Summary: Vascular Insulin Resistance through Fat

As a consequence of the continuous rise in the prevalence of obesity, metabolic diseases like insulin resistance, type 2 diabetes and associated conditions such as hypertension have reached epidemic proportions. This thesis focuses on the contribution of vascular insulin resistance to the development of type 2 diabetes and hypertension, in order to elucidate mechanisms and to identify possible therapeutic targets. Insulin has an important regulatory role in controlling vascular diameter in arterioles. Insulin determines vascular diameter by the production of both the vasodilator nitric oxide and the vasoconstrictor endothelin-1 and thereby controls blood pressure and delivery of nutrients, like glucose, to insulin-responsive tissues. Insulin resistance is defined as insensitivity to insulin-mediated glucose uptake in (mainly) skeletal muscle. In vascular insulin resistance, insulin's vasodilator effects are impaired resulting in insulin-mediated vasoconstriction, which can contribute to increased blood pressure (hypertension) and blunted glucose delivery to muscle, which can contribute to type 2 diabetes. An important risk factor for the development of metabolic and vascular insulin resistance is fat, or obesity. Obesity is characterized by an increased fat mass, vascular dysfunction and decreased glucose uptake in muscle. The increased secretion of endocrine substances (adipokines) by excessive adipose tissue, like tumor necrosis factor alpha (TNFα) and free fatty acids (FFA), is associated with impairment of vasodilator responses in arterioles.

Although obesity is strongly associated with the development of insulin resistance, type 2 diabetes and hypertension, the mechanisms behind obesity-induced vascular insulin resistance have not yet been elucidated.

This thesis focuses on the relationships between vascular insulin resistance and fat in a mouse study. Vascular insulin resistance was studied by the determination of the effect of insulin to induce vasodilation and vasoconstriction in isolated arterioles in the pressure myograph. Different aspects of fat were investigated: adipose tissue and increased adiposity (obesity) in general and acute exposure to FFA. In order to gain insight in insulin signaling, vasoreactivity and mechanisms that control blood pressure and insulin-mediated blood flow in skeletal muscle, vascular function in combination with these different aspects of fat was investigated in several strains of genetically modified mice.

Chapter II comprises two extensive reviews about the role of endothelial dysfunction and diabetes with a special focus on impaired insulin signaling and perivascular adipose tissue. The first review discusses the initial dysfunction of endothelial cells underlying metabolic and vascular alterations that contribute to the development of type 2 diabetes. The second review further emphasizes the role of perivascular adipose tissue and local production of adipokines on vasoregulation.
In Chapters III-VII, vascular function of arterioles of different mouse strains was investigated. It appeared that vascular responses to insulin in isolated arterioles of the mouse depends on genetic background. Arterioles isolated from mice with a Sv129*C57Bl/6 background, as used in chapter III and IV showed a vasoconstriction to insulin in control mice. In contrast, arterioles isolated from mice with a C57Bl/6 background showed no change in diameter to insulin (Chapter V-VII). To overcome discrepancies between different mouse strains, conclusions based on genetic mouse models were only made by using their control litter mates as reference.

In Chapters III and IV, the role of insulin receptor substrates (IRSs) in insulin-mediated vasoreactivity and their physiologic effects on vascular function was investigated. IRS proteins are important mediators of insulin signaling. IRS proteins are targeted by FFA causing disturbed intracellular insulin signaling, which could possible play a role in obesity-induced insulin resistance. However the exact role of IRS proteins in vascular insulin signaling is not known. The functional gene of either IRS1 or IRS2 was deleted in mice and arterioles were isolated to determine the functional effects on vascular insulin signaling. Previously, IRS proteins have mainly been linked to activation of the NO pathway of insulin, implying that IRS proteins have an important role in insulin-mediated vasodilation. However, the data presented in chapter III and IV show an important role of both IRS1 and IRS2 in the ET-1 pathway of insulin. The deletion of either IRS1 or IRS2 leads to insulin-mediated vasodilation in muscle arterioles, due to an impaired ET-1 activation. In IRS1 deficient mice, this vasodilation to insulin is accompanied by an altered muscle vascularization, compared to control siblings (Chapter III). Our data showed that reduced IRS1 expression may partly explain microvascular dysfunction associated with insulin resistance. In IRS2 deficient mice, the vasodilation to insulin was associated with a lower blood pressure, compared to control siblings. (Chapter IV). Blood pressure and cardiac function were measured with radiotelemetry and echocardiography. The decreased blood pressure in IRS2 deficient mice was caused by a decrease in cardiac output and a decrease in vascular resistance by a specific impairment of insulin’s vasoconstrictor effects. Our data suggest that decreased IRS2 activity protects against hypertension.

