Overview of current MD research projects / Medizinische Doktorarbeiten:

Project	MD doctoral thesis
1	Analysis of candidate targets in mouse and human blood at birth
2	Effects of hyaluronan (HA) and specific HA-synthase isoenzymes on differentiation of bone marrow adipocytes
3a	Effect of specific gene variants of mitochondrial complex one in recent-onset type 2 diabetes
3a	Tissue-specific energy metabolism in clusters of diabetes
3b	Roles of MIC26 and MIC27 in cellular lipid metabolism
4	Distinct signaling pathways of TBC1D1 and TBC1D4 in insulin-sensitive cells
5	IL-6 classical and trans-signaling in primary muscle cells
6	Insulin- and contraction signaling in skeletal muscle cells from individuals with insulin resistance and type 2 diabetes
7	Analysis of the role of NMDA receptors in mitochondrial function of pancreatic islets

Detailed description of projects:

Project 1: Mechanisms of sex-specific programming of obesity and type 2 diabetes risk by peri-conceptional exposure to a maternal obesogenic milieu

MD thesis: "Analysis of candidate targets in mouse and human blood at birth"

Background & own previous work: Maternal obesity continues to increase dramatically in developed countries. Preclinical and clinical data have demonstrated that the offspring born to overweight or obese mothers have an increased risk for metabolic disease such as obesity and type 2 diabetes, which also holds true in animal models. However, the molecular mechanisms linking prenatal obesogenic exposure to the later development of diabetes and obesity in offspring remain poorly defined. We previously established a mouse model of peri-conceptional maternal diet-induced obesity. We demonstrated that, even in the absence of any postnatal obesogenic influences, adult offspring developed adverse outcomes, i.e. increased body weight, insulin resistance and hepatic steatosis in males and impaired fasting glucose and altered adipose tissue expansion in females, indicating that peri-conceptional obesity induces metabolic disease in offspring in a sex-dependent manner. Moreover, intracellular signaling pathways can be altered by specific bioactive lipids or microRNAs.
Hypothesis & Aims: To investigate how exposure to maternal diet and diet composition in utero mechanistically affects obesity and diabetes-related traits.
Work program: Medical doctoral researchers will have the opportunity to contribute to this project by working on translational aspects. Following isolation of total RNA from dried blood spots already available from our previously established mouse model, transcript analysis of candidate genes identified in the project and based on literature will be conducted. Dysregulated genes and marker genes of dysregulated pathways will be selected and a literature search for homologs in human blood will be carried out. RNA samples from dried cord blood spots of offspring of the PEACHES (Programming of Enhanced Adiposity Risk in CHildhood – Early Screening) mother-child cohort may serve to verify possible blood-derived candidate markers found in the animal studies as indicators for the risk of developing overweight and/or type 2 diabetes.

Supervising investigators: Prof. Dr. Regina Ensenauer, Department of General Paediatrics, Neonatology and Paediatric Cardiology, Medical Faculty, HHU; Dr. Bengt-F. Belgardt; Institute for Vascular and Islet Cell Biology, German Diabetes Center, DDZ.

Project 2: Hyaluronan matrix in bone marrow adipose tissue: implications for the development and progression of insulin resistance

MD thesis: "Effects of hyaluronan (HA) and specific HA-synthase isoenzymes on differentiation of bone marrow adipocytes"

Background & own previous work: The glycosaminoglycan hyaluronan (HA), a major component of the extracellular matrix (ECM), is synthesized by three different HA synthases (HAS) HA assembly is dependent on activated sugar precursors, and therefore an interrelationship between HA and glucose metabolism is considered. We investigated functions of the HA-rich matrix as well as mechanisms of immunomodulation in metabolic and inflammatory disease models. Recently we could show that treatment with an inhibitor of HA synthesis (4-methylumbelliferone, 4-MU) but also in mice with a genetic deficiency in the two most abundant HA synthases (HAS), *Has2* and *-3*, prevented weight gain, AT hypertrophy, and inflammation of white and brown adipose tissue. Functionally, systemic glucose tolerance and insulin resistance were improved, suggestive of a role of HA in early stages of T2DM.
Hypothesis & Aims: Elucidating the so far unknown role of the HA-rich extracellular matrix for bone marrow adipose tissue.
Work program: Medical students will establish culturing primary adipocytes from bone marrow-derived preadipocytes from either C57BL6J wildtype mice or mice deficient in *hyaluronan synthase (Has)1,-2 or -3* fed with normal chow diet or a high fat, high sucrose diabetogenic diet. Pre-adipocytes from wildtype without and with obesity and insulin resistance will be characterized for mRNA expression of different *Has* isoforms during differentiation. In parallel, intracellular as well as extracellular HA will be quantified using HA-ELISAs. Further, cells will be treated during differentiation with the pharmacological inhibitor of HA synthesis, 4-MU, in order to characterize the effects of HA on preadipocyte differentiation. The specific functions of HAS isoenzymes will be addressed using lentiviral knockdown or overexpression of specific the respective HAS isoenzyme as well as using isolated cells from the above mentioned *Has1,-2 or -3* knockout mice. In a perspective, also the use of mice deficient in specific HA receptors such as CD44 or Rhamm can help to further clarify the functional importance of HA in bone marrow AT.

