-optical imaging, immuno-cytochemistry, molecular biology, pharmacology).

Lectureship (Physiology)

01.2007-08.2008  
Research associate at the University of Cambridge, Department of Pharmacology, UK (focus on cardiac Ryanodine Receptors, projects with Addenbrookes Hospital, Cambridge-electrophysiology, calcium imaging, pharmacology).

Lectureship (Pharmacology)

REDEEMER BIOSCIENCE LTD. founded

Further training at the Staff Development Division of the University of Cambridge (Project management, leadership development)

03.2005-12.2006  
Research associate of ESTEVE at the Medical University of Graz (focus on voltage-dependent sodium channels and GIRK channels, pain and cancer projects with Clinic of Neurology and Clinic of Dermatology and Venereology, both Graz, Austria-electrophysiology, confocal microscopy, molecular biology, pharmacology)

Research fellowship (BA price 2006) at Bochum University

Lectureship (Biophysics, Signal Transduction)

Education  
1994-2005  

APHAR price 2004

Lectureship (Pharmacology)
Curriculum Vitae

Angela Siegling, Ph.D

Current position
Patent & Licensing Manager in the Life Science area
Financing Administration and Technology transfer
Evaluation of new technologies and ideas, negotiation and deal making, business intelligence, due diligence

Organisation
Austria Wirtschaftsservice Gesellschaft mbH
Since
08.2008

Professional career
Vienna, Austria Eucodis GmbH
07.2004-07.2008
Chief Scientific Officer (CSO)
Management of the research program, application of grants
Establishment and supervision of all laboratories (22 FTE)
Responsibility for research budget
Management of intellectual property, Technology transfer
Interaction with collaborators, customers and investors
Participation with CEO and CFO in developing commercial strategies of the Company

Paris, France Mixis Fance S.A., subsidiary of PLIVA
05.2001-06.2004
Chief Scientific Officer (CSO)
Management of the company’s research program
Establishment and supervision of the laboratory (12 FTE)
Management of intellectual property, Technology transfer
Interaction with collaborators and steering of scientific cooperations
Establishment and management of the research budget

Wuppertal, Germany Bayer-AG
07.1996-04.2001
Research Scientist in Immunology and Neurology
Department of Anti-infective Research, Department of CNS Research
Molecular in vitro pharmacology in cell-based reporter assays
Identification and evaluation of new drug targets
Supervision of technicians and PhD students
Organisation of research collaborations with universities and industries

Project Manager directly reporting to the board of directors
Development of a drug candidate in the anti-infective and anti-cancer area
from basic research to preclinicals, Patent application development
Contracting and budget responsibility, Technology transfer
Berlin, Germany Charité, Berlin  
07.1994-06.1996  
RESEARCH SCIENTIST in Immunology,  
Institute of Medical Immunology, Prof. Dr. H.D. Volk  
Analysis of gene expression in transplantation models  
Supervision of PhD students, teaching assistant in immunology

New Orleans, USA Louisiana State University (study visit)  
02.1995-05.1995  
Department of Pulmonary and Critical Care Medicine, Prof. Jay Kolls  
Study visit granted by Boehringer Ingelheim GmbH  
Construction of adenoviruses for gene therapy in graft rejection

Erlangen, Germany Max-Planck-Institute  
09.1991-06.1994  
POSTDOCTORAL FELLOW  
Institute of Rheumatology, Prof. Dr. F. Emmrich, Prof. Dr. J.R. Kalden  
Establishment of molecular biological technologies for quantification of gene expression in immunological in vitro and in vivo models

Education  
1988-1991 PhD  
University of Leipzig  
Institute of Pharmaceutical Chemistry, Prof. Dr. G. Wagner  
Dr. rer. nat. in pharmaceutical chemistry: "magna cum laude"  
Thesis: "Synthesis of immunomodulating active 2,4-Dioxo-1,2,3,4-tetrahydro-quinazolines with aliphatic Thioether-, Sulfoxide-, Sulfonestructure in position 3 and tricyclic quianzoline-derivatives"

1983-1988 BASIC STUDIES  
University of Leipzig  
Study of biology and chemistry
**Curriculum Vitae**

**Jaap Wieling, Ph.D.**

**Current position**  
Sr. Vice-President QPS Holdings LLC, and General Manager, QPS Austria

**Organisation**  
QPS Austria GmbH

**Since**  
01.09.2012

**Professional experience**

07.2010-08.2012  
QPS Holding LLC  
Vice President of Bioanalysis and Technology R&D, Newark, Delaware, USA / Groningen, The Netherlands

05.2009-08.2012  
QPS Netherlands B.V.  
Managing Director, Bioanalysis & Drug Metabolism, Groningen, The Netherlands

09.1999-08.2012  
Xendo Holding B.V.  
Co-founder, Member of the Board, Groningen, The Netherlands

01.2006-05.2009  
Xendo Drug Development B.V.  
Managing Director, Groningen, The Netherlands

09.1999-12.2005  
Xendo Laboratories B.V.  
Managing Director, Groningen, The Netherlands

02.1992-09.1999  
Different research and management positions at contract-research and applied research organizations in the Netherlands (TNO, PRA, DSM)

**Other professional activities**

1995-2005  
Dutch Board of Certification  
Auditor for inspection of certified laboratories according to EN45001 / ISO17025

1995-2011  
University of Groningen  
Lecturer / teacher  
Lecturer / teacher GLP  
Lecturer / teacher Bioanalytical Mass Spectrometry
2001-present
KNCV (Dutch Royal Chemical Society)
Co-Founder, board member and President (since 2010) of FABIAN - bioanalytical society (representing Dutch and Flemish bioanalytical community)

2007-present
SBGG
Stichting Business Generator Groningen (Foundation)
Investment committee member Groningen
Assessment of patent, IP and / or business proposals

2010-present
Global CRO Consortium
Co-founder and member Global
Consortium for the advancement and protection of bioanalytical CRO in industry and regulatory environment

Education
1988-1993
University Centre for Pharmacy
Ph.D. in Mathematics and Natural Sciences

1997-1999
University Centre for Pharmacy
M.Sc. in Pharmacy

1983-1987
Institute of Technology, Faculty of Chemistry
B.Sc. in Analytical Chemistry
Hypochlorous acid induces the apoptotic machinery in yeast

Ali Alavian-Ghavanini¹, Didac Carmona-Gutierrez², Lukas Habernig², Maria A. Bauer², Christine Rossmann¹, Wolfgang Sattler¹, Frank Madeo², Ernst Malle¹

¹ Institute of Molecular Biology and Biochemistry, Medical University Graz, Austria; ² Institute of Molecular Biosciences, University of Graz, Austria;

Background:
The phagocytic enzyme myeloperoxidase, abundantly present in various inflammatory diseases, generates hypochlorous acid (HOCl) from H₂O₂ and physiological chloride ions. HOCl, an efficient microbicidal agent, is highly reactive with a biological molecules e.g. DNA, lipids and proteins.

Aims:
HOCl may induce apoptosis in eukaryotic systems although the underlying mechanisms are unclear. We now have used Saccharomyces cerevisiae as a well-established model organism, which also can undergo apoptosis, to study the core machinery executing cell death in response to HOCl.

Methods:
Wild-type yeast as well as different S. cerevisiae strains lacking several homologues of the mammalian apoptotic machinery were tested. Dihydroethidium was used to measure the production of reactive oxygen species (ROS). Clonogenic survival plating was performed to measure cell viability. Apoptosis was confirmed via TUNEL assay and annexin V/PI co-staining. Western-blot analysis and immunocytochemistry were performed to detect HOCl-modified proteins.

Results:
HOCl induces cell death in all yeast strains through generation of ROS. This could be confirmed by using different ROS scavengers and a yeast strain lacking mitochondrial DNA. HOCl elicits cell death via mitochondrial routes in all yeast strains lacking homologues of the mammalian apoptotic machinery. Apoptotic cell death induced by HOCl is relieved in Δkex1 cells but not in other deletion mutants of apoptotic pathway or known cell death-associated proteases. Using specific monoclonal antibodies we could demonstrate that cell lethality by HOCl is paralleled by formation of HOCl-modified proteins.

Conclusion:
Here we conclude that Kex1p may act in a beneficial manner to abrogate HOCl-mediated apoptosis in yeast.

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Telephone number: +43 (0)316 / 380-4198
Phospholipid transfer protein is expressed in cerebrovascular endothelial cells and involved in HDL genesis at the blood brain barrier

Anil Paul Chirackal Manavalan1, Alexandra Kober1, Jari Metso2, Tatjana Becker1, Karin Hasslitzer1, Cornelia Schweinzer1, Johannes Haybaeck3, Jasminka Stefulj4, Matti Jauhiainen2, and Ute Pazenkoebbeck1

1Institute of Pathophysiology and Immunology, Medical University of Graz, Austria; 2 National Institute for Health and Welfare, Biomedicum, Helsinki, Finland; 3Institute of Pathology, Medical University of Graz, Austria; 4Department of Molecular Biology, Ruder Boskovic Institute, Zagreb, Croatia.

Background:
Levels of phospholipid transfer protein (PLTP), a key protein involved in the biogenesis and remodeling of plasma HDL, are increased in brain tissue but PLTP activity is decreased in CSF of Alzheimer’s disease patients. Both, pro- or anti-atherogenic effects of PLTP have been reported and elevated plasma PLTP activity in insulin resistance is associated with obesity. We reported that live r X receptor (LXR) activation promotes cellular cholesterol efflux and formation of HDL-like particles in an established in vitro model of the blood-brain barrier (BBB) consisting of primary, porcine brain capillary endothelial cells (pBCEC).

Aim: We here aimed to investigate the expression, regulation and function of another LXR target gene, PLTP in pBCEC.

Methods:
Real-time PCR, immunoblotting and immunohistochemistry were applied. Assays for PLTP activity or cellular cholesterol efflux were performed using radiolabeled lipids.

Results: We found that PLTP is expressed and secreted by pBCEC and its activity and expression are enhanced by 24(S)-hydroxycholesterol (a cerebral cholesterol metabolite and endogenous LXR agonist) or TO901317 (a synthetic LXR agonist). When simulating diabetic conditions in vitro via glucose/insulin treatment, we detected a moderate increase in PLTP levels but a reduced PLTP activity. Pre-incubation with isolated active plasma PLTP enhanced the capacity of HDL3 to efflux cholesterol from pBCEC in a time dependent manner. Furthermore, RNA interference mediated PLTP silencing reduced apoA-I dependent cholesterol efflux from pBCEC.

Conclusion:
Based on our current findings, we propose that PLTP represents a key player in HDL genesis/remodeling at the BBB and in lipid transport between the brain and the circulation.

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Stretch-Induced Mechanotransduction in HL-1 Cardiomyocytes

Shaymaa, Bahnassy¹, Michael, Poteser², Hannes, Schleifer², Klaus, Groschner²

¹ Institute of Pharmaceutical Sciences - Pharmacology and Toxicology, University of Graz, Austria; ² Institute of Biophysics, Medical University Graz, Austria

Background:
Mechanical strain of cardiomyocytes is considered to be a major initiator of cardiac remodelling through mechanotransduction. Mechanical forces are efficiently converted into cellular signals to initiate structural and functional remodelling of cardiac myocytes. Molecular mechanisms underlying cardiac mechanotransduction are still barely understood. Nuclear Factor of Activated T-cells (NFAT) is an important calcium-responsive transcription factor and a key player in cardiac remodelling processes.

Aims:
In this study, we investigated the involvement of Ca²⁺ signalling molecules as a link between mechanical stretch and NFAT translocation in HL-1 atrial myocytes. We specifically focused on the potential role of receptor-operated and/or store-operated Ca²⁺ permeable channels of the TRPCs (classical transient receptor potential).

Methods:
Populations of HL-1 cells overexpressing GFP-NFAT were exposed to a single-stretch by 20% for 20 minutes on commercially available silicon membranes and NFAT localization was then analysed by fluorescence microscopy.

Results:
Results revealed that stretched HL-1 cells significantly increased NFAT nuclear translocation when compared to unstretched cells. Similar NFAT activation was obtained when HL-1 cells were stimulated by either endothelin-1 (100 nM) or thapsigargin (1 µM) in the absence of a stretch stimulus. HL-1 cells were found to express several TRPC that might contribute to mechanotransduction.

Conclusion:
These results demonstrate that similar levels of NFAT activation are induced in HL-1 cells by either mechanical stress, cell activation via Gq-coupled receptors or depletion of internal Ca²⁺ stores. Our data suggest a linkage between mechanical stretch and cardiac remodelling involving Ca²⁺ entry and NFAT activation.

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Characterization of circulating tumor cells and plasma DNA in patients with prostate cancer

Jelena Belic, Martina Auer, Ellen Heitzer, Stefan Gutschi, Katja Fischereider, Martin Pichler, Peter Ulz, Julie Geigl, Herbert Augustin, Florian Eisner, Gerald Höfler, Klaus Pantel, Sabine Riethdorf, Jochen B. Geigl, Michael R. Speicher

1 Institute of Human Genetics, Medical University of Graz, Austria, 2 Department of Urology, Medical University of Graz, Austria, 3 Division of Oncology, Medical University of Graz, Austria, 4 Institute of Pathology, Medical University of Graz, Austria, 5 Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, Germany

Background:
Recent studies show that analysis of circulating DNA and circulating tumor cells (CTCs) allow the detection of tumor-related genetic and epigenetic alterations that are relevant for cancer development and progression. Both plasma DNA and CTCs appear to be valuable candidates to serve as overall survival surrogate markers and could allow the early detection of patients with poor prognosis.

Aims:
We aimed to study the genomic landscape and mutation spectrum of CTCs and plasma DNA in early stage and metastatic prostate cancer patients.

Methods:
We analyzed blood from patients under an active surveillance protocol, prior to prostatectomy and metastatic patients. CTCs were isolated using the Veridex Cell Search system and PALM laser capture microdissection. For plasma DNA and CTCs we performed copy number variation analysis using array comparative genomic hybridization (aCGH) and Next Generation Sequencing.

Results:
Patients from the metastatic group showed significant differences in four analyzed parameters including Prostate Specific Antigen (PSA), number of CTCs, plasma DNA concentration and Gleason score compared to the other two groups. Furthermore, we detected copy number variations including a high level amplification on the X chromosome at the androgen receptor gene location. The amplification of the androgen receptor gene localization had previously been associated with metastatic prostate cancer. This gain was also observed in CTCs from these patients.

Conclusion:
It is possible to establish CNV profiles and to identify copy number changes associated with hormone treatment outcome, such as amplification of the androgen receptor on the X chromosome, from plasma DNA and CTCs. This might help to monitor patients with metastatic prostate cancer.

Contact:
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telephone number: +43 (0)316 / 380 - 7527
Implications of fibroblast growth factor 21 (FGF21) on cardiac energy metabolism

Manoja Brahma, Rene Adam, Kathrin Zierler, Rudolf Zechner, Günter Hämmerle

Institute of Molecular Biosciences, University of Graz, 8010, Graz, Austria

Background:
The heart depends on a constant source of energy such as fatty acids and carbohydrates in order to maintain its contractile function. Changes in myocardial substrate utilization have been reported under different pathophysiological conditions albeit the underlying mechanisms are poorly understood. Recently, we have found that cardiac fibroblast growth factor 21 (FGF21) mRNA expression was substantially increased in mice lacking adipose triglyceride lipase (Atgl(-/-)). FGF21 is predominantly expressed and secreted from the liver during prolonged fasting. Despite the importance of FGF21 as a regulator of hepatic energy metabolism, a role for FGF21 in cardiac energy metabolism has not been discovered so far.

Aim:
To elucidate the potential role of FGF21 in cardiac energy metabolism under physiological conditions.

Methods:
H9C2 cardiomyocyte cell line was infected with recombinant adenovirus expressing FGF21 (Ad−FGF21). Transgenic mice overexpressing FGF21 specifically in the heart (MHC-FGF21-Tg) were generated to elucidate the impact of FGF21 on cardiac energy metabolism.

Results:
Adenovirus mediated FGF21 expression (Ad−FGF21) resulted in increased triglyceride accumulation in differentiated H9C2 cardiomyocytes. Interestingly, fatty acid uptake was unchanged, while deoxyglucose uptake was significantly increased in Ad−FGF21 infected cells. Cardiac triglyceride content in MHC-FGF21-Tg mice was significantly increased compared to wild-type littermates. Furthermore, mRNA expression levels of PPARα target genes were in cardiac muscle of transgenic mice indicating a possible impairment in cardiac fatty acid utilization.

Conclusion:
Based on the above results in H9C2 cardiomyocytes and MHC-FGF21-Tg mice, we hypothesize that FGF21 could be involved in shifting the myocardial substrate preference from fatty acids to carbohydrates.

Contact:
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Molecular mechanisms of Nor-ursodeoxycholic acid (Nor-UDCA) in non-alcoholic fatty liver disease (NAFLD) and metabolically induced liver cancer

Anil Butchi Reddy\textsuperscript{1}, Robert Mc Mahon\textsuperscript{1}, Thierry Claudel\textsuperscript{1}, Melitta Penz-Oesterreicher\textsuperscript{1}, Christoph Oesterreicher\textsuperscript{2}, Michael Trauner\textsuperscript{1}

\textsuperscript{1}Hans Popper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III,\textsuperscript{2} Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria

\textit{DK-MCD}

\textbf{Background:}
Liver fibrosis is defined as the formation of excess fibrous connective tissue resulting from the deposition of extracellular matrix proteins as the result of a reactive or reparative process during liver injury. Resident hepatic stellate cells (HSC) are the major liver cell population contributing to fibrosis, which can ultimately disrupt liver architecture leading to cirrhosis and hepatocellular carcinoma.\textbf{Aims:}
The focus of our study is to elucidate the role of Nor-UDCA as novel therapeutic agent in the amelioration of liver fibrosis by directly affecting HSC activation.