Deficiencies in IRS expression and function in target tissues of insulin, like skeletal muscle are strongly associated with insulin resistance and type 2 diabetes. The additional role of IRS1 and IRS2 in insulin-mediated endothelin-1 production means that the roles of IRS1 and IRS2 in the insulin signaling are more complex than previously thought. This may have important physiological implications for our understanding of the regulation of blood pressure and muscle perfusion.

In Chapter V and VI, the mechanisms by which FFAs affect insulin-mediated vasoreactivity were investigated. In obesity, plasma levels of FFA are increased and are associated with impaired insulin signaling, impaired capillary recruitment and impaired insulin-mediated glucose uptake in muscle. The data described in Chapter V demonstrate that PKCθ is
present in the endothelium of muscle arterioles and that PKCθ is activated by FFA in muscle arterioles. FFAs activate PKCθ and induce insulin-mediated vasoconstriction, by a reduced activation of Akt and an increased activation of ERK1/2 by insulin (Chapter V). These data provide a new mechanism linking PKCθ activation to insulin resistance. The strong evidence of PKCθ activation in muscle arterioles by FFA was further investigated in adipose tissue arterioles in Chapter VI. This chapter shows that muscle and adipose tissue have a functionally distinct vasculature and that PKCθ is specifically activated in muscle arterioles, as opposed to adipose tissue arterioles. PKCθ activation in muscle arterioles was associated with insulin-mediated vasoconstriction in these arterioles and an increased blood flow towards adipose tissue in obese mice. These effects may promote increased nutrient storage in adipose tissue.

The data described in chapter V and VI show that PKCθ is present in muscle arterioles and is activated by FFA, resulting in insulin-mediated vasoconstriction and offers a new mechanism in which FFA can induce decreased muscle glucose uptake and insulin resistance.

In Chapter VII, the preliminary results of the effects of local secretion of adipokines on vascular function are described. This chapter examines the effects of perivascular adipose tissue (PVAT) isolated from lean and obese mice on insulin sensitivity and insulin-mediated vasoreactivity of vessels of lean mice. The data described in this chapter shows that PVAT is present around skeletal muscle arterioles in lean mice and that it is increased in obese mice. PVAT isolated from lean mice induces insulin-mediated vasodilation in a co-incubation with muscle arterioles of lean mice, by activation of AMPK. This vasodilator response to insulin was blunted in a co-incubation with perivascular adipose tissue from obese mice (Chapter VII). The blunted vasodilation was probably a result from the reduced production of adiponectin by PVAT isolated from obese mice.

Our data showed that PVAT influences vasoreactivity in-vitro by the local production of adipokines. In our set up, PVAT was not in direct contact with the arteriole and the effects on insulin-mediated vasoreactivity were entirely dependent on the secretion profile of the PVAT. The identification of specific adipokine secreted by lean and obese PVAT helps us to find specific treatment of vascular dysfunction to prevent increased blood pressure and blunted muscle perfusion in obesity.

In conclusion, adipose tissue and increased adiposity (obesity) in general, as well as fatty acids (FFA) contribute to vascular insulin resistance. In isolated muscle arterioles, we showed that these different aspects of fat induce insulin-mediated vasoconstriction and have important implications for muscle perfusion and blood pressure regulation. Both IRS proteins and PKCθ are important regulatory proteins in vascular insulin signaling. Together with newly identified adipose tissue round arterioles (PVAT), these are promising therapeutic targets for future studies on vascular dysfunction and insulin resistance.