Main supervising investigator: Prof. Dr. Maria Grandoch; Co-supervisor: Prof. Dr. Jens W. Fischer; Institute for Pharmacology and Clinical Pharmacology, Medical Faculty, HHU.

Project 3: Role of mitochondrial function in lipid-induced insulin resistance

MD theses: (1) "Roles of MIC26 and MIC27 in cellular lipid metabolism"; (2) "Effect of specific gene variants of mitochondrial complex one in recent-onset type 2 diabetes"; (3) "Tissue-specific energy metabolism in clusters of diabetes"

Background & own previous work: Altered mitochondrial functionality is frequently present in type 2 diabetes mellitus (T2DM). In particular, decreased capacity of oxidative phosphorylation (OXPHOS), altered formation of cristae and crista junctions and increased generation of reactive oxygen species (ROS) have been linked to the pathogenesis of T2DM. Improving mitochondrial functionality has been shown to restore insulin sensitivity and glucose homeostasis in certain model systems of T2DM. Previous work has focused on a) the mechanisms how lipids affect mitochondrial function and energy metabolism in a tissue-specific manner, and b) structure and function of the MICOS complex that is critical for determining cristae structure, formation of crista junctions, and OXPHOS. However, the underlying molecular mechanisms, the interplay between these processes and the causal relationships for diabetes development are largely unclear.
Hypothesis & Aims: Impairing oxidative phosphorylation combined with alteration of cristae morphology may result in insulin resistance, partially due to a systemic increase in fatty acid

levels and altered ROS production. The project aims to decipher the role of mitochondrial OXPHOS and cristae morphogenesis in the early steps of establishing IR.
Work program: (1) The MD candidate will be involved in determining the role of MICOS components (in particular of MIC26 and MIC27) resulting in insulin resistance (IR). For this we will apply suitable cell culture models for IR. It is planned to use a variety of established methods of testing IR after administration of free fatty acids, high glucose or high insulin to the culture medium. CRISPR-Cas9 methods will be used to generate specific gene deletions of MIC26/MIC27 in IR-sensitive cells. Moreover, direct analysis of lipid and energy metabolism will be performed using e.g. the fatty acid oxidation assays using a Seahorse Flux Analyser in these cells under IR-induced conditions. Concomitantly, changes in cristae structure will be determined in control cells and in cells depleted for MIC26/27. A broad range of biochemical, genetic and cell biology methods will be applied including advanced fluorescence imaging techniques. **(2) and (3**): soon to come.

Supervising Investigators: Prof. Dr. Michael Roden; Division of Endocrinology and Diabetology, Medical Faculty and German Diabetes Center, DDZ; Prof. Dr. Andreas Reichert; Institute of Biochemistry and Molecular Biology I, Medical Faculty, HHU.

Project 4: Metabolic flexibility in early diabetes development

MD thesis: "Distinct signaling pathways of TBC1D1- and TBC1D4 in insulin-sensitive cells"

Background & own previous work: TBC1D4 (AS160) and its close homologue TBC1D1 regulate insulin- and contraction-mediated glucose uptake and lipid metabolism in skeletal muscle and adipose tissue. Inactivating mutations in TBC1D4 or TBC1D1 result in an altered balance of glucose and fat as fuel and are associated with insulin resistance and increased risk for type 2 diabetes. We generated mouse models to study how lifestyle interventions like exercise and diet composition interact with genetic impairments of skeletal muscle insulin resistance.
Hypothesis & Aims: RabGAPs contribute to the impact of nutritional cues at early stages of life on systemic insulin sensitivity, insulin secretion and the risk for T2DM. We will use insulin resistant mouse models and specific experimental diets to further clarify the underlying mechanisms how nutrition and age affect metabolic flexibility and, as a consequence, insulin sensitivity.
Work program: MD projects focus on molecular work related to the analysis of glucose- and lipid substrate metabolism in insulin target tissues from (induced, tissue-specific/global) knockout mice at different time points in life by proteomics- and transcriptomics-based assays and validation of targets using Western Blotting and quantitative real-time PCR.

Main supervising investigator: Dr. Alexandra Chadt; Co-supervisor Prof. Dr. Hadi Al-Hasani; Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, DDZ.

Project 5: Role of Interleukin (IL-)6 trans-signaling in meta-inflammation and development of insulin resistance

MD thesis: "IL-6 classical and trans signaling in primary muscle cells"

Background & own previous work:
Obesity with type 2 diabetes mellitus (T2DM) is accompanied by a chronic, low- grade metabolic inflammation 'meta-inflammation', characterized by increased interleukin (IL)6 levels. Meta-inflammation may already occur in early developmental stages and impact metabolism and disease progression throughout different stages of life. IL-6 interacts with insulin signaling and controls metabolic processes such as lipid and glucose homeostasis. Interestingly, in skeletal muscle of T2DM patients, IL-6 signaling has been shown to be compromised, indicating links between IL-6- and insulin-dependent signaling pathways. We have developed novel mouse models that allow in-depth analysis of IR6 signaling in skeletal muscle and its contribution to diabetes development.