\textbf{Methods:}
Human HSC (LXII) and mouse HSC (mHSC) cell lines were treated with Nor-UDCA. Additionally, the ability of LPS or recombinant PDGF-C to initiate inflammatory or proliferative response was assessed in both cell lines pre-treated with Nor-UDCA. All samples were analyzed by qPCR, RT\textsuperscript{2} Profiler qPCR array, MTT assay and Western blotting.

\textbf{Results:}
Significant decreases in proliferation rates were observed in LXII and mHSC treated with Nor-UDCA for up to 96 hours/4 days. qPCR analysis showed increase anti-fibrotic and apoptotic markers (MMP2, MMP9, DR4, DR5 and Trail) in Nor-UDCA treated and Nor-UDCA - LPS or recombinant PDGF-C stimulated samples. RT\textsuperscript{2} Profiler qPCR arrays, where human HSC were treated for 24 hours with Nor-UDCA showed four-fold decrease expression of proliferation markers and four-fold increased levels of apoptotic markers.

\textbf{Conclusion:}
Preliminary results indicate that Nor-UDCA negatively impacts on proliferation rates and increases the expression of apoptotic markers in LXII and mHSC cell lines. At this point it is conceivable that Nor-UDCA could induce apoptosis or deactivation of activated HSC ultimately contributing to resolution of fibrosis.

\textbf{Contact:}
E-mail: anil.reddy@meduniwien.ac.at
Telephone number: +43 (0)1 40 400 4765
Altered protein expression in adipose triglyceride lipase knockout B-cell tumours

Katarina Fritz¹, Suman K. Das¹, Gerald Höfler¹, Britta Obrist², Stefan Spörk², Ruth Birner-Grünberger¹

¹Pathology; Medical University of Graz, Graz, Austria; ²Centre of Medical Research, Graz, Austria

Background:
Enhanced glucose consumption is well described in tumours (known as Warburg Effect) but also lipid metabolism is changed in cancer cells.

Aims:
Here we use murine tumour B-cells which are depleted for adipose triglyceride lipase expression (ATGL-KO) and compare their growth to WT tumour B-cells. In vitro as well as in vivo ATGL-KO tumour B-cells grow significantly faster than WT tumour B-cells. In order to depict the mechanisms responsible for this enhanced growth we performed differential proteomic profiling for the tumours grown in mice.

Methods:
Five mice each were sacrificed when ATGL-KO and WT tumours had the same size, the tumours were excised, five tumour lysates each, ATGL-KO and WT, were pooled and digested. For the quantitative comparison of proteins of the two different tumour species stable isotope dimethyl labelling was used.

Results:
The results show an up-regulation of glycolytic enzymes (e.g. phosphoglycerate mutase, fructosebisphosphate aldolase, phosphofructokinase) in ATGL-KO tumours. Furthermore proteins like adenylate kinase, creatine kinase, tumour protein D54 were found to be up-regulated in ATGL-KO tumours.

Conclusion:
The higher expression of glycolytic enzymes might compensate energy demands arising from the depletion of the lipase and beyond that could explain the increased growth rate of ATGL-KO tumours.

Contact:
e-mail: Katarina.Fritz@medunigraz.at
telephone number: +43 (0)676 / 67 73 015
Intact mitochondria despite triglyceride accumulation in CGI-58/- macrophages

Madeleine, Goeritzer¹, Silvia, Povoden¹, Dagmar, Kolb², Dagmar, Kratky¹

¹Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University Graz, Austria; ²Center for Medical Research and Institute of Histology, Medical University Graz, Austria

Background:
Comparative gene identification-58 (CGI-58) binds to lipid droplets by interaction with perilipin A in a hormone-dependent way. During lipolysis, hormone stimulation leads to phosphorylation of hormone sensitive lipase and perilipin, whereby CGI-58 is released to activate adipose triglyceride lipase (ATGL). Mutations of CGI-58 cause Chanarin-Dorfman-Syndrome resulting in TG accumulation in all tissues and in ichthyosis. Since CGI-58 knockout (-/-) mice die soon after birth due to a skin barrier defect we generated macrophage-specific CGI-58 (macCGI-58) -/- mice to elucidate the entire role of CGI-58 in macrophages.

Aims:
The aims of this project are to characterize macCGI-58/- mice, to investigate the role of CGI-58 in foam cell formation and atherogenesis and to study cell death in CGI-58/- macrophages. We also want to elucidate whether CGI-58 has ATGL-independent functions.

Methods:
qPCR, Western blotting, lipid parameters, immunofluorescence, electron microscopy

Results:
In contrast to whole-body CGI-58/- mice, macCGI-58/- mice are viable with no apparent changes in skin phenotype and plasma lipid parameters compared to wild type (WT) mice. We observed a massive increase of lipid droplets in CGI-58/- peritoneal macrophages as well as in CGI-58/- bone marrow-derived macrophages. In contrast to macrophages from WT and ATGL/- mice, CGI-58/- macrophages showed high mRNA levels of the pro-inflammatory cytokine Gro-1 which indicates that CGI-58/- macrophages have a M1-like phenotype. Furthermore, TG accumulation does not lead to mitochondrial dysfunction in CGI-58/- macrophages.

Conclusion:
We hypothesize that CGI-58 has not only ATGL-dependent functions but could also act independently or might activate other so far unknown lipase(s).

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Characterization of CTCs with Stem Cell Features from Patients with Breast Cancer

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Background:
Circulating tumor cell (CTC) analysis has the potential to provide novel insights into metastasis. In addition, it is a promising new diagnostic field for estimating the risk for metastatic relapse and progression in breast cancer patients. The persistence of CTC in breast cancer patients might be associated with cancer stem cells which have been suggested to be the active source of metastatic spread in primary tumors. Furthermore, CTCs may undergo epithelial-mesenchymal transition, which is essential for metastasis.

Aims:
The main aim of our study was to identify CTCs with stem cell features from patients with breast cancer. As a proof of principle, we have studied the expression of EMT and stem cell markers in two breast cancer cell lines including the claudin-low subtype SUM159 and the luminal subtype MCF-7.

Methods:
Using FACS we isolated three mammary epithelial cell states including stem-like (CD44hi CD24negEpCAMlo), basal (CD44hiCD24negEpCAMneg), and luminal (CD44loCD24hiEpCAMhi) from two human breast cancer lines SUM159 and MCF7. Moreover, cells were sorted by ALDH activity which is the universal cancer stem cell marker.

Results:
Even if expression of EMT and stem cell markers of all subpopulation of SUM159 differed significantly from MCF7 cell line, we were not able to observe any difference within the subpopulation using quantitative RT-PCR.

Conclusion:
As the results showed that there is no significant difference in mRNA expression level of stem cell and EMT markers in different population of SUM159, we decided to investigate expression level of miRNAs, which play an important role in metastasis and stem cell self-renewal and differentiation.

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Fenofibrate suppresses B-cell tumor via deprivation of serum lipids

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Background:
Nowadays, tumor lipid metabolism is regarded as an important topic. Increasing clinical and experimental data show the crucial link connecting lipid and tumor development such as the pathogenesis of cancer-associated-cachexia, which is accompanied with severe lipid loss. Supporting it, epidemiological studies indicate that overweight and obesity are associated with increased cancer risk.

Aims:
To test the hypothesis that aggressive B-cell tumor induces severe loss of white adipose tissue (WAT) in order to fuel itself. Using lipid lowering drug such as Fenofibrate to deprive lipid supply might suppress B-cells tumor.

Methods:
Using subcutaneously injection of oncogenic B-cells into C57/BL6 mice fed with Fenofibrate containing food; tumor weight, FFA uptake, TG/FFA content in tissues and serum were measured.

Results:
1. Compared to non-tumor control, WAT in tumor-bearing mice is reduced by 29.5%.
2. After Fenofibrate treatment, tumor weight decreases by 73.3% while serum triglyceride by 76.6%, FFA by 36.3%.
3. FFA uptake in liver increases but no apparent enhancement in tumor.
4. In vitro, lipid supplement promotes B-cell proliferation.

Conclusion:
B cell tumor, as one of the tumor with fastest proliferation, induces loss of WAT. After fenofibrate treatment, not only serum lipid concentration is reduced but also tumor is suppressed. Even though WAT weight decrease after fenofibrate treatment, liver fatty acids uptake increases while tumors do not change, it indicates fenofibrate drives lipid mobilization from WAT to peripheral tissues such as liver, followed by burning it or storing it in intracellular lipid droplet, finally lead to serum lipid deprivation and tumor suppression.

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A novel UCP interacting protein is involved in mitochondrial calcium uptake

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**Background:**
Mitochondrial Ca\(^{2+}\) sequestration plays a central role in cell signal transduction and is pivotal for important cellular functions. Though the phenomenon of mitochondrial Ca\(^{2+}\) accumulation is known since the 1950s, the molecular identity of underlying proteins has not been fully described so far. We showed that uncoupling proteins 2 and 3 (UCP2/3) fundamentally contribute to a certain mitochondrial uptake mode of intracellularly released Ca\(^{2+}\). Recently, other components of the mitochondrial uptake machinery, the mitochondrial calcium uniport protein (MCU), mitochondrial Ca\(^{2+}\) uptake 1 (MICU1) and leucine zipper EF hand-containing transmembrane protein 1 (LETM1) have been described.

**Aims:**
Aim of this study is to gain novel insights into the molecular mechanisms of mitochondrial Ca\(^{2+}\) uptake and to identify the individual contribution of UCP2/3, MCU, MICU1 and LETM1.

**Methods:**
Experiments are based on siRNA-mediated knockdown and overexpression of proteins, accomplished by cloning of new constructs as well as stable knock-down cell lines. Organelle Ca\(^{2+}\) measurements are accomplished by utilization of genetically-encoded biosensors in combination with high resolution fluorescence microscopy. Further characterization is done by genomic and proteomic approaches.

**Results:**
We identified a new, fundamental, component of the mitochondrial Ca\(^{2+}\) uptake machinery that contributes to UCP2/3-dependent but not MCU-dependent mitochondrial Ca\(^{2+}\) uptake (UCP2-interacting protein 3, UIP3). Furthermore, we found a membrane permeable small molecular weight inhibitor of the UCP2/3/UIP3-dependent uptake pathway.

**Conclusion:**
Mitochondrial Ca\(^{2+}\) uptake represents a complex phenomenon that most likely involves multiple channel-like proteins and their respective protein environment. Different routes of mitochondrial Ca\(^{2+}\) sequestration might be necessary to meet the demand of the individual cell type.

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Pathophysiological modifiers of intracellular Ca\textsuperscript{2+} handling and triggered arrhythmias

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\textbf{Background:}
Several studies have suggested abnormal Ca\textsuperscript{2+} handling plays a critical role in chronic atrial fibrillation (AF). However, changes in Ca\textsuperscript{2+} homeostasis in cardiomyocytes may occur early during atrial remodelling and promote arrhythmias and disease progression. However, little is known about time-dependent changes in intracellular Ca\textsuperscript{2+} homeostasis in atrial remodelling.

\textbf{Aim:}
Quantify time-dependent alterations in cardiomyocyte Ca\textsuperscript{2+} homeostasis and contractile function in atrial remodelling following rapid atrial pacing (RAP).

\textbf{Methods:}
In 12 pigs, a custom-made atrial pacemaker was implanted for RAP (600/min). Left atrial area size assessed by echocardiography. After 2 and 6 weeks (w) of RAP, cardiomyocytes were isolated from left (LA) or right (RA) atrium. Calcium transients (CaT) and cell shortening (CS) were measured during field stimulation using Fura-2 AM. SR Ca\textsuperscript{2+} content was measured as the peak of caffeine-induced CaT. Myofilament Ca\textsuperscript{2+} sensitivity was derived from simultaneous [Ca\textsuperscript{2+}]_i- and contraction measurements.

\textbf{Results:}
After 2 w of rapid pacing, LA size was increased and further increased after 6 w of pacing. In single cardiomyocyte isolated from LA after 2 w and 6 w pacing, the frequency-dependent Ca\textsuperscript{2+} response was blunted, and CaT amplitude from both sides tended to be decreased, despite time-dependent SR Ca\textsuperscript{2+} content increasing. However CS was not different between groups, which might be explained by increased Ca\textsuperscript{2+} myofilament sensitivity following RAP.

\textbf{Conclusion:}
In summary, the Ca\textsuperscript{2+} release process becomes time-dependently less efficient in atrial remodeling with RAP. SR Ca\textsuperscript{2+} load is increased, which heralds a potential risk of Ca\textsuperscript{2+} overload and arrhythmias.

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N-acetyltransferase 8-like, a mitochondrial protein, accelerates brown adipose tissue metabolism

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Background:
New aspects in fighting obesity arose with the proof that significant depots of brown adipose tissue (BAT) exist in adult humans. In contrast to white adipose tissue, the major fat storage organ, BAT burns fat to generate heat. Therefore, understanding the factors impacting BAT metabolism is of increasing interest.

Aims:
We aim to elucidate the function of N-acetyltransferase 8-like (Nat8l) in brown adipocytes. Nat8l has been described to be involved in lipid formation in the brain. However, until now implications in adipose tissue biology have not been studied.

Methods:
To elucidate the role of Nat8l in brown adipocytes, we stably overexpressed Nat8l in an immortalized brown adipose cell line (iBACs) and characterized these cells regarding triglyceride (TG) content, marker gene expression, mitochondrial mass and oxygen consumption rate. Furthermore, localization has been determined by western blot analysis of iBACs-fractions.

Results:
Endogenous Nat8l mRNA and protein levels increase during differentiation of iBACs. In differentiated iBACs it localizes in mitochondria. Stable overexpression of Nat8l in differentiating iBACs leads to impaired TG accumulation. Interestingly, we found that mRNA levels of brown adipogenic marker genes such as PPARα, PGC1α and PRDM16 are significantly increased in iBACs stably overexpressing Nat8l. Most strikingly, UCP1 mRNA and protein levels are up to 17-fold elevated. Furthermore, we show an increased mitochondrial mass. Consequently, basal and norepinephrine stimulated oxygen consumption is elevated.

Conclusion:
In this study we show that Nat8l overexpression boosts the brown adipogenic program and subsequently, energy expenditure in iBACs. The exact mechanism(s) by which Nat8l promotes this increased “brown” phenotype are matter of further investigation.

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ATP-binding cassette transporter A1 and apolipoprotein M are ‘novel players’ in cholesterol transport at the blood-brain barrier

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Background:
Impaired cholesterol/lipoprotein metabolism has been linked to AD. The BBB restricts exchange with plasma lipoprotein cholesterol and maintains cerebral cholesterol homeostasis via a mechanism of compensatory elimination. Unlike cholesterol, the oxysterols 24(S)-hydroxycholesterol (24OH-C) and 27-hydroxycholesterol (27OH-C) readily cross the BBB bidirectionally and promote expression of several targets including ATP binding cassette (ABC) transporter A1 and apo A-I.

Aims:
In light of our recent findings that modulation of cellular cholesterol metabolism alters APP synthesis and processing in porcine brain capillary endothelial cells (pBCEC), using the established in vitro model, we here aimed at identifying ‘novel’ transporters/proteins involved in cholesterol and oxysterol transport at the BBB.

Methods and Results:
RTQ-PCR analyses and immuno-blotting revealed that in addition to ABCA1 and SR-BI, porcine BCEC also express ABCG1 and ABCG4. ABCG1 but not ABCG4 expression was up-regulated (up to 10-fold) by LXR activation. To establish a function of ABCG1, RNA interference and sterol efflux assays were performed. ABCG1 silencing (by ~50%) reduced HDL mediated [³H]-cholesterol efflux (by ~50%) but did not influence [³H]-24(S)-hydroxycholesterol efflux from pBCEC. We show for the first time that, in addition to apoA-I, pBCEC express and secrete apoM, the levels of which were down-regulated upon treatment with natural LXR agonists. HDL enriched with apoM promoted cholesterol efflux from pBCEC more efficiently as compared to control HDL.

Conclusion:
Our present results imply that modulation of ABCG1 and apoM levels may influence cholesterol turnover at the BBB and adds two more targets for oxysterols that regulate several pathways of cholesterol metabolism at the interphase between the brain and the circulation.

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Signalling Cascades in cardiomyocytes in response to HOCl-modified LDL

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Background:
The microbicidal compound hypochlorous acid (HOCl), generated by the myeloperoxidase-H₂O₂-chloride system of activated phagocytes, reacts with the protein and lipid moiety of low-density lipoprotein (LDL), adversely affecting biological properties of this lipoprotein particle.

Aims:
HOCl-modified proteins/lipids are assumed to contribute to myocardial dysfunction. We propose that, compared to LDL, HOCl-LDL differently modulates gene expression and signalling pathways in cardiomyocytes. Therefore we aim to identify alterations in electrophysiological functions and molecular signalling in cardiomyocytes in response to HOCl-LDL, an in vivo occurring oxidative LDL modification.

