Summary and general discussion
Summary

In the present thesis we have investigated whether there is a role for microvascular dysfunction as a potential pathophysiological mechanism in linking obesity with insulin resistance and hypertension, all components of the metabolic syndrome and important cardiovascular risk factors. Defects in muscle microvascular function may contribute to insulin resistance through impaired availability of glucose and insulin to peripheral muscle cells and to elevated blood pressure through an increase in peripheral vascular resistance. In addition, insulin resistance and hypertension develop in situations of elevated cortisol levels. In this thesis we have studied whether associations between cortisol and these components of the metabolic syndrome may be, at least in part, explained by microvascular dysfunction. Finally, microvascular defects in the kidney are thought to play a role in the initiation and maintenance of salt sensitivity of blood pressure through the induction of renal vasoconstriction and interstitial inflammation. Salt sensitivity of blood pressure is a cardiovascular risk factor per se and is also closely related to insulin resistance and hypertension. Microvascular dysfunction not confined to a single organ may underlie these latter relationships. A better understanding of the role of microvascular function in associations between cardiovascular risk factors mentioned above may initiate further studies after microvascular function as a preventive or therapeutic target.

Several animal and human studies have suggested that insulin redirects blood flow from non-nutritive vessels to nutritive capillary beds, thereby enhancing the access of insulin and glucose to peripheral muscle cells. Insulin-mediated effects on microvascular perfusion have never been studied directly by intramuscular measurements in humans. In chapter 2, we showed that insulin affected human intramuscular microvascular perfusion as measured directly with the laser Doppler technique. Systemic hyperinsulinaemia increased two different measures of intramuscular microvascular perfusion: reactive hyperaemia after arterial occlusion and vasomotion. First, hyperinsulinaemia increased the maximal reactive response in intramuscular laser Doppler perfusion following arterial occlusion and reduced the time needed to reach this maximal response. Second, hyperinsulinaemia enhanced intramuscular microvascular vasomotion, in a way indicative of an increase in the contribution of endothelial and neurogenic activity. Also, we confirmed previous findings of stimulatory effects of insulin on total limb blood flow and skin microvascular perfusion. Taken together, these data show that the vascular effects of insulin in humans are not limited to total blood flow and the skin microvascular perfusion, but extend to microvascular perfusion in the muscle.

Obesity is associated with an increased risk of developing insulin resistance, hypertension and microangiopathy. We hypothesised that obesity is associated with microvascular dysfunction and that this contributes to the development of these obesity-associated disorders. In chapter 3, we demonstrated that obesity in humans is indeed characterised by defects in skin microvascular function, i.e. postocclusive capillary recruitment and endothelium-dependent vasodilation. Also, impairment of these measures of microvascular function was associated with elevated blood pressure and insulin resistance in both lean and obese women together. In addition, obesity-associated microvascular defects could be shown not only at resting conditions but also during
systemic hyperinsulinaemia. Furthermore, in chapter 4, we described that local and direct insulin-induced vasodilatory effects on the skin microvasculature are impaired in obesity. In the same chapter, we demonstrated obesity-associated defects in microvascular vasomotion. More precisely, obesity was characterised by reduced overall microvascular vasomotion in a way that is indicative of altered endothelial and neurogenic activity. Thus, our data demonstrate that obesity is clearly associated with defects in microvascular function. In addition, obesity is characterised not only by reduced peripheral glucose uptake, i.e. metabolic insulin resistance, but also by impaired insulin-induced microvascular effects, so-called microvascular insulin resistance. These phenomena may be functionally coupled, because the latter may limit availability of glucose and insulin to muscle cells and, consequently, may explain part of the obesity-associated reduction in peripheral glucose uptake. Also, obesity-associated defects in microvascular function may contribute to the development of hypertension and disease entities that are wholly or in part caused by microangiopathy, notably retinopathy, nephropathy and heart failure.

