The scientific background

Type-2-diabetes and the influence of obesity

Type 2 diabetes mellitus (T2D), with around six million patients and probably just as many people with unrecognized diabetes or high risk for this metabolic disorder is one of the largest common diseases in Germany. According to the International Diabetes Federation (IDF), the number of people with diabetes worldwide will rise by the year 2030 from the current about 382 million by around 55 percent to an estimated 592 million. In Germany, as well as the world, the incidence of diabetes mellitus has greatly increased in the past 50 years. Current projections suggest that the incidence will rise even faster. T2D is a chronic, progressive disease characterized by insulin resistance and impaired insulin secretion. These malfunctions may be acquired or inherited. T2D is important because of its high prevalence and incidence, the individual disease burden of patients due to macro- and microvascular complications, and the costs it generates for health system (Köster et al., 2012). The pathogenesis of T2D involves both genetic and environmental factors.

Obesity is the most common cause of developing T2D as it represents about 75 % of the causes (Wali et al., 2014). Obesity can lead to insulin resistance and impairment in energy metabolism of most tissues such as skeletal muscles, liver, adipose tissue, and pancreatic islets. However, insulin resistance does not lead to T2D unless it is accompanied by pancreatic beta cell dysfunction (Sakuraba et al., 2002; Marchetti et al., 2004; Cozar-Castellano et al., 2006). What is still unknown are the time points when beta cell dysfunction starts and the relative contribution of beta cell dysfunction in the development of T2D. Current evidence suggested that islet function was about 50 % of normal at the time of diagnosis (Matthews, 1999). The reduction of beta cell function most probably starts early about 10–12 years before diagnosis of diabetes (Holman, 1998). The major factors for causing progressive decrease in beta cell structure and function during the course of the disease are glucotoxicity, lipotoxicity, proinflammatory cytokines, and islet cell amyloidosis (Prentki et al., 2006). The combined increased flux of free fatty acids and glucose into the beta cell has detrimental consequences on beta cells (Wajchenberg, 2007). The excess free fatty acid entering beta cells inhibits proper glucose utilization in the mitochondria. Additionally, it is reported that these lipids are metabolized in a different way forming lipid intermediates that cause abnormal signaling and beta cell dysfunction. The glucotoxicity and lipotoxicity also may cause an increase in reactive oxygen species (ROS) which can damage the cell and then lead to beta cell death (Halban et al., 2014). Insulin and its downstream signaling pathway play important roles in the homeostasis of blood levels of glucose, and the disruption of the signaling pathway plays an important role in the pathophysiology of T2D. Insulin reduces blood glucose levels through increasing glucose uptake in muscle and fat and decreasing hepatic glucose production. Skeletal muscle consumes most glucose, accounting for about 75 % of insulin-dependent glucose uptake while adipose tissue accounts for only a small fraction (Klip et al., 1990). The adipose tissue, however, is crucial in the normal regulation of the insulin action all over the body. Adipocytes can store excess lipids in obesity but when they become saturated, lipids begin to accumulate inside other organs, and tissues making them insulin resistant. Adipocytes also can produce adipokines such as leptin and adiponectin which have been proved as insulin sensitizers due to their ability to decrease triglycerides (TG) synthesis, to stimulate beta oxidation of fatty acids, and thus to enhance insulin action in both skeletal muscle and liver (Shimomura et al., 1999; Ebihara et al., 2001; Hotta et al., 2000).

The scientific background

Aspects of the role of adipose tissue

Genetically modified animals deficient in white adipose tissue usually have severe insulin resistance in liver and muscle (Reitman et al., 2000). Transplantation of normal fat tissue into white adipose tissue deficient mice restores the insulin sensitivity (Gavrilova et al., 2000). Mice with a knockout of the insulin receptor in muscle have normal glucose tolerance (Bruning et al., 1998), whereas those with a knockout of the insulin-sensitive GLUT4 glucose transporter in adipose tissue have impaired glucose tolerance, apparently due to insulin resistance being induced in muscle and liver (Abel et al., 2001). Other recent studies reported that obesity promotes inflammatory signals especially in the adipose tissues that disrupt insulin action and mediate insulin resistance (Shu et al., 2012). Accordingly, the therapeutic targets of T2D will be preservation of beta cell structure and function, enhancing metabolic activity especially of the adipose tissues and decreasing lipid content in different tissues.