Methods:
The murine cardiomyocyte cell line (HL-1) and primary guinea pig cardiomyocytes were used. After treatment with HOCl-LDL, patch clamp and calcium transients were performed to evaluate electrophysiological properties and calcium imaging. Western-blots and qPCR were performed to follow underlying molecular signalling pathways.

Results:
We show that HOCl-LDL decreased L-type calcium and IK1 currents in guinea pig cardiomyocytes while an increased outward non-selective cationic (NSC) current was observed. Moreover, decreased cell shortening and calcium transient were found. Western-blots analysis showed decreased Cav1.2, NCX1, RyR2 and increased SERCA2a, phosphorylation of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) expression. Effects of HOCl-LDL were reversed by KN93, inhibitor of phosho-CaMKII.

Conclusion: Alterations in L-type calcium, IK1 and NSC currents as well as calcium transients and cell shortening have shown detrimental role of HOCl-LDL on cardiomyocytes. Moreover, CaMKII mediated changes in Cav1.2, SERCA2a, RyR2 and NCX1 expressions confirm modulation in Ca²⁺ homeostasis leading to myocardial dysfunction. Altogether, these data show physiologically harmful effects of HOCl-LDL on cardiomyocytes.

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p62/Sequestosome-1: A crucial player of Mallory-Denk body formation

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DK Metabolic and Cardiovascular Disease, number of semesters: 5

Background:
Enlarged hepatocytes in steatohepatitis contain cytoplasmic protein aggregates or “Mallory-Denk bodies” (MDBs). MDBs are primarily composed of misfolded keratins (K8 and K18), ubiquitin, chaperons and the stress adaptor protein p62/Sequestosome-1 (p62), but its pathogenesis is poorly understood. p62 is an important regulator of protein homeostasis and extends its role from cell survival to tumorigenesis.

Aims:
1. How does p62 modulates Mallory-Denk Body formation
2. Whether p62 has any influence over liver toxicity upon DDC induced MDB formation

Methods:
We have generated p62 total (p62-/-) and hepatocyte deficient (p62-hep-/-) mice to understand the role of p62 in MDB formation. These mice were intoxicated with DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) for 8 weeks to induce MDB formation. The formation of MDBs was assessed by immunofluorescence and immunohistochemistry with specific antibodies raised against p62 and MDBs (Mm120-1).

Results:
Our results demonstrate the absence of p62 positive aggregates in p62-/- and in p62-hep-/- mice. But, interestingly, keratin misfolded structures are observed in p62-/- mice and in p62-hep-/- mice featuring the cross beta-sheet confirmation of K8 (essential for the formation of MDBs). This observation provides us with the first evidence that p62 acts as glue in the MDB formation and that without its presence the dense classical structure of MDBs cannot be preserved. Liver function tests show a decrease in AST, ALT and AP serum parameters in both mouse lines as compared to wild types, suggesting a mending effect to liver toxicity.

Conclusion:
These results prove p62 to be an essential regulating component in the formation of MDBs. Furthermore, it demonstrates that MDB formation is a hepatocyte autonomous process.
Steatohepatitis- driven Hepatocellular Carcinoma: a Keratinopathy

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Background:
Steatohepatitis (SH) is morphologically characterized by steatosis, hepatocyte ballooning and cytoplasmic protein aggregates termed Mallory–Denk bodies (MDBs). MDBs are generally composed of misfolded keratin (K) 8, 18 and in part p62 and ubiquitin. It is well established that K aggregates are critical for MDB formation, thus Ks are associated with SH which is one of the main preconditions for the development of liver cirrhosis and hepatocellular carcinoma (HCC).

Aims:
In this study we aimed to clarify the cellular and molecular mechanisms of relative K8 excess over K18 on hepatocarcinogenesis in mice and its functional and clinical implication in human alcoholic and non-alcoholic SH (ASH/NASH)-induced liver cancer.

Methods:
18 month-old krt18-deficient (krt18-/-129P2/OlaHsd background) mice were investigated for the incidence of SH and SH-induced liver tumors by radiology, macroscopy, histology, immunohistochemistry, gene expression, lipidome analysis, and immunoblotting.
Results:
Aged krt18-/- mice developed the entire morphological spectrum of SH whereas aged wild-type (wt) mice showed simple steatosis. Aminotransaminase levels were also elevated in aged krt18-/- mice. Of note, 64% of male and 25% of female 18 month-old krt18-/- mice developed liver tumors revealing morphological and genetic features of HCC whereas 45% of male and 16% of female krt18+/-/ mice and 42% of wt mice developed HCC.

Conclusion:
Aged krt18-/- mice represent a novel spontaneous SH and SH associated HCC model with significant gender differences revealing features related to human HCC. Thus, variations of hepatocellular K18 seem to have an effect on the susceptibility regarding SH and SH-induced HCC.

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Analysis of Circulating Cell Free Tumor DNA in Plasma in Colon Cancer Patients.

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Background:
Circulating tumour cells (CTCs) and cell free DNA are released from tumours and may reflect tumour-specific changes. Therefore, the detailed analysis of mutant plasma DNA fragments and/or CTCs may represent a non-invasive patient-specific diagnostic, prognostic or predictive test.

Aims:
This study aims to characterize plasma DNA by establishing genome-wide copy number status and detailed mutation spectra in relation to the respective primary tumours and metastases from patients with stage IV colorectal carcinoma (CRC).

Methods:
Plasma DNA extracted from the blood of colorectal cancer patients and the corresponding primary tumours were subjected to array comparative genomic hybridisation (aCGH). Sequential analyses of plasma DNA was performed to address whether the evolution of the tumour genome is reflected in the circulation of patients with cancer. BEAMing was performed to test the presence of KRAS mutations in the plasma DNA to study the mode and rate of release of tumour DNA.

Results:
We detected tumour-specific copy number aberrations by aCGH in plasma DNA in 6 of 13 patients whereas in 7 patients we could not detect tumour specific CNVs indicating a low percentage of tumour DNA in the plasma. To verify this, we quantified the amount of KRAS mutated fragments in two patients with KRAS mutation in the primary. Consistent with aCGH the fraction of mutated fragment in one patient was close or below the detection limit of BEAMing, whereas the other patient showed up to 12% mutated fragments.

Conclusion:
The analysis of plasma DNA and CTCs may represent a valuable non-invasive tool for monitoring the tumor genome of patients with cancer.

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Regulation of G0S2 in acute and chronic hepatic inflammation

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Background and Aim:
Non alcoholic fatty liver disease (NAFLD) is becoming increasingly prevalent in industrialized countries and can progress to non alcoholic steatohepatitis (NASH), cirrhosis and cancer. Lipotoxicity and inflammation are important features in the progression to NASH. We hypothesized that G0/G1 Switch Gene 2 (G0S2), a negative regulator of adipose triglyceride lipase (ATGL), one of the key lipases regulating lipid partitioning, may play a critical role in NASH. We therefore aimed to address the impact of hepatic inflammation on G0S2 regulation.

Methods:
Wild type (WT) mice were injected lipopolysaccharide (LPS) 4μg/g body weight or fed a methionine and choline deficient (MCD) diet to induce NASH and study the impact of acute phase response and chronic hepatic inflammation on G0S2. Expression analysis was performed by qRT-PCR.

Results:
Hepatic G0S2 expression was repressed in WT mice 6 hours post LPS injection (0.11 ± 0.06 vs. 1.00 ± 0.19, p<0.05). Despite tendential recovery of G0S2 mRNA levels 12 hours post LPS injection, G0S2 analysis revealed significant repression.

In a model of long-term inflammation and NASH, 4 weeks MCD feeding resulted in increased tendency of G0S2 expression (1.63 ± 0.41 vs. 1.00 ± 0.40). Significant induction of hepatic G0S2 was reached in mice kept on MCD diet for 5 weeks. In line with elevated G0S2 mRNA results Western Blot analysis depicted increased protein amounts.

Conclusion:
Our findings unravel the differential regulation of G0S2 in acute and chronic inflammation. Future studies will address molecular mechanisms regulating G0S2 expression in different stages of inflammation.

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The N-acetylaspartate pathway: New implications in brown adipose tissue and a connection to PPARα signalling

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Background:
N-acetyltransferase 8-like (Nat8l) is the enzyme catalyzing the formation of N-acetylaspartate (NAA), the second most abundant metabolite in human brain. NAA “stores” acetyl-CoA and is supposed to be exported from neurons to oligodendrocytes where it is reutilized to synthesize lipids for axon myelination.

Aims:
As we found Nat8l highly abundant in brown adipose tissue we aim to elucidate the function of the NAA-pathway in brown adipose tissue biology and homeostasis.

Methods:
Immortalized brown adipocytes (iBACs) (controls and Nat8l overexpressing cells) were treated permanently with PPARα-antagonist GW6471 (10μM) from day 4 of differentiation, harvested on day 7. Mice were fed a 12-week high fat diet with water containing 10mM NAA or normal water. Plasma parameters were measured using the Accutrend®GCT-System (Roche).

Results:
Overexpression of Nat8l in iBACs led to delayed TG (triglyceride) accumulation and increased FFA (free fatty acid) release whereas glycerol release was not changed. Furthermore, the ultimate brown fat marker gene, UCP1, was significantly increased. This increase was depleted with PPARα-antagonist treatment. NAA treated iBACs also showed reduced TG accumulation. Additionally, when mice fed a high fat diet were consuming NAA-containing water, they showed significantly reduced weight gain compared to controls. Interestingly, FFAs were increased whereas glucose was decreased in the blood of these animals.

Conclusion:
With the obtained results we introduce a new biochemical pathway in brown adipocyte biology. Although the exact mechanism(s) by which Nat8l and/or NAA cause the observed phenotypes in iBACs/mice needs further investigation, the NAA-pathway seems to influence PPARα action and lipolysis, thereby influencing UCP1 expression and brown adipocyte homeostasis.

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Link between triacylglycerol synthesizing enzymes and cholesterol metabolism.

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Background:
Mice deficient in acyl-CoA:diacylglycerol acyltransferase1 (DGAT1), a key enzyme in triacylglycerol (TG) biosynthesis, are lean, resistant to high-fat diet (HFD) induced obesity and fatty liver disease. DGAT1-deficient (Dgat1−/−) mice exhibit increased energy expenditure, preserve insulin and leptin sensitivity and have normal quantitative absorption of dietary fat. However, they have abnormally high levels of TG stored in the enterocytes, and reduced postprandial triglyceridemic response. Currently, little is known about the consequences of DGAT1 deficiency on cholesterol homeostasis or how this TG synthesizing enzyme can regulate gut lipid metabolism. Recent report from our group showed that DGAT1 deficiency in apolipoprotein E knockout (ApoE−/−) mice markedly decreased intestinal cholesterol absorption and increased cholesterol efflux from macrophages compared with ApoE−/− mice. Concurrently, preliminary results from Dgat1−/− mice indicate similar observations when compared to wild-type (WT).

Aims:
We hypothesize that decreased lipid absorption either due to delayed or defective gastric emptying strongly contributes to the observed resistance of HFD-induced insulin resistance in Dgat1−/− mice.

Methods:
To clarify this we will compare fractional cholesterol absorption, fecal lipid extraction, and gastric emptying dependent and independent of gut transit between DGAT1−/− and WT mice. Further, to evaluate if DGAT1 deficiency in intestine is responsible for alterations in cholesterol homeostasis we have generated and are currently characterizing intestine specific DGAT1−/− (DGAT1i−/−) by crossing Dgat1floxed mice with transgenic mice expressing the Cre-recombinase in epithelial cells of the small intestine under control of the Villin promoter.

Results and Conclusion:
First results of our study will be presented at the meeting.

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qProteomics on PLIN1 Mutant Lipid Droplets using SILAC labelling

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Objectives:
Flies (Drosophila melanogaster) lacking all perilipins exhibit impaired yet functional body fat regulation, suggesting existence of ancient lipid homeostasis system. Genetic interaction of plin1 (perilipin1) with akhr (orthologue of mammalian beta-adrenergic receptor) and bmm (brummer, orthologue of mammalian, adipose triglyceride lipase) has been shown. PLIN1 seemed to modulate BMM activity. Additionally plin1 knock out (KO) flies are more sensitive to BMM dependent fat mobilization and the BMM localization is changed on plin1 mutant lipid droplets (LDs). Further, plin1 KO out causes reduced number and larger size of LDs. In this study we addressed quantitative and qualitative changes in LDs proteomes of plin1 KO flies as compared to control flies by using SILAC (Stable isotope labeling with amino acids in cell culture).

Methods
Lysine auxotrophic yeast was grown on either Lys-0 or Lys-8. From early embryonic stage plin1 KO and control flies were grown on Lys-0 and Lys-8 labeled yeasts. Fat bodies were dissected and mixed in equal numbers. Total protein from isolated LDs was enzymatically digested followed by capLC-SCX separation prior to RP-nLC-MS-MS on Thermo LTQ-FT. Data analysis was performed using proteome discoverer software.

Conclusion:
Total 71 LDs associated proteins were quantified, of which 11 were down- and 3 were up-regulated in the plin1 KO. Main finding from data supports the other proofs for partial redundancies among perilipins. Second, cyclophilin1, a microtubule associated protein was enriched in the plin1 KO, which might contribute to the giant LDs phenotype, since structural transformation might correlate with changes in LD-microtubule interactions.

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Characterization of platelet function in adipose triglyceride lipase-deficient mice

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Background:
Dyslipidemia is associated with an altered thrombotic phenotype indicating that circulating plasma lipoproteins have an effect on platelet function. Hyperlipidemic conditions lead to the promotion of a prothrombotic state, while the use of lipid-lowering drugs (e.g. statins or nicotinic acid) results in reduced platelet reactivity. To date, the molecular mechanism and pathway linking platelet function and dyslipidemia are poorly understood.

Aims:
Mice deficient in adipose triglyceride lipase (ATGL), the crucial enzyme for triacylglycerol (TG) hydrolysis, have an altered plasma lipoprotein profile due to their inability to mobilize TG from adipose and non-adipose tissues. Using this lipase-deficient mouse model we aim at studying the phenotype with respect to platelet function and thrombosis.

Methods and Results:
Blood from wild type (Wt) and Atgl-/- mice was drawn via retro-orbital plexus puncture and platelets were purified by negative selection using MACS column technology. RT-PCR analysis and immunoblotting of purified Wt platelets revealed the expression of ATGL and monoacylglycerol lipase (MGL) but not hormone sensitive lipase (HSL). This finding is in agreement with data obtained from platelet proteomic analysis. Platelet counts in whole blood were assessed by an automated cell counter and were comparable between Wt and Atgl-/- animals. To study platelet reactivity whole blood was perfused over collagen-coated chips and revealed that Atgl-/- mice had reduced thrombus formation.

Conclusions:
Atgl-/- mice showed a reduced thrombotic phenotype as assessed by collagen-induced thrombus formation. Whether this altered thrombotic phenotype is due to systemic or platelet-specific lack of ATGL is a major aim of future investigations.

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DK-MCD
The role of metabolic lipases in the pathogenesis of malignant tumours

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Background:
Our study currently focuses on modification of lipid hydrolysis by ATGL, a metabolic lipase results in alteration of aggressive properties of cancer and immunogenicity. Data from our research group indicated that loss of ATGL functional activity in vivo in B-cell tumors and Lewis lung carcinoma resulted in increased the rate of tumor growth compared to the control tumor by 5-10 folds. Recent studies instigate the importance of interaction/links between tumour and lipid metabolism with a considerable evidence through cytokine mediated signalling pathways of signal transducer and activator of transcription (STAT) proteins, a very well known transcription factor playing diversified roles in tumour metabolism.

Aims:
To study the i) expression and activation STAT proteins in the presence and absence of ATGL and to elucidate ii) Role of STAT proteins in the immune component of the tumour environment (Wild type and ATGL Knock-out/Knocked down) and understanding the iii) Role of ATGL in tumour initiation and progression.

Methods:
The project involved techniques like cell culture, tumour injection in 12 weeks old C57BL/6 mice, serum and tumour sample collection, real time quantitative PCR, western blotting, Cytokine profiling.

Results:
There was no significant change in the gene expression of STAT3 between B cell tumour phenotypes. The metastasis rate of ATGL knock out B16F10 melanoma was high compared to their normal counterpart. There cytokine levels of the sICAM-1, IL-16, M-CSF, CCL2, was high in the serum of mice injected with ATGL knockout B cell tumours than the wild type tumours.

Conclusion:
Cytokines such as TIMP-1, M-CSF, sICAM-1 up regulated in B cell tumours (ATGL-/-) increased migration and IL-16, sICAM-1 increased adhesion. There is a expected change in phosphorylation of certain STAT family proteins between B cell wild type and ATGL-/- tumours playing roles in tumour growth, T cell differentiation and lymph angiogenesis etc.,

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**Vms1 as a Novel Protector in Aging and Disease**

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**Background:**
Vms1 as a newly characterized protein acts as a cofactor of the ubiquitin-proteasome system (UPS) and might depict a yet unidentified link between the UPS and mitochondrial quality control.

**Aims:**
The aim of this work is to characterize Vms1 in terms of cellular quality control mechanisms by using the eukaryotic model organism *Saccharomyces cerevisiae*.

**Methods:**
Survival is determined by plating assays by the number of yeast cells (out of 500) that form a colony on agar plates at different time points during aging. DHE conversion to fluorescent ethidium, Mitotracker Red CMXRos and Mitotracker Green fluorescence are measured by flow cytometry. Western Blot analysis is used to determine the amount of total ubiquitylated cellular protein.

