

Chapter 9



Summary

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In this thesis we investigated whether, and through which mechanisms, PVAT affects insulin-induced microvascular recruitment. We did this to provide further insight into obesity-related microvascular dysfunction. Obesity leads to an increased risk for cardiovascular disease and insulin resistance, it has been proposed that this is due to microvascular dysfunction. Current models are unable to explain why microvascular dysfunction develops in obesity. Further insight into this pathogenesis might lead to better treatment strategies in the future. To study the contribution of PVAT to microvascular dysfunction we performed a number of studies.

First, in **chapter 3a** we showed that insulin-induced microvascular recruitment, or *microvascular* insulin sensitivity, in both skeletal muscle and skin, is directly related to the *metabolic* insulin sensitivity. The participants for this study were thus selected that they had a continuous range of BMI's. The reason for this selection was that we wanted the participants to have a broad range of metabolic insulin sensitivities, without creating distinct groups. Insulin was administered to achieve a postprandial insulin plasma concentration whilst keeping the blood glucose level constant at 5 mmol/l. Before, and after the insulin administration, microvascular measurements were performed in the skeletal muscle of the forearm as well as the dorsal skin of the finger. The former was measured with Contrast Enhanced Ultrasound (CEU), the latter with capillary videomicroscopy. The change in microvascular blood volume, or the amount of perfused capillaries, from baseline to hyperinsulinemia is the insulin-induced microvascular recruitment. The microvascular recruitment in skeletal muscle and in the skin are related with each other. This shows that in people who have insulin-induced microvascular recruitment in e.g. skeletal muscle, also recruit additional capillaries in their skin. Interestingly, both measures of microvascular insulin sensitivity are also related to the insulin-induced glucose uptake during the test day, i.e. metabolic insulin sensitivity. These findings are in line with previous observations that increases in microvascular blood volume contribute to glucose uptake due to increased delivery.

Microvascular perfusion not only contributes to insulin sensitivity, but is also an important determinant of the systemic blood pressure. In **chapter 3b** we studied, in the same group of participants as chapter 3a, whether microvascular perfusion in skeletal muscle as measured by CEU is related to office blood pressures. Contrast Enhanced Ultrasound is currently frequently employed to study microvascular responses in relation to insulin sensitivity, but has surprisingly not yet been applied for the research of blood pressure regulation or hypertension. Therefore, we studied whether this technique might have a future application in this field of microvascular research. As hypothesized, microvascular perfusion is inversely related to blood pressure. This relation holds true for systolic, diastolic, and mean blood pressure.

In chapter 3a and 3b we have established the relevance of insulin-induced microvascular responses for metabolic insulin sensitivity. In **chapter 4** we went on to study the contribution of perivascular adipose tissue (PVAT) to insulin-induced microvascular responses. To do so, we isolated resistance arteries from the hindlegs of C57Bl/6 mice, and studied insulin-induced changes in diameter in the presence and absence of PVAT and various agonists and antagonists. By doing so, we identified that insulin induces microvascular vasodilation in the presence of PVAT from lean C57Bl/6 mice, but not in the absence of PVAT or in the presence of PVAT from obese db/db mice. The difference in microvascular responses with the different types of PVAT is explained by the differing amount of secretion of the AMPK-agonist adiponectin. Moreover, inhibition of the inflammatory mediator JNK restores the vasodilator phenotype of obese PVAT. When we again scavenged adiponectin after the inhibition of inflammation in obese PVAT, insulin does not induce vasodilation anymore. Absence of the energy-sensing protein AMPK α 2 in the microvessels also inhibits insulin-induced vasodilation in the presence of PVAT.

The last observation was further explored in **chapter 5**, here we studied insulin-induced vasoreactivity mice, both in-vivo with CEU and ex-vivo with pressure myography. In this chapter we studied how AMPK α in microvessels affects the balance between the two vasoactive cascades initiated by the binding of insulin to its receptor. We found

that AMPK in the endothelium is activated by adiponectin, one of the adipokines secreted by PVAT as found in chapter 4, as well as AICAR, a pharmacologic agent. Activation of AMPK leads to enhanced activation of eNOS, but also inhibition of ERK1/2, which is involved in the vasoconstrictor pathway. Furthermore, AMPK specifically enhances Ser¹¹⁷⁷ phosphorylation of eNOS through Acetyl-CoA-Carboxylase. Pharmacologic or genetic inhibition of AMPK results in blunted insulin-induced vasodilation, even in the presence of adiponectin or AICAR. To test the relevance of these findings in-vivo, we performed microvascular blood volume measurements before and during insulin administration in mice with regular expression of AMPK α 2 and mice with AMPK α 2 deficiency. The latter show a decreased microvascular blood volume during hyperinsulinemia, which is in line with the vasoconstriction as observed in ex-vivo in the resistance arteries from these mice.

In **chapter 6** we studied both whether short nutrient excess leads to microvascular dysfunction in mice, and whether this may be caused by altered PVAT function. We further explored the observation in chapter 4 that enhanced JNK activation causes PVAT dysfunction. After two weeks Western Diet, C57Bl/6 mice do not show insulin-induced microvascular recruitment, as studied by CEU, whereas C57Bl/6 mice on a normal diet do show insulin-induced microvascular recruitment. Ex vivo, resistance arteries from C57Bl/6 mice on a normal diet vasodilate on increasing doses of insulin when they are incubated together with PVAT, similar to chapter 4. However, when resistance arteries from C57Bl/6 mice on a normal diet are incubated with PVAT from mice on a Western Diet, they do not vasodilate anymore. This demonstrates that the PVAT phenotype changes during these two weeks diet. Moreover, resistance arteries from Western Diet mice do not vasodilate in response to increasing doses of insulin, even in the presence of PVAT from chow mice. This demonstrates that the resistance arteries become resistant to the factors secreted by PVAT. Indeed, resistance arteries from the Western Diet mice do not show insulin-induced vasodilation when they are incubated with adiponectin either. The PVAT dysfunction is caused by infiltration into PVAT by cells from the bone marrow that express the inflammatory mediator c-Jun N-terminal Kinase 2. Surprisingly however, the latter does not affect the resistance artery properties.

Finally, in **chapter 7** we set out to study the contribution of altered PVAT phenotype to blunted insulin-induced microvascular recruitment in women. In this chapter we confirm previous findings by others that obese women are metabolically insulin resistant in comparison to lean women, and have a blunted microvascular recruitment, as studied with CEU. We could demonstrate that the metabolic insulin resistance is mediated in part by the blunted insulin-induced microvascular recruitment. The perivascular adipocyte cross-sectional area is increased in obese women, compared to the lean women. The increase in adipocyte cross-sectional area at least partially explains the blunted microvascular recruitment in the obese versus the lean women. The PVAT phenotype was also studied ex-vivo, where insulin-induced vasodilation was seen in the presence of PVAT from lean women, but not in its absence in resistance arteries from lean women. In contrast, the resistance arteries from the obese women vasoconstrict in the presence of their own PVAT, and do not do so in absence of PVAT.

In conclusion, PVAT enhances insulin-induced microvascular recruitment and vasodilation in- and ex-vivo, and this effect is blunted in obesity or after short-term nutrient excess. The change in effect of PVAT on microvascular function is mediated by decreased secretion of adiponectin, and is caused by the infiltration of bone marrow derived cells. The results presented in this thesis warrant further research to explore the contribution of PVAT to blunted insulin-induced microvascular recruitment.