Central adiposity is associated with cardiovascular risk independently of overall adiposity. Part of this association may be explained by enhanced secretion of cytokines by visceral adipocytes resulting in a general proinflammatory state. Microvascular dysfunction may link specifically central adiposity with cardiovascular risk factors, such as insulin resistance and hypertension. In chapter 5, we showed that in adults visceral adiposity as measured with MRI was inversely associated with capillary recruitment, independently of total body adiposity. Also, in both adults and children, truncal subcutaneous adipose tissue using skinfold measurements was inversely related to capillary recruitment. Furthermore, in adults, the association between visceral adiposity and capillary recruitment could, at least statistically, be partly explained by a composite inflammatory score, containing information about plasma C-reactive protein, interleukin-6 and tumour necrosis factor-α. These data in adults suggest a role for visceral adiposity and its associated proinflammatory state in capillary perfusion. Also, our findings in children and adults suggest that not only visceral but also truncal subcutaneous adiposity is detrimental for capillary perfusion and that this process may start before puberty. These observations underline the necessity for interventions in childhood to prevent truncal obesity and its associated cardiovascular risk.

Obese individuals are exposed to daylong increased systemic FFA concentrations. Previous studies have shown that elevation of FFA concentrations resulted in insulin resistance and elevated blood pressure whereas lowering of FFA concentrations increased insulin sensitivity. Therefore, FFA-induced modulation of microvascular function may contribute to obesity-associated insulin resistance, hypertension and microangiopathy. In chapter 6, we demonstrated that in lean women, acute elevation of FFA levels impaired microvascular function, i.e. postocclusive capillary recruitment and endothelium-dependent vasodilation, both in the basal state and during hyperinsulinaemia. In parallel, overnight lowering of FFA levels in obese women improved capillary recruitment both in the basal state and during hyperinsulinaemia. This suggests that elevated FFA levels in obesity may contribute to microvascular dysfunction. Furthermore, in lean and obese women, changes in FFA levels were inversely associated with changes in capillary recruitment and insulin-mediated glucose uptake. Moreover, in lean and obese women, these changes in microvascular function could statistically explain
approximately 29% of the association between changes in FFA levels and changes in insulin-mediated glucose uptake. These data further support functional coupling between insulin’s stimulatory microvascular and metabolic effects by concomitant changes in both effects during FFA elevation and lowering. Furthermore, they underline the importance of microvascular dysfunction as a partial explanation for FFA-induced insulin resistance in obesity.

Situations of endogenous and exogenous cortisol access are characterised by elevation of blood pressure and insulin resistance. Cortisol-related impairments of microvascular function may explain part of the cortisol-induced hypertension and insulin resistance. In chapter 7, we provided evidence of an inverse association between urinary cortisol and skin capillary recruitment in healthy women, but not in men. Furthermore, linear regression analyses showed that, in women, capillary recruitment explains approximately 37% of the cortisol-blood pressure relationship. These findings in healthy women suggest that interventions directed at preservation or improvement of microvascular function may be worthwhile in women in whom cortisol excess is to be expected.

Microvascular injury to the kidney is thought to play a role in the initiation and maintenance of salt sensitivity of blood pressure. Therefore, generalised microvascular dysfunction may contribute to the development of salt sensitivity, insulin resistance and hypertension, and may thus link these cardiovascular risk factors. In chapter 8, we demonstrated a strong inverse linear association between capillary recruitment and endothelium-dependent vasodilation in the skin microvasculature on the one hand and salt sensitivity of blood pressure on the other hand. Also, we confirmed previous findings that salt sensitivity of blood pressure is inversely associated with insulin sensitivity and positively associated with blood pressure. More importantly, we demonstrated that the relationship of salt sensitivity with both insulin sensitivity and blood pressure is largely, at least statistically, dependent on microvascular function. These data are in agreement with a potential central role for generalised microvascular dysfunction as a link between salt sensitivity, insulin resistance and hypertension.
Discussion