**Results:**
Vms1 overexpression in yeast results in reduced oxygen stress and increased survival. Consistently we observe improved mitochondrial quality, i.e. a rise in membrane potential and mitochondrial biomass. HttQ97, which is a hallmark protein of Huntington’s disease, is known to impair mitochondrial function. Vms1 overexpression can rescue the death phenotype of HttQ97 in yeast probably in a ubiquitin-dependent manner.

**Conclusion:**
Mitochondrial function declines with age and age-related diseases. In this work we can show that Vms1 reduces cellular stress and increases mitochondrial quality during aging and in HttQ97 expression strains. We further presume that Vms1 represents a poorly characterized factor of the ubiquitin-proteasome-pathway. Vms1 might act as yet unknown link between the UPS and mitochondria.

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Spatio-temporal correlations between Ca$^{2+}$ and ATP in the lumen of the endoplasmic reticulum

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**Background:**
The ER performs myriad of pivotal cellular functions associated with survival, proliferation, and, protein synthesis, folding, modification, transport and degradation. Due to the energy demand of these processes a continuous supply of ATP into the ER is vital. So far no data about the real time ATP dynamics in the ER exists.

**Aims:**
- a.) To develop a genetically encoded ATP sensor targeted to the lumen of the ER.
- b.) To understand the dynamics of ATP in the lumen of ER.
- c.) To study the correlation between ATP and Ca$^{2+}$ signalling.

**Methods:** An ER-targeted genetically encoded FRET-based ATP biosensor was generated using standard cloning procedures and characterized using array confocal laser scanning microscopy. Cellular Ca$^{2+}$ and ATP were measured using epifluorescence microscopy.

**Results:** ATP in the ER ([ATP]$_{ER}$) is lower than in the cytosol and mitochondria. Cell stimulation yields ER Ca$^{2+}$ depletion that subsequently leads to a transfer of ATP into the ER yielding significant increases of [ATP]$_{ER}$. Cytosolic Ca$^{2+}$ signaling regulates the ATP transfer into the ER.

**Conclusion:** During cell stimulation, the decrease in ER Ca$^{2+}$ triggers enhanced ATP transfer into the ER, thus, resulting in increased [ATP]$_{ER}$. The transfer of ATP is tightly coupled to ATP generating processes while cytosolic Ca$^{2+}$ is crucial. These findings, for the first time, enlighten the dynamics of cellular ATP control and reveals ER ATP as being tightly regulated by the cellular Ca$^{2+}$ signalling. Alterations in spatial ATP transfer might induce cellular stress leading to cellular dysfunction.

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DK-MCD
Discovery of Novel Polyesterases using Activity Based Proteomics

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Background:
Polyethylene terephthalate (PET) is a polymer currently used to produce items such as plastic bottles, fleece jumpers, thermal blankets and medical devices such as sewing cuffs, surgical mesh and vascular prostheses. PET is a relatively inert substance and to achieve certain property changes, e.g. coloring or addition of a fine metallic layer on the surface, the polymer has to be modified. This modification is currently done by chemical degradation which often removes a large amount of mass from the polymer to get the desired effect. However, this modification can also be achieved using enzymes that can cleave internal ester bonds on the polymer’s surface (polyesterases).

Aims:
Few polyesterases are currently known and our goal is to discover more of them using a screening technique based on activity based proteomics.

Methods:
We have designed and are synthesising an activity based probe based on the chemical structure PET which will allow us to selectively target and isolate enzymes which are active on internal ester bonds in PET. The probe will include a p-nitrophenyl phosphonate which mimics the tetrahedral transition state of a carboxyl ester attacked by and active site serine, but will instead of being cleaved covalently bind to the enzyme. As we also add a bio-orthogonal linker group to the probe, in the form of an azide, we will be able to pull our polyesterases out of a complex mixture to identify them by LC-MS/MS or label them with fluorescent probes allowing for identification on gels.

Results:
Currently we are optimizing the synthesis of our activity based probe.

Conclusion:
In the end we expect to detect the polyesterases produced by microorganisms already known to have PET-modifying/degrading capabilities.

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Influence of an open ductus arteriosus on cerebral tissue oxygenation during the first day of life in preterm and term neonates

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Background:
Ductus arteriosus (DA) plays an important role in hemodynamics and oxygenation.

Aim:
To analyse the influence of open DA on cerebral tissue oxygenation (crSO2) during the first day of life.

Methods:
In this prospective observational study near-infrared-spectroscopy (NIRS) measurements were performed on the right forehead during the first 24 hours of life in preterm/term infants. Cardiac ultrasounds were done after beginning and cessation of NIRS measurements. Based on the second ultrasound infants were divided into “open-DA” and “closed-DA” group. In case of open DA the diameter, DA-ratio (time left-to-right/total shunt time) and Vmax/ Vmean(m/s) (in both shunt directions) were assessed. crSO2 was divided into 4 periods, 6 hours each, and means of both groups were compared to each other.

Results:
44 neonates (35±3weeks, 2392±833g) were included: 22 in each group. Cardiac ultrasounds were performed at 4±2 and 25±5 hours after birth. All infants had an open DA at the first ultrasound without differences concerning ductal diameter and DA-ratio. All infants had a bidirectional shunt, with a predominant “left-to-right” shunt. In “open-DA” group Vmax of “right-to-left” shunt was significantly higher than in “closed-DA” group. crSO2 of “closed-DA” group was significantly higher in all periods (1-6: 78 versus 70; 7-12: 78 versus 70; 13-18: 78 versus 69; 19-24: 78 versus 72) compared to “open-DA” group.

Conclusion:
Infants with a closed DA at the end of the first day of life have a significantly higher crSO2 during the first 24 hours of life than infants with an open DA.

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Organspecific pO\textsubscript{2} and pCO\textsubscript{2} metabolisms of a novel, closed, extracorporeal mini-perfusion circuit in a pediatric animal model

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Background:
Open cardiopulmonary bypass (CPB) has important adverse effects, especially in a paediatric setting.

Aim:
We hypothesized that a closed CPB system in an animal model would have better organ specific parenchymal pO\textsubscript{2}/pCO\textsubscript{2} changes and metabolic results detected from the parieto-temporal lobe of cerebrum, the left ventricular myocardium and the right hepatic lobe.

Methods:
The animals were randomized to the P-MEC\textsuperscript{®} group (Paediatric Mini Extracorporeal Circuit, yet not available on free market, Medtronic™, Minneapolis, USA, 7 pigs, 10.6±2.9 kg) and to the CPB group (conventional cardiopulmonary bypass, 7 pigs, 10.5±2.7 kg); the perfusion systems were minimized (priming volume of 202.8±37.3 ml) and adjusted to basic variables comparable to paediatric extracorporeal perfusion settings. Newly developed fibre-optical sensors, combined with a phosphorescent dye were used for pO\textsubscript{2} and pCO\textsubscript{2} measurement. Haemodynamic and respiratory variables as well as samples of arterial, central-venous and intracranial bulbo-venous metabolic variables were recorded.

Results:
There were no differences in preoperative metabolic and haemodynamic variables. Significantly higher need for both blood transfusion (395.7±47.2 ml vs. 28.5±14.8 ml) and higher lactate levels were detected in the CPB group (p<0.001). ANOVA revealed significantly higher cerebral pO\textsubscript{2} levels (p=0.007) in the P-MEC group, while pCO\textsubscript{2} levels appeared similar. In contrast, both hepatic and myocardial pCO\textsubscript{2} levels were higher in the CPB group (p=0.004), while pO\textsubscript{2} levels were similar.

Conclusion:
Under standardized conditions in an animal model, P-MEC\textsuperscript{®} produced less lactate and better results concerning O2/CO2 metabolism and transfusion requirements without compromising safety.
Mitochondrial Ca$^{2+}$ uptake 1 (MICU1) and Mitochondrial Ca$^{2+}$ Uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic β-cells

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PhD Molecular Medicine

Background:
Mitochondrial Ca$^{2+}$ uptake in pancreatic β-cells modulates the organelle’s production of ATP and that of essential coupling factors facilitating sustained glucose-stimulated insulin secretion (GSIS). Therefore, any interference with mitochondrial Ca$^{2+}$ signals may disrupt the metabolism-secretion coupling and compromise GSIS. MICU1, MCU, UCP2/3 and LETM1 are proteins in the inner mitochondrial membrane that are proposed to contribute to mitochondrial Ca$^{2+}$ uptake.

Aims:
This study was designed to verify the individual contribution of MICU1, MCU, LETM1 and UCP2 to mitochondrial Ca$^{2+}$ uptake and metabolism-secretion coupling in pancreatic β-cells.

Methods:
Expression analysis of MICU1, MCU, LETM1 and UCP2 was performed in a rat insulinoma cell line (INS-1 832/13). Mitochondrial and cytosolic Ca$^{2+}$ was measured using FRET-based genetic sensors after siRNA-mediated knock-down of target proteins. Luciferase assay was used to estimate cytosolic ATP while GSIS was measured with ELISA. Mitochondrial morphology was analysed using array confocal laser scanning microscopy.

Results:
This study revealed a crucial contribution of MICU1 and MCU to mitochondrial Ca$^{2+}$ uptake in response to intracellular Ca$^{2+}$ release and D-glucose-induced Ca$^{2+}$ influx which is a major physiological stimulant for insulin secretion. In contrast, UCP2 and LETM1 distinctively contributed to mitochondrial Ca$^{2+}$ sequestration of either intracellularly released or entering Ca$^{2+}$, respectively. GSIS was significantly reduced in MICU1 and MCU silenced cells without any changes in total cellular insulin content, thus, affirming their fundamental role in metabolism-secretion coupling in pancreatic β-cells.

Conclusion:
This data emphasize a crucial role of MICU1 and MCU in mitochondrial Ca$^{2+}$ uptake and, thus, metabolism-secretion coupling in pancreatic β-cells.

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Phospholipid scramblase 1 is not involved in human trophoblast fusion

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PhD Molecular Medicine

Background:
The outermost layer of the human placenta facing maternal blood is called syncytiotrophoblast, a multinucleated epithelial layer. It grows by fusion with underlying mononucleated cytotrophoblasts. During the complex fusion process the cell membrane loses its regular phospholipid asymmetry by phosphatidylserine exposure to the cell surface. Phospholipid scramblase 1 (PLSCR1) is one of the transporters involved in phosphatidylserine transport. Dysregulation of the fusion process might lead to severe pregnancy complications such as preeclampsia and fetal growth restriction.

Aims:
In this study we investigated the involvement of PLSCR1 in trophoblast fusion.

Methods:
Placental tissues of different gestational ages were immunostained for PLSCR1. RNA interference and a scramblase inhibitor (R5421, ethaninidiothioic acid) were used for functional studies in BeWo cells, a trophoblast fusion model cell line, as well as first trimester placenta explant cultures. Silencing of PLSCR1 and fusion efficiency were evaluated by immunostaining, Western blot and β-hCG-measurement in the supernatant.

Results:
Syncytiotrophoblast, macrophages and endothelial cells showed abundant expression of PLSCR1, while cytotrophoblasts showed only weak expression.

Using RNA interference in BeWo cells resulted in effective knockdown of the PLSCR1 protein (up to 70%); however, no changes in the fusion pattern were observed. First trimester explants and BeWo cells treated with scramblase inhibitor did not show any visible effect on PLSCR1 protein function. Further it did not provoke a change in β-hCG release.

Conclusion:
PLSCR1 is localized in the placental villous trophoblast compartment. However, there is no evidence for its functional role in the fusion process evaluated by two independent approaches.

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Contribution of Fra-2 transcription factor to pulmonary hypertension

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Background:
Vascular remodeling is a hallmark of pulmonary hypertension (PH), and is characterized by proliferation and migration of pulmonary smooth muscle cells (PASMC). The underlying molecular mechanisms that lead to vascular remodeling are still not fully understood.

Aims:
Our aim is to characterize the involvement of Fra-2 in the development and progression of PH in vivo and in vitro.

Methods and Results:
A time course analysis of mice over-expressing Fra-2 (Fra-2 tg) was undertaken. At all investigated time-points (8, 12 and 16 weeks) Fra-2 tg mice exhibited pronounced lung inflammation mainly due to increase in T cell subsets. This inflammatory profile was accompanied by vascular remodeling, increased right ventricular systolic pressure (RVSP) and Fulton index. Echocardiography revealed decrease in the pulmonary artery acceleration time (PAAT) in 16 weeks old mice. On molecular level, increased phosphorylation of AKT and p38 pathway was observed in Fra-2 tg lung homogenates in comparison to controls. Expression of downstream AP-1 target genes such as collagens and TGF-β, known to be involved in remodeling processes, were also up-regulated. Accordingly, lung sections from IPAH patients revealed pronounced expression of Fra-2 in remodeled vessels. In vitro, platelet-derived growth factor (PDGF-BB) up-regulated Fra-2 expression in PASMC and silencing of Fra-2 together with each of the jun component, led to reduced proliferation of hPASMC.

Conclusion:
Our study identified a novel role of Fra-2 in vascular remodeling. These data suggest that the early activation of the AP-1 transcription factors could be responsible for initiating the remodeling process, through activation of genes such as collagens or TGF-β.

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Regulation of angiogenesis in the human placenta by trophoblast cells and tissue macrophages

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PhD Molecular Medicine

Background:
An adequate placental vasculature is essential for the appropriate development and growth of the fetus during gestation. Thus, the vessel formation from pre-existing vessels (angiogenesis) plays a crucial role for placental and thus fetal development.

Several studies indicate that trophoblast cells and placental tissue macrophages (Hofbauer cells) are involved in the process of placental angiogenesis.

Aims:
The main goal was to examine whether trophoblast cells (TB) or Hofbauer cells (HBC) have an effect on angiogenesis of feto-placental endothelial cells. Therefore, we investigated paracrine effects as well as cell-to-cell interactions.

Methods:
Placental arterial endothelial cells (AEC), TB and HBC were cultured at 21% or 8% oxygen for 24h or 48h. The respective conditioned media were used for in vitro angiogenesis assays, at 21% or 8% oxygen.

For coculture experiments AEC were mixed with TB or HBC and seeded on Matrigel to observe their influence on network formation.

Results:
The 48h conditioned media of trophoblast cells at 21% oxygen reduced network formation by 30% and the 48h TB conditioned media cultured at 8% oxygen resulted in a decrease of AEC tube formation of 70%. The HBC conditioned media cultured for 48h at 8% oxygen revealed a reduction of 13% on network formation.

Coculture of AEC with trophoblast cells as well as with Hofbauer cells showed an increased network formation of 30%.

Conclusion:
Cell-to-cell interactions and monocultured condition media show opposite effects regarding network formation of endothelial cells.

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The myeloperoxidase product hypochlorous acid generates irreversible high-density lipoprotein receptor antagonists

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PhD Molecular Medicine

Background:
End stage renal disease (ESRD) is associated with persistent low-grade inflammation and elevated levels of plasma advanced oxidation protein products (AOPPs). AOPPs arise from reaction of plasma proteins with the myeloperoxidase product hypochlorous acid (HOCl) released by activated neutrophils. Recent findings revealed that AOPPs are antagonists of the major high-density lipoprotein (HDL) receptor, scavenger receptor class B type I (SR-BI) and effectively block reverse cholesterol transport.

Aims:
Specific modifications that render AOPP-albumin an SR-BI ligand are not identified. We investigated oxidation-induced structural alterations that convert plasma albumin into an HDL-receptor ligand.

Methods:
Albumin from ESRD-patients and controls was isolated from serum by affinity chromatography. Mass spectrometry was used to study structurally defined lysine modifications on albumin. Flow cytometry served to investigate the role of lysine residues for binding of HOCl-albumin to SR-BI and to determine binding kinetics.

Results:
We observed that exposure of albumin to hypochlorous acid generates irreversible HDL-receptor antagonists. Of particular interest, N-chloramine residues within oxidized albumin promoted irreversible binding to SR-BI, resulting in permanent receptor blockade. Importantly, the levels of the myeloperoxidase specific oxidation product 3-chlorotyrosine of albumin, isolated from ESRD patients, correlated with its SR-BI inhibitory activity.

Conclusion:
Given that several potential atheroprotective activities of HDL are mediated by SR-BI, the present results raise the possibility that oxidized albumin, through irreversible SR-BI blockade, contributes to the pathophysiology of cardiovascular disease in ESRD-patients.

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Uremia alters HDL composition and anti-inflammatory properties

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PhD Molecular Medicine

Background:
Cardiovascular disease, stroke, and peripheral vascular disease are notorious problems in patients with chronic kidney disease. Accelerated atherosclerosis is thought to be caused by increased inflammation, oxidative stress, and impaired triglyceride and HDL metabolisms. Functional impairment of HDL may contribute to the excess cardiovascular mortality experienced by patients with renal disease.

Aims:
Recent studies have shown that HDL exerts anti-inflammatory effects on leukocytes. The aim of this study was to investigate whether anti-inflammatory properties of HDL on leukocytes are impaired in ESRD patients in comparison to control subjects.

Methods:
HDL was isolated from end stage renal disease patients (ESRD) and age matched healthy controls. Biochemical analyses were used to study alterations in the proteome and lipid composition of HDL. Human polymorphonuclear leukocytes (PMNLs) were isolated from peripheral blood from healthy volunteers. The ability of HDL from ESRD patients and control subjects to suppress LPS-induced neutrophil activation was assessed by flow cytometric measurement of the cell shape change. Migration assays with THP-1 monocytes were performed using migration plates.