The gain of adipose tissue in the body, which can result to overweight and obesity, is linked with the increase in the number of adipocytes (hyperplasia) as well as the increase in the volume of adipocytes (hypertrophy). Hypertrophy is seen as the initial feature of incipient obesity (Brook et al., 1972). However, since the adipocytes grow only to a limited extent and store triglycerides (lipids) to a certain limit, the formation of new adipocytes is imperative. Under normal conditions adipocytes develop from progenitor cells (a process called adipogenesis) to ensure proper fat cell turnover; approximately 10% of the fat cells are renewed annually (Spalding et al., 2008). Recently, it was shown that these progenitor cells reside in a perivascular niche of adipose tissue (Tang et al., 2008). Besides regular turnover, the expression of certain fat-depots due to overfeeding not only results from hypertrophy but also from an increase in their number (hyperplasia). In particular lower body fat seems to respond to overfeeding by hyperplasia, with the gain of approximately 1.6 kg lower body fat corresponding to roughly 2.5 billion new adipocytes within 8 weeks (Tchoukalova et al., 2010). This enormous gain of adipocytes, as well as the regular turnover rate, reflects the ability of preadipocytes or mesenchymal stem cells (MSC) to proliferate and differentiate upon appropriate stimuli. The mechanisms underlying this process are still poorly understood. A study conducted by Widberg et al. (2009) showed that the growth factor, fibroblast growth factor 1 (FGF1) is involved in the proliferation of preadipocytes and plays an important role in their differentiation into mature adipocytes (Newell et al., 2006). In addition, a high-fat diet in mice induces a high FGF1 activity in adipose tissue (Jonker et al., 2012). Interestingly, FGF1 is a major inductor of the High-Mobility-Group-AT-hook 2 (HMGA2) gene in immortalized murine pre-adipocytes (Ayoubi et al., 1999), as well as in primary human MSC (Markowski et al., 2011). Further studies (Markowski et al., 2013; Thies et al. 2014) confirm a highly significant association between obesity and an increased *HMGA2* expression, and a connection between HMGA2 and hyperplasia of white adipose tissue.

Transgenic mice constitutively expressing a truncated HMGA2, still containing the three AT-hook domains, exhibit a giant phenotype including an increased amount of body fat (Battista et al., 1999). In contrast HMGA2-knock-out mice not only show a pygmy phenotype but also do not gain weight after high-caloric diet which is linked to hypoplasia in white adipose tissue (WAT) (Zhou et al., 1995). Likewise, a lack of HMGA2 impairs the determination of MSC towards preadipocyte (Pasquali et al., 2004). Another relevant gene for adipogenesis is peroxisome-proliferator-activated receptor-ɣ (PPAR-ɣ). PPAR-ɣ is a ligand-binding nuclear

transcription factor of the PPAR subfamily of nuclear hormone receptors (Chawla et al., 2001) and promotes gene expression when it heterodimerizes with retinoid X receptor α. PPAR-ɣ expression is both necessary and sufficient for ongoing adipogenesis, in particular for the differentiation of preadipocytes to mature adipocytes (Darlington et al., 1998; Gregoire et al., 1998; Lowell, 1999). A complete absence of white and brown adipose tissue was noted in PPAR-ɣ deficient mouse (Barak et al., 1999). During human adipogenesis the expression of PPAR-ɣ-mRNA increases and assumes a key regulator function during adipogenesis and maintaining the adipocyte phenotype. Further investigations show a significantly lower expression of PPAR-ɣ in overweight groups, both in human and dogs, than in normal weight groups (Thies et al., 2014; Thies, 2014). This suggests a lower level of maturation of adipocytes in the studied canine and human adipose tissue. This assumption is supported by the highly significant inverse correlation between the expression of *PPAR-ɣ* and the Body Mass Index (BMI) (Thies, 2014). However, the lower mRNA expression of *PPAR-ɣ* in subcutaneous abdominal WAT from individuals with overweight and the inverse correlation between *PPAR-ɣ* and *HMGA2* expression suggest a role of *PPAR-ɣ* and *HMGA2* as antagonists in the differentiation of adipocytes and the proliferation and self-renewal of their progenitors, respectively. A significant correlation between *HMGA2* and BMI in combination with a highly significant inverse correlation between *PPAR-ɣ* and BMI indicate that the decrease of *PPAR-ɣ* with increasing BMI represents a gain of body weight through immature adipocytes in WAT (Thies, 2014). As a result of augmentation of immature adipocytes the ability of WAT to store or mobilize triglycerides and to respond to insulin could be diminished.