Methodological considerations

Intramuscular laser Doppler measurements
Several techniques have been used in the past to measure human muscle blood flow, such as isotope clearance, venous occlusion plethysmography, and dye dilution or thermodilution [1]. These methods have the disadvantage that they measure segmental or total muscle blood flow, but do not give any information about blood flow distribution within the muscle vascular bed. Other methods such as positron emission tomography [2,3] and the recently introduced contrast enhanced ultrasound [4,5] are able to give us some information about microvascular blood flow distribution, because they are able to visualise microvascular blood volume in human skeletal muscle. However, these methods do not measure microvascular perfusion directly and continuously, and, therefore, do not allow analysis of microvascular vasomotion. In chapter 2, we used an implanted laser Doppler probe to measure effects of a systemic insulin infusion directly in the muscle. This technique has several advantages. First, the intramuscular laser Doppler probe measures the average perfusion in all arterioles, venules and capillaries of a certain measured volume of muscle tissue. Previous studies in rats have demonstrated that discrete regions of nutritive and non-nutritive blood flow could be identified by this method [6]. Therefore, changes in intramuscular laser Doppler perfusion may give us information on microvascular blood flow distribution independently of total blood flow [6]. Second, the intramuscular laser Doppler technique allows continuous registration of muscle perfusion and, thus, is able to measure dynamic changes in perfusion elicited by provocational manoeuvres or the administration of vasoactive substances [7-10]. Third, the laser Doppler technique can easily be applied simultaneously at different sites of a single tissue, such as several intramuscular probes, or microvascular perfusion in different tissues can be measured concomitantly, such as in muscle and skin [7,8]. This allows the comparison of relative changes in laser Doppler perfusion between different sites within one tissue or between different tissues. Finally, intramuscular laser Doppler measurements may give information about microvascular vasomotion and influences of vasoactive substances thereon by analysis of the contribution of different frequency intervals to the laser Doppler signal [11]. Nevertheless, our and previous experiences also reveal several limitations of the intramuscular laser Doppler technique. Table 1 demonstrates the calculated coefficients of variation derived from the data described in chapter 2. In agreement with previous studies [7,8], intramuscular laser Doppler perfusion at rest exhibited considerable variability on different days. Part of this is likely to be explained by a different probe position. Other factors, such as a different degree of tissue trauma after puncture, may also contribute. Therefore, comparison of laser Doppler perfusion at rest between different days with different probe positions is not feasible. The reproducibility of the relative increase in laser Doppler perfusion and the time needed to reach peak perfusion after arterial occlusion were acceptable between days and these measures may be calculated when comparisons between days (or different probe positions) are investigated. In contrast to our finding, a previous study demonstrated a
poor reproducibility of time to reach peak perfusion between different days [8], which may partly be explained by differences in laser Doppler probe design.

Table 1. Coefficients of variation of various laser Doppler measures during postocclusive hyperaemia before and after 2 hours of saline infusion and on two separate days