Results:
The results revealed that in comparison to controls, HDL from ESRD patients was less efficient in suppressing LPS induced neutrophil activation. Distinct compositional alterations in uremic HDL impaired it’s ability to suppress LPS induced neutrophil activation. However, neither control HDL nor uremic HDL were able to inhibit the migration of monocytes.

Conclusion:
Our observations suggest that the compositional alterations observed in uremic HDL reflect a shift to a pro-inflammatory profile that impairs anti-inflammatory activity of HDL.

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In utero programming of endothelial function

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PhD Molecular Medicine

Background:
There is growing evidence that environmental factors in pregnancy act in utero to program offspring’s future health. Despite its short duration, gestational diabetes mellitus (GDM) has long term consequences for offspring and confers increased risk for endothelial dysfunction. Placental endothelium is continuous with fetal endothelium and hence a model to study fetal endothelial function.

Aims:
We hypothesized that primary human arterial endothelial cells isolated from term placentas after healthy pregnancies (normal AEC) and GDM affected pregnancies (diabetic AEC) would differ in their intrinsic biological program. We focused on two key endothelial processes i.e., proliferation and angiogenesis.

Methods:
To determine proliferation, viable and dead cells were counted. In vitro angiogenesis (2-D network formation) was studied in media containing 2% normal or diabetic cord blood serum (CBS). The global DNA methylation profile was determined by 450k methylation arrays.

Results:
Diabetic AEC had reduced proliferation (ANOVA p<0.003) with 36±10% fewer viable and 33±8% fewer dead cells. In normal CBS total tube length was increased by 45±10%, number of branching points by 311±28% and number of meshes by 163±50% in diabetic vs. normal AEC (ANOVA p<0.001). Diabetic CBS did not influence network formation. Epigenome analysis revealed differences in global methylation pattern between normal and diabetic AEC.

Conclusion:
Altered proliferation and network formation of diabetic AEC, even when cultured under identical conditions as normal AEC, argues for changes independent of the acute environment. These changes appear to reflect an intrinsic program to which epigenetic modifications contribute. Thus GDM-associated programming of endothelial cells in the offspring may begin in utero.

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25-hydroxycholesterol: a novel mediator of glioblastoma growth?

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Abstract:
Glioblastoma multiforme (GBM; astrocytoma grade IV) is the most common malignant brain tumor with a very poor prognosis even under the current maximal therapy. Therefore, new therapeutical approaches are urgently needed. Since oxysterols were recently demonstrated to enhance proliferation of medulloblastoma cells, exert suppressive effects on the adaptive immune system and tumor promoting effects on tumor associated macrophages (TAMs), we aimed to explore 25-hydroxycholesterol (25-OHC) as a potential mediator of glioma progression. During ongoing studies we found that human glioblastoma cell lines (A172 and U87) express cholesterol-25-hydroxylase (Ch25H). Ch25H mRNA and protein levels were upregulated in response to the proinflammatory cytokine TNFα and IL1β stimulation. This was reflected on product level, i.e. increased intra- and extracellular concentrations of 25-OHC indicating responsiveness to stimuli from the immune system. Moreover, our data demonstrate that 25-OHC is able to induce migration of THP-1 monoblasts, which is mediated at least in part by GPR183, a G-protein coupled receptor. In summary our findings suggest that 25-OHC could be a potent signaling molecule in glioblastoma and may play an important role as chemoattractant for TAMs.
Pulmonary hypertension is associated with decreased number of circulating CD34+CD133+ cells

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PhD Molecular Medicine

Background:
Endothelial progenitor cells (EPCs) may cause pulmonary arterial remodelling and can be detected in the circulation of PH patients. They might serve as biomarker of the disease. EPCs can be defined as CD34+CD133+ cells.

Aims:
We aimed to characterize the circulating EPCs in PH patients as well as in healthy controls using a nine-colour staining assay for the Fluorescent Activated Cell Sorting.

Methods:
Peripheral venous blood was taken from 22 patients with PH and 19 healthy controls. Mononuclear blood cells were freshly isolated by means of density gradient centrifugation. The obtained fresh vs. fixed cells were stained with fluorescent conjugated antibodies against the following cell surface markers: CD117, CXCR2, CD309, CD34, CD14, CD31, CD133, CD16, CD45.

Results:
CD34+ cells were significantly decreased in the PH as compared to control samples (p<0.001). A CD34+CD133+ circulating EPC population was detected in every subject. Fixation neither affected the cell count nor the fluorescence intensities of conjugates as checked using single stained samples. The percentage of EPCs was significantly lower in PH patients as compared to controls (p<0.01). CD34+CD133+ EPCs were positive for CD45 suggestive for their bone myeloid origin.

Conclusion:
These preliminary results suggest that CD34+CD133+ EPCs are significantly reduced in PH patients. In order to confirm these results and to define the clinical relevance of EPC number, the investigations must be extended to a larger patient population.

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Cerebral Open Flow Microperfusion (cOFM): a histological study.

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Background:
Neural probe implantation in the central nervous system elicits an encapsulation reaction that involves microglia, astrocytes, meningeal cells and oligodendrocyte precursors. The ensheathing reaction is a major factor to decrease device performance. The gold standard for neurochemical measurement is microdialysis, is also limited due to probe encapsulation in scar tissue.

Aim:
Recently our group has established cerebral open flow microperfusion (cOFM), another relatively new probe based technique to monitor transport over intact blood-brain barrier following 15 days of probe implantation. It is developed combining push-pull perfusion and open flow microperfusion (OFM). OFM is capable of sampling a variety of substances including lipophilic and higher molecular weight substances. The aim of our present study was to assess morphological changes of normal brain parenchyma after cOFM probe implantation by quantitative histology.

Methods:
cOFM probe were implanted in the left prefrontal cortex of Sprague dawley rats. After 15 days, the rats were euthanized and brains were extracted and fixed in paraformaldehyde. Serial sectioning of the implantation site was done and stained with H&E and for activated microglia and reactive astrocytes.

Results:
Microscopic examination revealed slight edema and significant increase in Iba-1+ microglial cells within 140 µm of cOFM probe implantation site. The number of GFAP+ astrocytes was counted near the implantation site and no significant difference was found in comparison to contralateral hemisphere.

Conclusion:
In the present study, GFAP+ astrocytes were detected near cOFM probe implantation but a continuous sheath of GFAP+ cells was not found to surround the cOFM probe insertion site.

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Quanitative Analysis of regulatory T cells in tissue using Immunohistochemistry

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PhD Molecular Medicine

Background:
Naturally occurring CD4(+) CD25(high) FOXP3(+) regulatory T cells (nTregs) are key mediators of immunity, which orchestrate and maintain immunological tolerance. Depletion of nTregs has been shown to result in multiorgan autoimmune disorder and abundant evidence exists that either their count or their activity plays a critical role in diseases such as Type 1 diabetes mellitus, multiple sclerosis, inflammatory bowel disease and many more. Thus, their imaging and quantification in tissue is of vast medical and scientific interest.

Aims:
The current method for quantitative determination of nTregs is analysis by flow cytometry. This study aims to establish immunohistochemistry of nTregs for two reasons: (1) to provide an alternative method of quantification additional to FACS analysis and (2) to assess the localization of nTregs in situ.

Methods:
Formalin-fixed paraffin-embedded intestine biopsies were cut and deparaffinized, followed by antigen retrieval through HIER. In the beginning, single-stainings with specific primary antibodies for the following three markers of nTregs were carried out using fluorescence-labelled secondary antibodies: CD4 (cell surface), CD25 (cell surface) and FoxP3 (nucleus). Subsequently, the three markers were combined into a triple-staining.

Results:
Single- as well as triple-stainings were successfully established concerning the depiction of nTregs in tissue biopsies with a confocal Laser Scanning Microscope.

Conclusion:
With the highly sensitive Laser Scanning Microscope a detection of triple-stained regulatory T cells in paraffin-embedded tissue is possible. Yet, the future task will be the optimization of the staining for automated quantitative analysis with TissueFaxes.

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Investigation of vascular reactivity in mouse tertiary pulmonary arteries

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PhD Molecular Medicine

Background:
Pulmonary hypertension is characterised by vascular remodelling, vasoconstriction and hypertrophy. Increased vascular resistance contributes to disease progression. Currently vasodilators are the predominant therapeutic strategy.

Aim:
To investigate vascular dynamics in tertiary pulmonary arteries of mice by applying an ex vivo model

Method:
Changes in the pulmonary arterial tone were assessed using a Multi Wire Myograph system 620M in response to different vasoconstrictors.

Results:
Three different types of vasoconstrictors were used; phenylephrine, serotonin and the thromboxane analogue U46619. A dose dependent vasoconstriction was observed with all three vasoconstrictors. Phenylephrine and U46619 produced a prolonged vasoconstriction (~60 minutes) while arteries treated with serotonin self-relaxed within 30 minutes. The potency of contraction was 2 fold greater for U46619 in comparison to phenylephrine and serotonin.

Iloprost, a clinically used prostacyclin analogue, was used as vasodilator for vascular reactivity studies. A dose dependent relaxation was observed for iloprost when arteries were pre-constricted using U46619, but not phenylephrine.

Conclusion:
Wire myograph is an appropriate and reproducible method to study vascular tone in mice pulmonary arteries. Signalling differences in the vasodilatory response to iloprost will be the subject of future studies.

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The metabolic effects associated with CD8-depletion in a murine model of type 2 diabetes fail to influence diabetic nephropathy

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PhD Molecular Medicine

Background:
In an earlier study, we evaluated the effect of regulatory T cells (Tregs) on a mouse model of type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN). The depletion of Tregs was associated with deterioration in insulin resistance and worsened DN, while Treg substitution significantly alleviated insulin resistance and improved DN. Metabolic effect of treatment with Tregs were associated with a significant decrease in the number of CD8+CD69+ activated T cells in the visceral adipose tissue (VAT) of obese mice, as well as with a decrease of CD4+ and CD8+ T cells in the kidney.

Aims:
The following study was designed to evaluate the effects of depletion of CD8+ T cells on insulin resistance and renal end-points.

Methods:
Five-week old male db/db mice were uninephrectomized and treated with a CD8-depleting antibody. Metabolic parameters and urinary albumin were measured weekly, insulin sensitivity testing was performed and mice were sacrificed after 21 and 56 days.

Results:
Treatment with CD8-depleting antibody resulted in a decreased number of CD8+ T cells and CD8+CD69+ activated T cells in the spleen. After 8 weeks, CD8-depleted mice performed significantly worse in insulin sensitivity testing as compared to control mice, whereas there was a trend for a better insulin sensitivity after 3 weeks. Assessment of renal end points failed to document an effect of CD8-depletion on DN.

Conclusion:
While CD8-depletion appears to ameliorate the early stages of insulin resistance and continuing depletion deteriorates insulin sensitivity in later stages, this does not influence the progression of DN in this setting.

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T-cells modulate ectopic calcification and soft tissue remodelling in mice

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PhD Molecular Medicine

Background:
Ectopic visceral and vascular calcifications are a pathophysiological hallmark of several diseases including chronic kidney disease, nephrocalcinosis, and calciphylaxis. Immune cells modulate soft tissue remodelling, yet the contribution of specific immune cells subsets to different aspects of calcification are unclear.

Aims:
To further evaluate the specific pathophysiological role of regulatory T cells in ectopic calcification, we use dba2 mice that have a natural splice variant in the ABCC6 gene and are thus prone to develop ectopic soft tissue calcifications and vascular calcification.

Methods:
Dba2 mice were depleted of T cells or regulatory T cells using either an anti-CD3 or an anti-CD25 monoclonal antibody, respectively. Successful depletion was confirmed by flow cytometry. After this immunomodulation, the female dba2 mice are set on a phosphorus-rich diet for 10 days and sacrificed for radiological and histopathological analyses.

Results:
Feeding a high phosphate diet to dba2 mice induced a clear phenotype of ectopic calcification entailing cardiovascular and renal calcification. Both, T-cell depletion and regulatory T cell depletion significantly increased renal calcification as shown by higher calcium score in micro-computed tomography as well as by histological sections.

Conclusion:
In summary, our data suggest a pivotal role of regulatory T cells in cardiovascular remodelling by limiting the pro-inflammatory milieu and thus preventing the ectopic renal calcification.

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Transcriptional regulation of intestinal fatty acid translocase/CD36 expression by GATA4

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PhD Molecular Medicine

Background:
CD36 is a multifunctional membrane receptor found on various cell types, including luminal surface of enterocytes where it facilitates fatty acid uptake. GATA4 is a zinc finger transcription factor involved in early endoderm development. Both CD36 and GATA4 are mainly expressed in the duodenum and jejunum of adult mice. Recently, it was found that GATA4 intestine specific knock out mice (iKO) show a markedly decreased expression of the CD36 gene.

Aims:
Prompted by markedly decreased CD36 gene expression in GATA4iKO mice we aimed at assessing the role of GATA4 in the transcriptional regulation of the mouse CD36 gene.

Methods
Deletion mutants of the proximal mouse CD36 promoter (-1790bp - +13bp) were cloned into a luciferase reporter plasmid. Luciferase activity was monitored in transfected HepG2 cells in the presence or absence of adenoviral GATA4 overexpression or in the presence or absence of plasmid-based overexpression of a GATA4 co-repressor, FOG2.

Results:
Basal luciferase activity was similar for all tested deletion mutants. Importantly, GATA4 overexpression led to a marked increase in luciferase activity of all deletion mutants. The induction of promoter activity by GATA4 overexpression was most pronounced for the mutant -1079 (26-fold), followed by -837 (23-fold), -1536 (13-fold), -609 (9-fold) and -1790 (7-fold). While GATA4-induced activity of the promoter -1079 was markedly attenuated by FOG2 co-expression, the activity of the promoter -837 remained unchanged.

Conclusion:
Results show that GATA4 is a potent activator of CD36 gene promoter activity, whereby the promoter region -1079 to -837 harbours the most responsive GATA4 elements.

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Lung cancer cells utilize gluconeogenesis under glucose depletion

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PhD Molecular Medicine

Background:
Gluconeogenesis is the generation of glucose from smaller carbon substrates. The key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK), was shown to provide metabolites for cell growth, however the role of gluconeogenesis in cancer cells is unknown.

Aim:
To analyze the role of gluconeogenesis in lung cancer cells.

Methods:
Expression of both PEPCK isoforms was assessed in three NSCLC cell lines under different glucose concentrations. ¹³C₃ enrichment in gluconeogenesis metabolites in ¹³C₃-lactate treated cells was assessed using liquid chromatography/mass spectrometry (LC-MS/MS) in collaboration with the Core Facility Mass Spectrometry (CMR). Spheroids were generated from a suitable cell line (NCI-H23) and served as a model for nutrient depletion. Apoptosis was assessed using a caspase-3-activity assay.

Results:
In A549, NCI-H23, and NCI-H1299 cells the mitochondrial isoform of PEPCK (PCK2) was expressed on the mRNA and protein level. In A549 and H1299 cells PCK2 expression peaked at 1mmol/L glucose. Levels of PCK2 mRNA were significantly higher in lung cancer samples compared to corresponding normal lung. In order to analyze, whether lung cancer cells may metabolize lactate in the direction of gluconeogenesis, cells were incubated with medium containing ¹³C₃-lactate. Under low glucose conditions, 3-P-glyceraldehyde, a central gluconeogenesis/glycolysis metabolite, exhibited a label-enrichment of 25-60%. The PEPCK inhibitor 3-mercaptopicolinate significantly enhanced apoptosis of A549 cells under glucose depletion (0.2 mmol/L D-glucose) but not under high glucose (20mmol/L). Further, 3-mercaptopicolinate significantly reduced the growth of multicellular spheroids.

Conclusion:
Lung cancer cells utilize at least some steps of gluconeogenesis to overcome the detrimental metabolic situation during glucose deprivation.

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The role of ABCG2 transporter in pulmonary circulation

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PhD Molecular Medicine

Background:
Pulmonary hypertension (PH) is a devastating vascular disease leading to right heart failure and premature death. Homeostatic abnormalities of cyclic nucleotides, prostaglandins and phosphodiesterases are reflected in an increase in pulmonary vascular tone and pulmonary arterial smooth muscle cell (PASMC) proliferation which are hallmarks of PH. Members of the ABC (ATP-binding cassette) transporters can actively efflux these molecules. However their role in the progression of the pulmonary vascular remodelling is unclear.

Aims:
We aimed to investigate the expression of the ABC transporters in the human pulmonary arteries and in idiopathic pulmonary arterial hypertension and to clarify the role of ABC transporters for the development of PH.

Methods:
Expression profiling of ABC transporters in human pulmonary arteries and in idiopathic pulmonary arterial hypertension (IPAH).
Characterisation the role of ABCG2 inhibitors in PASMC- and PAEC proliferation.
Investigation of ABCG2⁻/⁻ mice in chronic hypoxia which is a trigger of PH.

Results:
Amongst other ABC transporters ABCG2 was upregulated in samples of IPAH patients compared to control samples. Mitogen treatment altered the expression level of this transporter and pharmacological inhibitors of ABCG2 attenuated the proliferation of PASMCs and PAECs. Nevertheless mice lacking ABCG2 were not protected against hypoxia induced PH.