The reinforced increase of immature preadipocytes compared to mature insulin-sensitive adipocytes in subcutaneous adipose tissue has a huge impact on lipid and glucose metabolism and the endocrine function of adipose tissue (Danforth, 2000). In the field of glucose metabolism plasma glucose cannot, or only to a small extent, be stored in the preadipocyte. This is due to the lack of insulin-dependent glucose transporter type 4 (GLUT4), which is expressed only in the subsequent stages of adipogenesis in adipocytes (Avram et al., 2007). This means that with an increasing proportion of immature preadipocytes in adipose tissue also enhances insulin resistance and thus increases the risk of chronic hyperglycemia. In the field of lipid metabolism free fatty acids cannot, or only to a small extent, be stored in the preadipocyte due to absence of fatty-acid-binding proteins 4 (FABP4). The free fatty acids are deposited in other tissues outside the fatty tissue such as liver or the insulin-producing beta cells in the pancreas (Kirkland et al., 2002). The increased storage of free fatty acids results in lipotoxicity in these cells, which ultimately leads to apoptosis of beta cells and thus to a decrease in insulin production (Gehrmann et al., 2010). The endocrine function of adipose tissue, particularly with regard to the production of adipokines is disrupted or severely impaired as a result of a high fraction of immature preadipocytes. Adipokines are peptides that signal the functional status of adipose tissue to targets in the brain, liver, pancreas, immune system, vasculature, muscle, and other tissues (Fasshauer et al., 2015). Secretion of adipokines is altered in adipose tissue dysfunction and may contribute to a spectrum of obesity-associated diseases.

A study in adipose tissues and lipomas, benign tumors of adipose tissue demonstrated that the HMGA2-induction both in adipose tissue and in lipomas is accompanied with a concomitant activation of p14Arf-p53-p21-axis (Markowski et al., 2013), which is an indication of the increasing cellular senescence of adipocytes and preadipocytes in adipose tissue. An

accumulation of senescent cells triggers inflammation in adipose tissue, which ultimately leads to insulin resistance (Ahima, 2009; Minamino et al., 2009) and generally promotes the pathogenesis of metabolic syndrome and T2D. In moderately obese individuals, there is an increase in the fraction of small adipocytes, including preadipocytes which are associated with insulin resistance (McLaughlin et al., 2007) and inflammatory reactions in subcutaneous adipose tissue (McLaughlin et al., 2010). Consistent with the results of et al., Minamino et al. und McLaughlin et al. a highly significantly increased expression of HMGA2 in subcutaneous white adipose tissue of individuals with T2D could be observed (Markowski et al., 2013,Thies 2014), likely due to a high fraction of preadipocytes. A further link between the genes of HMGA2 and PPAR-ɣ and the pathogenesis of T2D is the occurrence of single nucleotide polymorphisms (SNPs) in these genes (Ohshigo et al., 2011; Voight et al., 2010; Zeggini et al., 2007; Dayeh et al., 2013). Because of these SNPs, the methylation status of the genes can be modified and thus the activity of the corresponding gene can be altered. The Pro12Ala polymorphism in the human PPAR-ɣ gene is associated with a lower BMI, decreased insulin resistance and decreased risk of T2D (Deeb et al., 1998; Altshuler et al., 2000).