<table>
<thead>
<tr>
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<th>CV (%) after saline infusion (unchanged probe position)</th>
<th>CV (%) between days (different probe position)</th>
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<tr>
<td>Baseline perfusion</td>
<td>17.2 ± 10.9</td>
<td>30.8 ± 25.6</td>
</tr>
<tr>
<td>Peak perfusion</td>
<td>21.4 ± 15.2</td>
<td>22.7 ± 16.6</td>
</tr>
<tr>
<td>Absolute increase</td>
<td>24.4 ± 19.2</td>
<td>27.2 ± 18.0</td>
</tr>
<tr>
<td>Relative increase</td>
<td>18.6 ± 23.5</td>
<td>18.8 ± 21.7</td>
</tr>
<tr>
<td>Time to peak</td>
<td>11.4 ± 14.4</td>
<td>10.1 ± 10.9</td>
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Also, intramuscular laser Doppler measurements in conscious individuals are susceptible to movement artefacts. A slight change in angle or depth of the laser Doppler probe, for example due to (spontaneous) muscle fasciculations, induces a shift in the measured muscle volume and, thus, in the basal laser Doppler signal. These artefacts can be minimised by adequate fixation of the probe in the muscle and immobilization of the leg in a vacuum cushion. Finally, the introduction of a laser Doppler probe in muscle tissue may alter microvascular perfusion due to tissue trauma. Staxrud et al. [12] found that insertion of a laser Doppler probe in the skin induced a significant vasodilatory response as measured by a laser Doppler probe placed on the skin surface above the puncture site. One hour after puncture, skin laser Doppler flow was still markedly elevated compared with baseline. To our knowledge, the contribution of puncture trauma to intramuscular laser Doppler flow has never been studied and the variation in vasodilatory response due to muscle puncture is not known. In chapter 2, we started the laser Doppler measurements 45 minutes after muscle puncture, because within 30 minutes stable laser Doppler readings had been achieved. This suggests, but does not prove, that the vasodilatory response due to the introduction of the probe in the muscle faded away within 30 minutes. The true contribution and duration of muscle puncture to the intramuscular laser Doppler signal remains to be studied. Taken together, our and previous studies demonstrate that the intramuscular laser Doppler technique can be applied for measurement of intramuscular microvascular perfusion if restrictions due to limited reproducibility, movements artefacts and influences of muscle puncture are taken into account.

Skin microvascular measurements in obesity

In the present thesis endothelium-dependent and endothelium-independent vasodilation of the microcirculation were determined by iontophoretic application of acetylcholine and sodium nitroprusside in combination with laser Doppler flowmetry. In addition, skin capillary recruitment after arterial occlusion, which is an estimate of the functional capillary reserve available, was measured by direct intravital capillary microscopy. Measures of obesity may be related to characteristics of the skin, such as of stratum corneum thickness and dermal capillary depth [13]. These skin characteristics may interfere with the microvascular measurements we performed in the present thesis in several ways.
In obesity, the visibility of skin capillaries with direct microscopy may be influenced by alterations in the depth at which they are located or the composition of the above lying skin. In that case, one would expect that capillary density in the resting state and after arterial occlusion are equally affected. In chapter 3, capillary recruitment after arterial occlusion was markedly impaired in obesity. Capillary density in the resting state, however, was similar in obese and lean women. Also, the depth at which the microvascular vessels are located and the composition of the above lying skin may be hypothesised to interfere with laser Doppler measurements. The laser beam has to penetrate the skin and a fraction of the light is scattered back by moving red blood cells. The frequency shift in the returned signal is proportional to tissue perfusion. If penetration of the skin is more difficult and/or blood vessels would be situated at a greater depth in skin of obese compared with lean individuals, the basal signal would be reduced. However, in chapter 3 and in several other studies, it has been demonstrated that lean and obese individuals do not differ in basal laser Doppler skin perfusion [14,15]. Thus, these data demonstrate that obesity-associated alterations of skin anatomy are not likely to have interfered with measurements of postocclusive capillary recruitment and laser Doppler perfusion.

In order to estimate microvascular endothelium-dependent and endothelium-independent vasodilation, acetylcholine and sodium nitroprusside were administered to the skin microvasculature by anodal and cathodal iontophoresis, respectively. Also, the direct microvascular effects of insulin administered by cathodal iontophoresis were studied in lean and obese women. Iontophoresis is a non-invasive method of introducing charged substances across the skin by means of a small electric current. It is based on the fact that when an electrical potential difference is applied to a solution, solute ions will migrate towards an electrode of opposite charge. A major disadvantage of the method of iontophoresis is that the amount of drug delivered at the site of the microvasculature in the skin cannot be measured directly. The amount of drug delivered through skin may differ between lean and obese individuals. However, both groups demonstrated comparable microvascular vasodilatory responses to cathodal iontophoresis of sodium nitroprusside, a direct nitric oxide donor. This suggests, but does not prove, that the amount of sodium nitroprusside delivered to the level of the microvasculature is similar in obese and lean individuals. Measurements of the drug concentration remaining at the application site after the iontophoresis procedure or, even more precise but also more invasive, direct measurement of the drug concentration at the level of the microvasculature through micropuncture studies could further elucidate this issue.