Conclusion:
Despite literature data and our in vitro results suggest an adverse contribution of ABCG2 to PH, the absence of this transporter could not avoid the development of PH in hypoxia-mouse model. Therefore we cannot conclude whether the observed upregulation of ABCG2 in PH is a causative or a consequence of the disease.

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Loxl2 May Represent a Novel Therapeutic Target in Primary Sclerosing Cholangitis

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PhD Molecular Medicine

Background:
Lysyl oxidase-like 2 (LOXL2) catalyzes the cross-linking of collagens and elastin and participates in angiogenesis, matrix remodelling, and epithelial-mesenchymal transition. However, little is known on the role of LOXL2 in human liver fibrosis, especially in cholangiopathies and biliary type of liver fibrosis.

Aim:
To determine LOXL2 expression in different mouse models of cholangiopathies and human cholestatic liver disease.

Methods:
Loxl-2 mRNA and protein expression was studied in Mdr2 knockout mice, common bile duct ligated (CBDL) male C57/Bl6 mice, Swiss Albino (SA) mice fed an 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-supplemented diet, and SA mice fed 1% lithocholic acid (LCA)-supplemented diet. In addition, we determined LOXL2 expression in human explant livers, including primary sclerosing cholangitis (PSC) and disease controls using immunohistochemistry and immunofluorescence labelling.

Results: LOXL2 mRNA was significantly overexpressed in 4 and 8 weeks-old Mdr2⁻/⁻ mice (8-fold increase in 4 and 10-fold increase in 8 weeks-old), mice subjected to CBDL (up to 7-fold increase), DDC-fed (up to 11-fold increase after 4 weeks), and LCA-fed mice (13-fold increase) compared to controls. LOXL2 protein expression was predominantly found in the portal region. In PSC, LOXL2 expression was found nearby proliferating and injured bile ducts and in association with fibrotic septa. In disease controls, similar staining results were detected.

Conclusions: LOXL2 is overexpressed in mouse models of cholangiopathies and human chronic cholestatic liver disease. As allosteric inhibition of LOXL2 proved successful in experimental liver and lung fibrosis, therapeutic implications in regard to cholangiopathies should be considered.

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Age dependent defect in suppressive capacity of regulatory T cells in patients with type 1 diabetes

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PhD Molecular Medicine

Background:
Regulatory T cells (Treg) play a pivotal role in the control of immune responses and reduced number and/or defects in function of these cells are involved in the development of type 1 diabetes (T1D). Restoration of Treg function may potentially prevent and/or delay disease progression.

Aims:
In the present study, we aimed to assess number, suppressive capacity and susceptibility to apoptosis of peripheral Treg in children and adults with T1D compared to healthy controls.

Methods:
Peripheral Treg from 27 T1D patients (age: 13±3 years) with a diabetes duration of 1.1±1.3 years, 24 T1D patients (age: 33±11 years) with a diabetes duration of 1.2±1.1 years and healthy, age-matched individuals were quantified by FACS analysis. Apoptosis and suppressive potential of Treg were measured in vitro.

Results:
The percentage of Treg was similar between patients and healthy controls but apoptosis was significantly increased in children with T1D compared to healthy controls [1.04%±0.76% vs 0.45%±0.46%, p=0.029] and suppressive capacity was significantly decreased [44.4%±19.7% vs 59.4%±16.6%, p=0.033]. Apoptosis of Treg in adult T1D-patients was increased compared to controls [2.0%(0.8-3.6%) vs 1.0%(0.5-2.1%), p=0.007] whereas the suppressive function of Treg was similar in adult T1D and healthy subjects [mean±SD: 38.2%±21.1% vs 39.8%±15.6%, p=0.721].

Conclusion:
Our results show functional differences with increased apoptosis in children and adults with T1D. Suppressive capacity of Treg seems to decrease with age and in children with T1D the decrease of suppressive activity of Treg appears accelerated, suggesting an age dependent defect of Treg function in the pathogenesis of T1D.

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Impact of endothelial lipase on vascular reactivity

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PhD Molecular Medicine

Objectives:
Endothelial Lipase (EL) is a phospholipase produced mainly by vascular endothelial cells. EL acts primarily on phospholipids of circulating high density lipoprotein (HDL) yielding various bioactive lipolytic products and decreased HDL plasma levels. Both lipolytic products and decreased HDL may impair endothelium dependent vascular relaxation.

Aims:
The aim of the present study was to examine the effect of EL overexpression on acetylcholine (Ach) induced relaxation of mouse aorta.

Methods:
C57BL/6 mice were injected i.v. with a total of 2.4*10⁹ particles EL or LacZ adenovirus. After 48h aortae were collected immediately or 30 minutes after i.v. injection of human HDL. To address the role of nitric oxide, mice were treated with L-NNA. Nitrite concentrations in organ bath, indicative of NO levels were determined by HPLC. In order to study the metabolism of HDL in vivo mice were injected with EL or LacZ adenovirus followed by HDL re-isolation from serum by ultracentrifugation.

Results:
Mice overexpressing EL had markedly decreased both HDL-cholesterol plasma levels and Ach-induced relaxation compared with LacZ control mice. Injecting human HDL restored HDL plasma levels and Ach-induced relaxation. Importantly, the improvement of relaxation by injected HDL was'nt observed in mice treated with eNOS inhibitor L-NNA. HDL isolated from EL overexpressing mice was found to promote poor tissue relaxation as compared to control HDL.

Conclusion:
Our results indicate that EL mediated depletion of HDL and consequently decreased NO production, but not EL-generated lipolytic products, is a major cause of impaired vascular relaxation in aortae of EL overexpressing mice.

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Acyl chain-dependent effect of lysophosphatidylcholine on endothelium-dependent vasorelaxation

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PhD Molecular Medicine

Objective:
Endothelial Lipase (EL) is a phospholipase produced mainly by vascular endothelial cells. Hydrolysis of HDL-phosphatidylcholine by EL generates lysophosphatidylcholine (LPC) and free fatty acids. palmitoyl-lysophosphatidylcholine (16:0 LPC), oleoyl-LPC (18:1 LPC), linoleoyl-LPC (18:2 LPC) and arachidonoyl-LPC (20:4 LPC)

Aims:
In the present study we aimed to determine the effect of LPC 16:0, 18:1, 18:2 and 20:4 on acetylcholine (Ach) induced relaxation of mouse aorta using wire myography.

Results:
All tested LPC impaired Ach-induced relaxation, with rank order of potency as follows: 18:2>20:4>16:0>18:1. Both indomethacin, a nonselective cyclooxygenase (COX)-inhibitor and SQ29548, a thromboxane A₂ (TXA₂) receptor antagonist, improved relaxation impairment evoked by LPC 20:4 and 18:2, but not by LPC 16:0 and 18:1. The effect of LPC 20:4 could also be improved by TXA₂- and prostacyclin (PGI₂)-synthase inhibitors. As determined by EIA assays, the tested LPC promoted secretion of PGI₂, TXA₂, PGF₂α, and PGE₂, however, with markedly different potencies. LPC 16:0 was the most potent inducer of superoxide anion production by mouse aortic rings, followed by LPC 18:2, 20:4 and 18:1, respectively. The strong antioxidant tempol recovered relaxation impairment caused by LPC 18:2, 18:1 and 20:4, but had no effect on LPC 16:0.

Conclusion:
Collectively, the tested LPC impair Ach-induced relaxation through induction of proconstricting prostanoids and superoxide anions. The potency of attenuating relaxation and the relative contribution of underlying mechanisms are strongly related to LPC acyl-chain length and degree of saturation.

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Effect of photodynamic therapy (PDT) on immune regulation

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PhD Molecular Medicine

Background:
Photodynamic therapy (PDT) is an antitumor approach carried out by administration of a photosensitizer and light exposure, leading to the damage of tumor tissue. Moreover it seems to induce innate and adaptive anti-tumor immune responses. Interestingly, previous work in experimental models has revealed that depleting regulatory T cells (Tregs) can potentiate the efficacy of PDT.

Aims:
We are investigating the immunological changes induced by PDT and its effect on level and function of Tregs (CD4+CD25highCD127lowFoxP3+ cells).

Methods:
Blood is collected from patients with ESCC or patients with actinic keratoses (AK, an in situ condition that can develop into squamous cell carcinoma) before PDT, 7 and 14 days after treatment. Tregs levels are quantified by FACS and Tregs function by co-culture proliferation assays with T effector cells (CD4+CD25lowCD127highFoxP3- cells).

Results:
Our results so far indicate that PDT may inhibit the suppressive capacity of systemic Tregs from ESCC but not from AK patients. Tregs levels, however, appear to be highly variable among patients. In parallel, we are investigating the effect of PDT in a mouse tumor model of squamous cell carcinoma (SCC VII in syngeneic C3H/HeN mice). Our preliminary data from the animal work revealed that higher numbers of Tregs are present in the spleen of tumor bearing mice compared to control mice. Currently we are optimizing the PDT treatment regimen and delineating the effect on level and function of Tregs in the mouse model.

Conclusion:
A better understanding of the immunological aspects linked to PDT will be of great importance for optimization of the approach.

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A mouse model for polymorphic light eruption

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PhD Molecular Medicine

Background:
Evidence indicates that polymorphic light eruption (PLE) patients are resistant to the immune suppressive effects of sunlight, including an impaired migration of Langerhans cells out of the skin and a reduced infiltration of neutrophils and macrophages into the skin upon UV exposure. The exact mechanisms by which UV leads to the foresaid immunological disturbances in PLE remain to be determined.

Aims:
To study the pathogenic mechanisms of PLE and to elucidate a potential involvement of mast cells and their mechanisms we were looking for a useful mouse model.

Methods:
KitW-Sh/W-Sh mice and their wild-type controls were subjected to a chronic UVB regime over 4 weeks, as well as a rechallenge irradiation. Thickness of shaved dorsal skin was measured by a spindle-loaded pocket thickness gauge. Inflammation and cellular infiltration was evaluated via H&E staining, RT-PCR, ELISA, FACS and immunohistochemical stainings.

Results:
KitW-Sh/W-Sh mice displayed a reduced tolerance to UVB radiation compared to wild-type controls, determined by hyperplasia, inflammation and cellular infiltration into the skin. Immediately after irradiation KitW-Sh/W-Sh mice exhibited an excessive scratching behaviour that was absent in control mice. The minimal scratching dose of KitW-Sh/W-Sh mice was below their inflammatory threshold dose, determined by UVB dose response studies. Although photohardening did not affect the abnormal UV tolerance and scratching behaviour, the phenotype was dependent on a deficiency of mast cells, since engraftment of wild type mast cells rescued KitW-Sh/W-Sh mice.

Conclusion:
This model is useful to study the pathogenic mechanisms of photodermatoses (such as PLE). Mast cells may be required for disease desensitization through photohardening.

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Responses of Osteocalcin to Oral Glucose Load in Insulin-resistant and Non-insulin-resistant Women

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PhD Molecular Medicine

Background:
Osteoblast-produced osteocalcin (OC) might play a role in energy metabolism and in the regulatory circuit between pancreas and osteoblasts.

Aims:
The aim was to evaluate the effect of a 75g oral glucose tolerance test (OGTT) on total OC, undercarboxylated osteocalcin (ucOC), and carboxylated osteocalcin (cOC) in insulin-resistant (IR) and non-insulin-resistant (nonIR) premenopausal women. Further, the relationship of changes in total OC, ucOC, and cOC with AUCinsulin and Matsuda index were examined.

Methods:
In this cross-sectional study, 105 premenopausal women underwent OGTT (21 IR (HOMA-IR > 2) and 84 nonIR). After glucose load, changes in total OC, ucOC, and cOC were evaluated after 30, 60, and 120 minutes.

Results:
At baseline, IR women had significantly lower levels of total OC, cOC, and ucOC. In nIR women, total OC decreased by 20.7% from 18.4ng/ml [14.8-24.9] at baseline to 14.6ng/ml [10.9-17.8] after 120 minutes; ucOC decreased by 21.9% from 3.2ng/ml [2.3-4.6] to 2.5ng/ml [1.7-3.5]; cOC decreased by 27.0% from 15.2ng/ml [12.3-20.6] to 11.1ng/ml [9.0-15.1] (p<0.001, respectively). In IR women, neither decreased significantly. In stepwise linear regression analyses, after adjusting for age and BMI, the declines in OC and cOC upon glucose load were predictors of AUCinsulin (ΔOC: beta=-0.301, p=0.001; ΔcOC: beta=0.315, p<0.001) and Matsuda index (ΔOC: beta=-0.235, p=0.003; ΔcOC: beta=-0.245, p=0.002).

Conclusion:
Glucose intake in nonIR women has a short-term lowering effect on the levels of OC, ucOC, and cOC. In IR women these parameters seem constantly suppressed, suggesting insulin resistance in osteoblasts.

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Fetal HDL-associated apoM-S1P complex mediates vasoprotective action on the feto-placental endothelium. In Gestational Diabetes Mellitus (GDM) endothelial barrier integrity is impaired

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PhD Molecular Medicine

Background:
Fetal high density lipoprotein (fHDL) has a unique composition when compared to maternal HDL and GDM causes quantitative and qualitative alterations of maternal and fetal HDL proteome (Sreckovic, 2012). In adult, HDL-associated sphingosine-1-phosphate (S1P) is bound mainly to apolipoprotein M (apoM) and maintains vascular integrity.

Aim:
The aim of this study was to identify whether fHDL also carries S1P and its regulatory effect on feto-placental endothelium in GDM.

Methods:
HDL was isolated by ultracentrifugation from control and GDM maternal/fetal donors (n=11). ApoM, S1P were quantified by ELISA kits. S1P receptor (S1PR1) expression was determined by qRT-PCR and immunoblotting. Transendothelial electrical resistance of human placental endothelial cells (HPEC) was measured by an impedance sensor (ECIS). Cells were treated with S1P, HDL, S1PR1, Rho and Rac inhibitors. Rearrangement of cytoskeleton by S1P on HPEC was visualized by phalloidin and vinculin staining.

Results:
ApoM-S1P levels are equal in healthy, maternal and fHDL. In GDM, circulating S1P bound to fHDL is decreased to 20% (p<0.01), due to the 40% lower association of apoM to fHDL (p<0.01). S1PR1 is highly expressed on HPEC. Healthy, fHDL increases barrier integrity in HPEC, while GDM fHDL attenuated permeability to 50-60% (p<0.01). fHDL and S1P protective mechanism in HPEC is induced by S1PR1/Rac pathway. S1P cytoskeletal rearrangement was confirmed by immunofluorescence staining.

Conclusion:
fHDL exhibits S1P and mediates protective actions on the feto-placental endothelium. S1P metabolism is affected by GDM and corroborated with endothelial barrier dysfunction resulting in marked increases in vascular permeability that is one of the central features of inflammation.

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Inhibition of monoacylglycerol lipase (MAGL) improves DSS-induced colitis but not colitis-associated colon cancer

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Background:
Endocannabinoids such as anandamide and 2-arachydonoylglycerol (2-AG) play an important role in reducing intestinal inflammation. They exert their actions via cannabinoid and GPR55 receptors, which are part of the endocannabinoid system.

Aims:
Here we tested whether inhibition of monoacylglycerol lipase (MAGL), the key enzyme for degradation of the (2-AG) could lead to an improvement of DSS-induced colitis and to a reduction of tumor growth in a model of colitis-associated cancer. In addition, we tested weather genetic knockdown of GPR55 could influence severity of colitis.

Methods:
In the colitis models, mice were given 2.5% dextran sulfate sodium (DSS) for a week. JZL184 (16 mg/kg twice daily s.c.) was used to pharmacologically knock down MAGL. Additionally, a group of MAGL and GPR55 knock out and wild types mice received 2.5% DSS over a period of a week. To study a potential inhibiting effect of JZL184 (16 mg/kg once daily s.c. 15x) on tumor growth in the colon, a model of colitis-associated cancer was employed. Tumors were evaluated after 12 weeks.

Results:
In JZL184-treated mice, inflammatory scores (diarrhea, colon shortening, colon weight, ulcerations), COX-2 and Il1β expression were reduced (n=8; ANOVA; p<0.01). GPR55 knockout mice showed macroscopic and microscopic improvement of colitis (n=8) In the colon cancer mice treated with the MAGL inhibitor, tumor area was macroscopically evaluated but revealed no improvement as compared to vehicle treated mice.

Conclusion:
Manipulation of the endocannabinoid system in acute intestinal inflammation has protective effects but may not be of protection in inflammation-driven colon cancer.

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Identity – Existentiality – Burnout:
An empirical study on Sense of Coherence and Stress-coping in nursing staff

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Background:
The ability to cope with a stress situation adequately is described as Sense of Coherence (SOC). In recent years the concept of SOC has received increased attention in social and medical science. Recent studies have also shown a relationship between SOC and perceived job stress and accordingly a link between coping strategies and job satisfaction in nursing staff.

Aims:
The objective of this study is to investigate the sense of coherence in relation to self-reported health and job strain among nursing staff in hospital.

Methods:
70 female and male nurses working in hospital have been measured using following questionnaires: The Sense of Coherence Questionnaire (SOC), Temperament and Character Inventory (TCI), Health Behaviour Questionnaire (HBQ), Three-Factor Eating Questionnaire (TFEQ), Freiburg’s Questionnaire for Disease Coping (FKV), Multidimensional Inventory for Religious/Spiritual Well-being 48 (MI-RSWB 48), Structure of Religiosity Test (S-R-T), Stress-Coping-Questionnaire (SVF 120) and a questionnaire measuring recovery and workload (EBF 55).