Hypothesis & Aims:
IL6 signalling may play an important role in modulating insulin sensitivity in skeletal muscle. High-fat diet-induced obesity in young genetically modified mice will uncover the role of IL-6 classic and trans-signaling in obesity-induced meta-inflammation with respect to the early transition from the healthy state to insulin resistance.
Work program: Medical doctoral researchers will establish satellite muscle cells from transgenic mouse strains to obtain permanent cells with typical muscle-like characteristics for subsequent analysis of IL-6 related pathways and metabolic behavior. The cell will be metabolically characterized using state-of-the-art technologies including high-throughput transcriptomics and proteomics.

Main supervising investigator: Prof. Dr. Jürgen Scheller; Institute for Biochemistry and Molecular Biology II, Medical Faculty, HHU; Co-supervisor Prof. Dr. Hadi Al-Hasani; Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, DDZ.

Project 6: Exercise-triggered mechanisms contributing to beneficial metabolic responses and type 2 diabetes protection

MD thesis: “Insulin and contraction signaling in skeletal muscle cells from individuals with insulin resistance and type 2 diabetes”

Background & own previous work: Physical exercise is associated with positive metabolic effects on glucose- and lipid metabolism, insulin sensitivity and protection against type 2 diabetes. However, the mechanisms for these effects are not well understood. We established in vitro methods for contracting rodent skeletal muscle and cultured human muscle cells by electrical pulse stimulation (EPS; “training in a petri dish”). We identified secreted muscle proteins and metabolite patterns associated with insulin sensitivity using high-resolution mass spectrometry.
Hypothesis & Aims: Hormone-like proteins (myokines) and metabolites secreted from skeletal muscle are thought to mediate beneficial effects of exercise in multiple organs. We will use models of exercise and muscle contraction to identify regulatory factors, myokines and metabolites that improve insulin action in cultured skeletal muscle and adipose cells, and insulin secretion in beta cells.
Work program: Cultured muscle cells (metabolically healthy or insulin resistant) will be subjected to EPS training in vitro, and analyzed before and after contraction for transcriptional control of glucose and lipid metabolism and contraction-related signaling by RNASeq, real-time qPCR, phosphoproteomics and Western blotting. After the contraction protocol, conditioned skeletal muscle cell media will be analyzed for novel secreted factors that improve insulin signaling and secretion in an autocrine or paracrine manner.

Supervising Investigators: Prof. Dr. Hadi Al-Hasani; Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, DDZ; Co-supervisor Prof. Dr. Regina Ensenauer; Paediatrics, Neonatology and Paediatric Cardiology, Medical Faculty, HHU.

Project 7: Investigation of alternative mechanisms involved in the anti-diabetic effects of dextromethorphan

MD thesis: "Analysis of the role of NMDA receptors in mitochondrial function of pancreatic islets”

Background & own previous work: T2DM develops when insulin resistance in organs, such as liver or skeletal muscle, is accompanied with a failure in the insulin-secreting pancreatic beta cells. The latter cells decline in their function, meaning they do not secrete sufficient insulin, and in their survival, meaning that the beta cells start to die or to dedifferentiate. This decline in beta cell function, survival and differentiation is progressive upon onset of T2DM and is believed to contribute to diabetes progression in humans with T2DM. Notably, most oral anti-diabetic medication cannot stop this disease progression. Therefore, after some years of

medical treatment, blood glucose concentrations return to those before anti-diabetic treatment. Thus, it is essential to identify and explore pathways within beta cells that protect them from dysfunction, death and dedifferentiation, so that current oral anti-diabetic medication is successful for a longer duration, thus avoiding the need for insulin injections.
We have previously found that blocking NMDA receptors both genetically and pharmacologically increases glucose-stimulated insulin secretion and beta cell survival in vitro and in vivo.
Hypothesis & Aims: Here we aim to dissect the molecular mechanisms underlying the anti-diabetic effects of dextrorphan in pancreatic beta cells.
Work program: *Xenopus laevis* oocytes will be used to heterologously express NMDA receptors and interacting channels to investigate the effects of dextrorphan and other NMDA receptor antagonists on ion currents by electrophysiological recordings. In addition, INS1E cells and primary mouse or human pancreatic islets will be employed to investigate how dextrorphan affects mitochondrial function.

Supervising Investigators: Prof. Dr. Eckhard Lammert; Institute for Metabolic Physiology, Institute for Metabolic Physiology, Faculty of Mathematics and Natural Sciences, HHU and Institute for Vascular and Islet Cell Biology, German Diabetes Center, DDZ; Prof. Dr. Nikolaj Klöcker; Institute for Neuro- and Sensory Physiology, Medical Faculty, HHU.

Further project information can be found at our website. **If you are interested to join us**, please visit the "Online-Sprechstunde" (see link at **www.vivid.hhu.de**) and/or contact the coordination office (**vivid(at)hhu.de**).