Results:
Statistical analyses showed a significant correlation between SOC and job related stress (r = -.589, p < .05) as between SOC and the ability to activate individual resources in stress situations (r = .586, p < .05). Furthermore there is a relationship between SOC and consumption of alcohol (r = -.394, p < .05), sleep disorders (r = -.369, p < .05) and self reported physical complaints (r = -.325, p < .05).

Conclusion:
SOC seems to be a health promoting resource in the observed group of individuals by coping with a stressful situation adequately and activating available resources.

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Genetic Loss of Muscarine-3 Receptor Decreases Biliary Bicarbonate Secretion and Aggravates Cholestatic Liver Injury in Mice

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DS General and Clinical Pathophysiology

**Background:**
The muscarine 3 receptor (M3-R) represents the primary cholangiocyte receptor for afferent vagal innervation (T Roskams et al., 2004). Experimental vagotomy negatively affects bile flow and liver regeneration (D Alvaro et al., 1997). We hypothesized that the M3-R plays a critical role in bile formation and cholangiopathies.

**Aims:**
To determine the role of the M3-R in bile formation and in mouse models for cholangiopathies.

**Methods:**
Bile flow and composition were compared between M3-R^-/-^ and wild type (WT) control mice. Liver injury and degree of cholestasis were compared between genotypes in response to 5 days common bile duct ligation (CBDL) and 17 days feeding diethoxycarbonyl-1, 4-dihydrocollidine (DDC).

**Results:**
M3-R^-/-^ showed significantly reduced bicarbonate-dependent bile flow compared to WT controls (bile flow 73±19 vs. 87±10 µl/30min and biliary bicarbonate secretion 38±2 vs. 41±2 nmol/l, M3R^-/-^ vs. WT, p<0.05). After CBDL M3-R^-/-^ showed significantly increased ALT (3500±564 vs. 564±243 U/l, M3R^-/-^ vs. WT, p<0.05) and serum bile acid levels (3624±664 vs. 1341±480 µmol/l, M3R^-/-^ vs. WT, p=0.06) compared to WT controls. In response to DDC-feeding M3-R^-/-^ demonstrated significantly increased ALT levels (2324±613 vs. 1823±257 U/l, M3R^-/-^ vs. WT, p<0.05), as well as bile acid levels (1138±358 vs. 370±208 µmol/l, M3R^-/-^ vs. WT, p<0.05) compared to WT controls.

**Conclusions:**
The M3-R is essential for biliary bicarbonate secretion. Genetic loss of the M3-R accelerates cholestatic liver injury after CBDL as well as DDC-feeding, suggesting that M3-R-dependent biliary bicarbonate secretion may represent an important protective mechanism for bile ducts.

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**norUDCA protects Common Bile Duct Ligated Mice from Collecting Duct Tubular Epithelial Lesions**

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**DS General and Clinical Pathophysiology**

**Background:**
The pathophysiology of renal failure in jaundiced patients is enigmatic. We recently demonstrated tubular injury with cast formation in kidneys of common bile duct ligated (CBDL) mice. Additionally, CBDL FXR⁻¹ mice with a more hydrophilic bile acid pool were protected from kidney injury, suggesting a causative role for bile acids. We therefore hypothesized, that hydrophilic *nor*ursodeoxycholic acid (*norUDCA*) may be a novel therapeutic approach for this problem.

**Aims:**
1. To determine whether *norUDCA* protects CBDL mice from collecting duct tubular epithelial lesions.
2. To determine the tubulotoxic effects of different bile acids *in vitro*.

**Methods:**
PAS-stained kidney sections of 7d *norUDCA* (0.5%) - fed and chow - fed CBDL mice were compared. MDCK cells (a well characterized collecting tubular duct epithelial cell line) were incubated with taurocholic acid (TCA), cholic acid (CA), chenodeoxycholic acid (CDCA), and *norUDCA* at increasing concentrations. Viability was assessed using WST-1 assay.

**Results:**
Prefeeding of *norUDCA* prevented tubular epithelial injury in CBDL mice. Only CDCA and GCDA induced cell death *in vitro* in a dose - and time dependent manner.

**Conclusion:**
*norUDCA* inhibits tubular epithelial lesions in CBDL mice. Potentially toxic bile acids induce tubular epithelial cell death in a dose dependent manner. Consequently toxic bile acids presumably represent the culprits for collecting duct tubular epithelial injury in cholestasis. Urinary excretion of hydrophilic bile acids such as *norUDCA* may be protective.

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Seasonal differences in nasal mucus proteome between allergic rhinitis patients and healthy controls

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Background:
Nasal mucus contains a variety of proteins which play a role in the defense against various allergens.

Aims:
The aim of this study was to analyse the nasal mucus proteome and discover its pathophysiologic impact on allergic rhinitis.

Methods:
Nasal mucus was collected with a special suction device in and out of pollen seasons from allergics and healthy controls. The samples were then sent for LC MS/MS mass spectrometry. Experimental protein spectra were compared to theoretical spectra via public protein databases. Gene enrichment analyses were performed by Cytoscape 2.8.1/BINGO 2.44 software tools.

Results:
In total 245 proteins could be identified where 201 different proteins were identified in allergics and 134 proteins were identified in healthy controls out of season. In season 190 different proteins were identified in allergics and 192 were identified in healthy controls. Biological processes in allergics in season compared to off season showed up-regulation in collagen metabolism and blood coagulation cascade as well as intracellular pH regulation, whereas in season Interleukin 1 metabolism was up-regulated. Nasal mucus proteome in healthy controls in season compared to off season showed significant up-regulation in proteins responsible for superoxide radical removal, interleukin 1 metabolism, natural killer cytotoxicity, innate immune response and defense response.

Conclusion:
Nasal mucus differs between allergics and healthy controls in and out of season. Allergics seem to show less response as fewer protein pathways are. Healthy controls, however, show more immune activation of humoral and innate immune system. These data reflect the important role of mucus as first line defense to allergens.

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Electrophysiological Characterization of K⁺ channels in different breast cancer cell lines

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Background:
GIRKs (G-protein Activated Inwardly Rectifying K⁺ channels) occur in about 30% of breast tumours, correlating with metastasation and progression of disease.

Aims:
Functional K⁺ channels are characterized in native breast cancer cell lines and in cell lines overexpressing GIRK proteins to identify whether GIRK channels have pivotal functional roles in cancerogenesis and metastasis.

Methods:
Patch Clamp; Single channel recording.

Results:
K⁺ channels were recorded from the native MCF7 cell line (MCF7WT), MCF7 cells overexpressing GIRK variants (MCF7hGIRK1c#4, MCF7hGIRK1d#7, MCF7hGIRK1d#9, MCF7hGIRK1d#17) and native MDA231 cells. The cells frequently exerted mechanosensitive channels, permeable to K⁺, Na⁺ and Ca²⁺ (MSC) and a constitutively active K⁺ selective “Big Channel” (BC). MCF7 transgenic cell lines exerted in addition a unique inwardly rectifying, constitutively active, K⁺ channel (nMSC) with equal conductance and ion selectivity to MSC (mostly found in MCF7hGIRK1d#9 cells). So far, there is only one observation in MCF7hGIRK1d#17 whose single channel conductance is in the range of GIRK channel.

Conclusion:
MSC was not blocked by 200 µM Gd³⁺, as the classical MSC protein, trp1. Antisense k.o. experiments are projected in order to identify its molecular nature. BC shows ion-selectivity and inward rectification properties similar to constitutively active Kᵦ’s. Since both MSC and BC appear both in MCF7 cells with and without G³/ᵦ overexpression, they are G-protein independent. Since nMSC activity is found predominantly in MCF7hG1d cell line, we conclude that overexpression of GIRK1 splice variants interferes with membrane anchoring of MSC. Analysis of other cell lines and GIRK1 k.o. cell lines will reveal whether functional GIRK channels exist in breast cancer cells.

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Thyroid dysfunctions have an impact on oxLDL levels in Sprague Dawley rats

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DS Translational Molecular and Cellular Biosciences

Background:
Thyroid dysfunctions might be implicated in the pathophysiology of metabolic disorders like obesity and atherogenic lipid profiles. Oxidized LDL (oxLDL) has been described to be strongly associated with the incidence of metabolic disorders.

Aims:
We analysed the impact of thyroid dysfunctions on oxLDL levels in hypothyroid (HYPO), hyperthyroid (HYPER) and control rats, either fed with normal rodent diet or high-fat diet (HFD).

Methods:
Sixty female Sprague-Dawley-rats were divided into controls, HYPO and HYPER groups. HYPO state was induced with propylthiouracil in drinking-water, HYPER state via intraperitoneal injections of triiodothyronine (T3) over twelve weeks. Further, groups were sub-divided into normal-diet and high-fat diet (HFD) fed animals. Analyses included T3 status, oxLDL, nitric oxides (NOX) and triglycerides (TG).

Results:
Serum T3 levels confirmed successful induction of thyroidism. Investigations of oxLDL revealed that both diet HYPO groups had significantly decreased oxLDL levels, whereas both HYPER groups showed a significant increase. Further, we found that normal diet HYPOs and HYPERs exhibited declined NOX levels. Regression analysis revealed a highly significant association of oxLDL and NOX ($P = 0.675$, $r = 0.032$) in the normal diet control group. Analyses of TG, a further biomarker of lipid metabolism, revealed that the HFD had no effect on TG levels. The HYPER rats showed increased TG levels regardless of diet, the HYPO state did not influence TG levels.

Conclusion: Our results indicate that thyroid dysfunctions are associated with metabolic risk factors like oxLDL.

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G-Protein activated inwardly rectifying potassium channel and cancer biology: Breast cancer as a model

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Background:
Since RNA encoding GIRK1 is heavily over-expressed in breast tumours, GIRKs (G-protein Activated Inwardly Rectifying K⁺ channels) potentially play an eminent role in cancerogenesis and metastasation.

Aims: The thesis project aims to characterize functional roles of GIRK1 proteins and splice variants thereof on vital parameters of different breast cancer cell lines that are important for tumour biology.

Methods: Stable, genome integrated over-expression and silencing of the gene encoding GIRK1 in connection with cell biological assays, such as proliferation, wound healing, invasion, and cell adhesion and cell motility.

Results: Cell lines, based on MCF7, MDA231 and MCF10A, stably over-expressing the human GIRK1a, c, d, e and variant and dimeric G are currently engineered. In addition several constructs, based on the pSIREN plasmid (with and w/o DsRed2), containing shRNAs encoding different regions of GIRK1 have been fabricated and tested for their silencing efficiency. Efficiency of these constructs, so far, ranged between 10 and 100% reduction, depending on shRNA and splice variant. Our preliminary experiment on vital parameters showed that MCF7hG1c has the highest invasive potential in comparison to the control group. MCF7hG1d had the highest rate of wound healing. MCF7hG1e had the highest rate of adhesion when compared to the control.

Conclusion: Cell lines, shRNAs and the influence of GIRK1 overexpression/silencing will be assessed.

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B vitamins and MRI-detected ischemic brain lesions in patients with recent transient ischemic attack or stroke: The VITATOPS MRI-Substudy

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PhD Neuroscience

Background:
Elevated concentrations of homocysteine are associated with cerebral small vessel disease (CSVD). B-vitamin supplementation with folate and vitamins B12 and B6 reduces homocysteine concentrations.

Aims:
In a sub-study of the VITAmins TO Prevent Stroke (VITATOPS) trial, we assessed the hypothesis that the addition of once-daily supplements of B-vitamins would reduce the progression of CSVD-related brain lesions.

Methods:
A total of 359 patients with recent stroke or TIA, who were randomly allocated to double-blind treatment with placebo or B-vitamins underwent brain MRI at randomisation and after 2 years of B-vitamin supplementation. MR images were analysed blinded to treatment allocation. Outcomes related to the pre-specified hypothesis were progression of white matter hyperintensities (WMH) and incident lacunes.

Results:
After two years of treatment with B vitamins or placebo, there was no significant difference in WMH volume change (0.08 vs 0.13 cm³; p=0.419) and incidence of lacunes (8.0% vs 5.9%, p=0.434). In a sub-analysis of patients with MRI evidence of severe CSVD at baseline, B-vitamin supplementation was associated with a significant reduction in WMH volume change (0.3 vs 1.7 cm³; p=0.039).

Conclusion:
Daily B-vitamin supplementation for two years did not significantly reduce the progression of brain lesions due to presumed cerebral small vessel disease in all patients with recent stroke or TIA, but may do so in the subgroup of patients with recent stroke or TIA and severe cerebral small vessel disease.

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The endocannabinoid N-arachidonoyl glycine (NAGly) inhibits store operated Ca\(^{2+}\) entry by abrogating STIM1/Orai1 interaction

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PhD Neuroscience

Background:
The endocannabinoids anandamide (AEA) and its derivate N-arachidonoyl glycine (NAGly) are endogenous signaling lipids exhibiting a broad spectrum of physiological effects, which are induced by both binding to receptors or receptor-independent modulations of ion channels and transporters. The impact of these endocannabinoids on STIM1/Orai1 dependent store-operated Ca\(^{2+}\) entry (SOCE), a ubiquitous Ca\(^{2+}\) entry pathway regulating multiple cellular functions, is unknown.

Aims:
As endocannabinoids have also been described to be modulators of the cellular ion homeostasis in different cell types, this study was designed to elucidate the potential contribution of endocannabinoids to the regulation of SOCE.

Methods:
Single-cell Ca\(^{2+}\)-imaging on various cell lines (endothelial, leukaemia) was performed with fluorescence microscopy using the fura-2 technique. In order to follow the key molecular events involved in SOCE activation, we studied STIM1 cluster formation and its subsequent coupling to Orai1 with confocal microscope, while CFP/YFP based FRET measurements allowed us to monitor STIM1 oligomerisation and STIM1-Orai1 interaction dynamics.

Results:
Unlike AEA, NAGly reversibly inhibited Ca\(^{2+}\) entry via SOCE in a time- and concentration dependent manner, and this effect could be recapitulated when STIM1 and Ora1, the molecular constituents of SOCE were overexpressed to enhance Ca\(^{2+}\)-entry. Confocal microscopy showed that NAGly disturbs neither STIM1 clustering nor its co-localisation with Ora1. Surprisingly, dynamic FRET measurements revealed that NAGly strongly diminished STIM1-Orai1 interaction, while it had no impact on STIM1 oligomerisation.

Conclusion:
Our findings unveiled the STIM1/Orai1-mediated SOCE machinery as a so far unknown molecular target of NAGly, which might have multiple implications in cell physiology.

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Association of cardio-respiratory fitness and cognition in elderly: Results of the Austrian Stroke Prevention Study

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PhD Neuroscience

Background:
Cognitive decline is an inevitable phenomenon accompanying ageing, which leads to disability and decreased quality of life. The pace of this degeneration is subject to many influences, including life-style decisions. A beneficial effect of cardio-respiratory fitness on cognition has been described, but large scale cohort studies with extensive setup are rare.

Aims:
This study investigates the association between maximal oxygen consumption (VO₂ max) as a measure of fitness level, and cognitive measurements of all domains in a large population based cohort.

Methods:
The cohort consisted of 726 participants (mean age 64±8 years, 58% females) of the Austrian Stroke Prevention study, who underwent cognitive measurements and graded exercise stress test on a bicycle ergo-meter. VO₂ max was calculated using weight and the maximum and resting heart rate. Statistical analysis was performed using SPSS v20.

Results:
VO₂ max was significantly associated with memory (p=0.036; β=0.068), conceptualization (p=0.030; β=0.078), visual memory (p=0.035; β=0.076), attention (p=0.047; β=0.069), and g-factor (p=0.004; β=0.086). The observations were independent of age, sex, educational level. Addition of cardiovascular risk factors (hypertension, diabetes, smoking, BMI, cholesterol) to the model increase the effect and significance of the observation.

Conclusion:
The results suggest a protective role of cardiovascular fitness on cognition and may ameliorate age-related decline and disability in old age.

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Psychosocial stress counteracts behavioral changes associated with murine colitis, but not colitis itself

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PhD Neuroscience

Background:
Inflammatory and functional visceral pain is associated with many psychosocial and psychiatric disturbances. In preclinical research, evaluating behavioral changes associated with pain may improve the face and predictive validity of animal models of chronic visceral pain.

Aims:
The study objective was to assess emotional and social behavior in male C57Bl/6 mice following induction of mild colitis with dextran sodium sulfate (DSS) and repeated exposure to water avoidance stress (WAS).

Methods:
Four groups of mice were studied: control mice, mice treated with DSS (2% added to drinking water), mice exposed to WAS for 1 hour daily, and mice treated with DSS+WAS. During these treatments, the disease activity score was evaluated daily. After 1 week of treatments, the mice were subjected to behavioral tests that included the open field (OF), social interaction test (SIT), and tail suspension test (TST). The data was analysed with two-way ANOVA.

Results:
DSS caused some weight loss, decreased colon length and enhanced colon weight; these changes remained unaffected by WAS. In the OF, DSS reduced the time spent in the central zone (p<0.01). Similarly, the time of social interaction with a novel mouse was shortened by DSS (p<0.01). In both OF and SIT the effect of DSS was counteracted by WAS (p<0.05). DSS and WAS had no significant effect on immobility time in the TST.

Conclusion:
DSS-induced colitis is associated with enhanced anxiety-like behavior and decreased social interaction. The behavioral changes associated with colitis, but not colitis itself, are counteracted by exposure to psychosocial stress.

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Hypersensitivity to thermal pain in peptide YY knockout mice

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PhD Neuroscience

Background:
Peptide YY (PYY) and neuropeptide Y (NPY) are enteroendocrine and neuronal messengers. While NPY participates in nociception and cognition, the implication of PYY in these functions is little known.

Aims:
Male wild-type, PYY knockout, and NPY+PYY double-knockout mice were studied for their sensitivity to noxious heat in the plantar test and for their learning and memory performance in the Barnes maze.

Methods:
In the plantar test, the plantar side of the hind paw was exposed to an infrared source at 2 intensities and the withdrawal response recorded. In the Barnes maze the animals received training to find a target hole and then studied for short-term and long-term memory performance.

Results:
In the plantar test at the higher infrared intensity, both PYY and NPY+PYY knockout mice showed a significant decrease in withdrawal latency (p < 0.01 and p < 0.05) whereas at the lower infrared intensity only PYY knockout mice exhibited a decrease in withdrawal latency (p < 0.01) relative to wild-type mice. In the Barnes maze, significant differences were only found in short term memory, as NPY+PYY knockout mice visited the target hole less often (p < 0.01) and showed a lower number of total visits (p < 0.01) than wild-type mice.

Conclusion:
Genetic deletion of PYY decreases the pain threshold to noxious heat, which is not enhanced by additional knockout of NPY. The decrease in target hole visits in the Barnes maze is probably due to reduced locomotion and does not reflect an impairment of short term memory.

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Effects of β-Synuclein overexpression on development of brain pathology

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PhD Neuroscience

Background:
Synucleins are a highly homologous protein family. The best known member, α-Synuclein, is abnormally accumulated in neurodegenerative-disease-related aggregates, so called Lewy Bodies, one hallmark of Parkinsons disease. Notably, parts of the peptide backbone can be found in amyloid plaques of Alzheimer’s disease patients. Interestingly, there is evidence that endogenously expressed β-Synuclein, another family member, prevents α-Synuclein aggregation and it seems to interfere with several regulatory and signalling pathways.

Aims:
Effects of β-Syn overexpression on brain pathology are evaluated in a transgenic animal model of AD.

Methods:
Lentiviral particles are directly injected into the hippocampus of transgenic mice overexpressing the human Amyloid Precursor Protein with Swedish and London mutations (hAPPSL) via stereotactic intracerebral injection. The viral particles contain one of three different constructs: an empty lentiviral vector as negative control, one with human β-Synuclein and another one with a GFP construct.

Results:
First, lentiviral particles were tested in different cell lines including SH-SY5Y and HEK293T cells. Transfection efficiency for all constructs was approved on protein and nucleic acid levels. Afterwards, particles were injected into the hippocampus of transgenic mice. Injection site, particle distribution and protein expression were studied after 1 and 3 months. Based on these results large scale studies are planned including behavioural studies as well as histological and biochemical examination of treated animals.

Conclusion:
Combining intracerebral injection and lentiviral protein expression in primary neurons is perfectly suited for analyzing neurodegenerative diseases in vivo. With this method potential therapeutic peptides can be directly distributed in brain regions of interest.

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Introducing a Vascular Risk Factor into an AD Mouse Model:

Effect of ApoB-100 overexpression on pathology and behavior of APP-transgenic mice

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PhD Neuroscience, number of semesters: 5

Introduction:
Increasing evidence suggests that vascular factors may contribute to the pathogenesis of Alzheimer’s disease (AD). It is still under debate to what extent vascular factors participate to the onset of the disease and its deterioration.

Aims:
Crossbreeding of an established hAPP transgenic mouse line with hApoB-100 overexpressing mice, a model of hyperlipidemia, introduced a vascular risk factor into the existing AD model. Possible synergistic effects were investigated over age.

Methods:
Learning paradigms (Morris-water-maze: MWM, contextual-fear-conditioning: CFC), biochemical (Aβ38, Aβ40, Aβ42 levels, lipid peroxidation, mRNA expression and plasma lipid levels) and histological readouts (GFAP, CD11b, 6E10, LOC, ApoB).

Results:
ApoBxAPP mice display progressive learning and memory impairments in MWM and CFC and an early onset of brain amyloid-pathology similar to hAPP single-transgenic animals. Notably, also single-transgenic hApoB mice developed cognitive impairment compared to non-transgenic littermates at higher ages. Overexpression of hApoB changed the plasma lipid profile, increased cerebral lipid peroxidation and led to accumulation and extravasation of hApoB in cerebral vessels of ApoBxAPP and hApoB mice. Astrogliosis in hippocampus and corpus callosum was only detected in old double-transgenic animals but not in littermates. Additionally, measurement of cerebral mRNA revealed changes in expression levels of many lipid metabolism associated genes in these animals.

Conclusion:
Although crossbreds did not develop more distinct behavioral deficits than single-transgenic hAPP littermates, several additional features indicate that this combination might better reflect the situation of elderly humans than hAPP overexpression alone. Additionally, this work gives insight into the interplay between cerebrovascular lesions, AD specific pathology and lipid metabolism.

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Assessment of the uptake of magnetite labeled nanoparticles in the rat brain using MRI

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PhD Neuroscience

Background:
The blood brain barrier (BBB) protects the brain from circulating harmful substances and it often hinders therapeutic intervention. That may be an explanation why some treatment concepts fail. Transportation of drugs via nanoparticles (NPs) appears as new and promising approach to cross BBB and to develop new therapeutic models.

Aims:
This experimental study with MRI wanted to explore the potential of magnetite labelling for making NPs traceable in rat brain.

Methods:
MR studies were performed on a clinical 3 Tesla scanner (Tim Trio, Siemens Healthcare, Germany) using a 2-element surface coil for signal reception. For in vivo measurements only the NPs were used that showed the strongest R1 relaxivity effect in phantom measurements. Following anaesthesia, baseline brain scans were performed and repeated after injection of NPs in the leg vein. After calculation of T1 maps of the brain, brain tissue was segmented and analyzed with a histogram technique. Correlations between relaxivity changes due of NPs in brain and auto-fluorescence imaging were used for MRI confirmation data.

Results:
NP could not be visually assessed in the T1 brain maps but histogram analysis revealed that there was a NP related R1 increase of approximately 3% in brain. T1 changes in gray matter versus auto-fluorescence in corpus callosum were statistically relevant.

Conclusion:
Our results indicate that even small amounts of NP can be visualized in vivo by incorporating magnetite. Only a small amount of NP seems to cross the BBB. However, with a robust histogram technique it is possible to detect even these small variations in R1.

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Establishment of neuronal differentiation in vitro and beginning to explore the role of microRNA-451 in neuronal development

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PhD Neuroscience

Background:
Micro-RNAs (miR) are small, endogenous noncoding RNA molecules, which regulate gene expression posttranslationally. miR-451 regulates tumorigenesis as well as the self-renewal and/or multipotential differentiation capacity of stem cells.

Aims:
We aim to analyse the role of miR-451 and/or miR-144 (its cluster gene) in neuronal differentiation in vitro and to identify respective miRNA associated regulatory mechanisms.

Methods:
NT-2 (Ntera-2 cl.D1) cells were transduced with lentiviral vectors encoding miR-451 and/or miR-144 under the control of the EF1α promoter. Successful transduction was established by miR-451 and/or miR-144 as well as target gene (for example Dicer, Drosha) expression profiles. The effect of miR-451 and/or miR-144 on the neuronal differentiation potential of NT-2 was analysed.

Results:
We were able to successfully transfect NT-2 with miR-451 and/or miR-144 expressing lentiviral vectors. Significant up-regulation of miR-451 and miR-144 was accompanied by a pronounced down-regulation of the miR-451 target gene Dicer. Morphological changes of transduced cells were only observed following transduction with miR-451/144. There was also a pronounced reduction in the number of neurospheres following miR-451/144 overexpression in NT-2 cells. The expression of neural differentiation markers is currently being investigated (MAP2, Tuj1, NeuN, Doublecortin, Nestin, GFAP, Sox2, Neurofilament).

Conclusion:
The clustered expression of miR-451 and miR-144 seems to have a pronounced inhibitory effect on early retinoic acid induced neural differentiation of NT2 cells. Dicer, a key factor in micro-RNA processing is down-regulated during miR-451/144 induced inhibition of early neuronal differentiation and might therefore play an important part in early neuronal differentiation process.

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Nitric Oxide as a Fast Responder to Brain Trauma: Post Traumatic NO levels in Brain and the Body and Gene Expression in Hippocampus

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PhD Neuroscience

Background & Aims:
Nitric oxide, a gaseous neurotransmitter and a short-lived free radical, has been related to both protective and detrimental processes in central nervous system.
Our primary aim is to elucidate the post-traumatic NO production, to reveal protective and detrimental levels in rat brain.

Methods:
TBI was induced by fluid percussion injury on Sprague Dawley adult male rats (moderate ≤2.5 atm ≤severe). DETC-Fe spin trapping was used for detection of NO 4h, 1-2-3 days after trauma. Paramagnetic nitrosyl iron complex was quantified by electron paramagnetic resonance. Hippocampal nNOS, iNOS, GLUT1, BDNF and NT4 were analyzed by RT-PCR. G6PDH was used as internal control.

Results:
NO levels increase in cortices, hippocampi and cerebella of injured animals. The increase in hippocampi was restricted to ipsilateral site, whereas in cortices and cerebella contralateral site was also found to be affected. Cortical and hippocampal NO levels were similar to the sham levels 1d post-TBI. Severity dependency in NO increase was apparent in all there brain regions. Further, NO levels were found to be increased also in liver and heart tissues, with a profile very similar to brain.
Hippocampal nNOS expression increased on the day of trauma and was halved 1d later as compared to sham, yet higher again next two days up-to 2-fold. Similar profile was seen with iNOS and GLUT1. NT4 showed up to 5-fold decrease in all time points in severe trauma compared to the sham while BDNF remained unaffected.

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Effects of Biodegradable Magnesium Implants on the Epiphyseal Growth Plate: Investigations of a Transepiphyseal Rat Model

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Background:
Excellent mechanical properties and the ability to corrode in living organism make magnesium a promising candidate for application as biodegradable osteosynthetic device.

Aims:
For the purpose of osteosynthesis in paediatrics, this study aims to investigate the biodegradation process of two magnesium alloys and their effects on the epiphyseal growth plate in an in vivo rat model.

Methods:
Twelve male Sprague-Dawley rats underwent a surgery with drill holes performed transepiphyseal through the distal femoral growth plate. Each rat received either a ZX50 or a WZ21 magnesium pin per femur. The contralateral leg was also drilled and served as a control. With µCT monitoring the pin degradation rate and bone growth discrepancies within 6 months were observed.

Results:
ZX50 pins degraded fast with high amounts of hydrogen gas inside the bone marrow leading to a local destruction of the growth plate. The bone growth was consecutively significantly diminished (Mann Whitney U p<0.05). WZ21 implants did not induce adverse reactions of the growth plate and showed no leg length discrepancies. The release rate of gas was low and hydrogen could be easily resorbed by the surrounding tissue.

Conclusion:
The fast degrading ZX50 led to massive gas formation according to the chemical reaction \( \text{Mg} + 2\text{H}_2\text{O} \rightarrow \text{Mg(OH)}_2 + \text{H}_2 \). The high amounts of gas destroyed the entire growth plate. WZ21, however, achieved good results and the effect on the growth plate did not differ from the contralateral drill hole lesion. Therefore WZ21 fulfils a prerequisite for a use as osteosynthetic device in paediatric orthopaedics.

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Femoral Bone Length Differences after epiphyseal growth plate injury in a growing Rat model

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**Background:**
Growth plate injuries in children can result in severe growth disturbances such as axial or leg length differences. These bone length discrepancies can be due to posttraumatic growth stimulation or inhibition. Growth inhibition may occur because of ossification of the cartilaginous growth plate.

**Aims:**
The aim of this study was to investigate the healing process and tolerance level of an injured epiphyseal growth plate in a growing rat model.

**Methods:**
The distal femoral growth plate of growing Spraque Dawley\textregistered rats were subjected to a drilled standardized defect of 1.5 cm in diameter (n=6/group). The right femur was left untreated as control. After 1 week, 1, 3 and 6 months \textmu-CT scans were performed monitoring the healing process and measuring the femoral bone length gain. At the same time points, histological analysis was made by HE and Safranin-O-Green stainings.

**Results:**
Both, \textmu-CT monitoring and histological analysis, confirmed the formation of a physeal bone bridge. By month 6, however, a significant bone length difference (p=0.024) was determined between the experimental and contralateral bone.

**Conclusion:**
Our study showed that a growth plate injury of 1.5 cm, which is a defect of a 3rd of the entire size of the growth plate, leads to the formation of a physeal bone bridge which was observed after one month. After a period of 6 months a significant bone length discrepancy was documented. Interestingly, the shorter length of the experimental femur affected also the reference bone in the way that the bone adapted via visible bending.

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Matrix metalloproteinases’ expression in human growth plate chondrocytes is enhanced at high levels of mechanical loading – a possible explanation for overuse injuries in children

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Background:
Overuse injuries are an emerging clinical problem as greater numbers of children do sport activities of increased levels of training and competition at earlier ages. The molecular mechanisms activated within the growth plate, when mechanical stimuli go above a physiologic level, are unknown.

Aims:
As matrix metalloproteinases (MMPs) are critically important for remodeling of the extracellular matrix and angiogenesis within the growth plate, this study aims to investigate the expression of MMPs in human growth plate chondrocytes in response to various loading durations and intensities.

Methods:
Primary human growth plate chondrocytes were subjected to mechanical forces equal to either physiologic loads, near detrimental or detrimental loads for 2 h. Additionally, chondrocytes were exposed to physiological loads for up to 24 h. Changes in expression of MMP-2, -3 and -13 were investigated.

Results:
MMP expression was linearly dependent on loading duration and intensity, with physiologic loads having the same effect as detrimental loads when applied long enough.

Conclusion:
Our study confirmed the involvement of physeal MMPs in the response to mechanical loading. Based on these findings training intensities and especially regeneration intervals should be chosen carefully in young athletes in order to prevent overuse injuries.

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Importance of HEY1/2 in Notch4 dependent regulation of Slug and Twist1 in Melanoma

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Background:
Notch signaling is involved in cell fate determination, stem cell potential, and lineage commitment. Aberrant regulation of Notch is observed in a growing number of malignant tumours and the biological function of this pathway is critically context dependent. Slug and Twist1 gene expressions were found to be active in multiple carcinomas which are associated with poor prognosis, metastasis and invasion. Overexpression of Slug and Twist1 and subsequent downregulation of E-cadherin facilitate an epithelial to mesenchymal transition Phenotype which eventually leads to dissemination of cancer cells.

Aims:
To investigate on the molecular mechanisms of Notch4 signaling mediated regulation of Slug and Twist1 in melanoma.

Methods:
Lentiviral gene overexpression, gene silencing (siRNA), Electrophoretic mobility shift assay (EMSA), chromatin Immunoprecipitation assay (ChIP), Immunoblotting, Real Time RT-PCR.

Results:
Overexpression of the intracellular domain of Notch4 (N4ICD) resulted in a decrease of Slug and Twist1 expression levels and real-time RT-PCR showed a decrease in RNA transcripts in cells overexpressing N4ICD. EMSA demonstrated a binding of Notch signaling key mediator CSL to consensus sites upstream the transcription start site of the Slug and Twist1 promoter. N4ICD transduction led to a significant transcriptional upregulation of the Notch target genes HEY1 and HEY2 which was confirmed by real-time RT-PCR. Selective knockdown of HEY genes using targeted siRNAs resulted in upregulation of Slug and partially Twist1 in non-transduced cells.

Conclusion:
HEY1/2 seems to be involved in Notch4 mediated regulation of Slug and Twist1 in melanoma.

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Gene Linkage Study Of Cpl And Msx1 In Indigenous Communities Of Tarapaca And Chorrera In Amazonas Colombia

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**Background and Purpose:**
Cleft Lip and/or Palate (CLP) are the most common congenital craniofacial malformation in humans, the prevalence rate varies according to the population; In the etiology are involved risk factors environmental and genetic. Studies of linkage have showed an important role for MSX1 gene. We explored the probably of linkage of CPL with MSX1 gene in families from indigenous communities of Amazonas.

**Methods:**
Blood samples was obtained from patients with CLP and their parents to DNA isolation, regions of MSX1 was studied for PCR and direct sequencing to evaluate possible intra-family linkage.

**Results:**
In Chorrera 57 samples were collected 11 was patients with CLP 46 relatives, 10 were of other pathologies to a total of 15 families, 12 with members affected with CLP, we found that there are relationships between families 1-2-9-8-12, 3-5-6-8-11-12-13, 4-10, and 7-15. In Tarapacá 37 samples were collected, we identified 13 patients with CPL, 30 relatives, 10 other pathologies. We studied 11 families with members affected with CLP, we found that there were some relationships among 8,9,10,11 families and between 2-3 families.

**Conclusions:**
we assess the probability of relationship between families of Chorrera and Tarapacá we found that there is a relationship between the family 6 of Tarapacá with families 1,2,9,8 and 12 of Chorrera. The molecular results showed that genotype and allele frequencies for markers MSX1 showed Hardy Weinberg balance.

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