Non-specific reactions during iontophoresis
During iontophoresis procedures part of the measured vascular response may be elicited by non-specific effects of the employed current or the drug vehicle [16-18]. During anodal iontophoresis these non-specific effects are weak, but several studies report substantial effects during cathodal iontophoresis [16-18]. Previously, we showed that both anodal iontophoresis of the vehicle of acetylcholine and cathodal iontophoresis of the vehicle of sodium-nitroprusside elicited small but significant responses [19]. In addition, we demonstrated that systemic hyperinsulinaemia did not influence these responses [20]. We confirmed these findings in lean and obese women together in chapter 2. Insulin infusion
did not affect the responses to the acetylcholine-vehicle (median (interquartile range), before vs. during insulin infusion, 67 (25–174) vs. 95 (34–163)%, P=0.8; tested in 3 obese and 11 lean women) or the sodium-nitroprusside-vehicle (40 (-16–139) vs. 67 (16–171)%, P=1.0; tested in 6 obese and 10 lean women). Unfortunately, the number of, in particular obese, participants in these extra studies was too small to determine whether responses differed between lean and obese women. In chapter 4, we demonstrated that skin vasodilatory responses induced by cathodal iontophoresis of the vehicle of insulin did not differ significantly between 40 lean and 40 obese women (Figure 1 chapter 4; ANOVA for repeated measures F=0.73 and P=0.4 obese vs. lean women). Remarkably, the microvascular vasodilation induced by cathodal iontophoresis was considerable in both lean and obese women. Therefore, in this chapter, additional analyses with correction for the responses to cathodal iontophoresis of the vehicle of insulin were performed. Thus, our current and previous data suggest that iontophoresis of vehicles can induce a significant and considerable microvascular vasodilatory response. As a consequence, in my opinion, microvascular responses to iontophoresis of vehicles should be measured and, if necessary, taken into account when evaluating microvascular effects of vasoactive substances administered through the iontophoresis procedure.

Influence of gender on (associations with) microvascular function

Previous studies have shown gender differences in skin blood flow responses to provocational manoeuvres [21,22] and in endothelial nitric oxide production in skin microvasculature [23]. In these studies, women are characterised by higher skin microvascular reactivity than men. In the present thesis we used different ways to deal with possible gender differences. In chapter 3, 4 and 6 of the present thesis, we studied only one gender. We studied healthy women instead of men because obesity-associated or FFA-induced impairments are more likely to be detected in a study population that is characterised by a non-compromised microvascular function at baseline. Evidently, caution should be taken in extrapolating these findings in women to men. In chapter 5 and 8, relationships were adjusted for gender differences by statistical analyses. Regression analyses demonstrated that results in these chapters and in chapter 2 did not differ significantly between men and women. In chapter 7, the results were analysed and presented for men and women separately, because there were significant differences in associations of urinary cortisol with capillary recruitment and other variables between both genders. In general, in studies including microvascular function a possible gender difference should always be considered.

Measurement of free fatty acids

Previous studies showed that ongoing in-vitro lipolysis during Intralipid and heparin infusion can produce falsely high plasma FFA concentrations [24,25]. In chapter 6 of the present thesis we used several methods to prevent in-vitro lipolysis in blood samples taken during Intralipid and heparin infusion in the lean women. First, previous studies showed that in samples frozen immediately after collection, FFA concentrations were significantly lower than in samples incubated on ice or at room temperature [24,25]. Therefore, prechilled tubes were used to collect blood samples, plasma was instantly separated from cells by centrifugation (4°C), plasma was frozen immediately (-20 °C) and
transported to a -80 °C freezer within 24 hours. Second, the addition of lipoprotein lipase inhibitors (tetrahydrolipostatin (THL) or paraoxon) to blood samples has been shown to significantly reduce in-vitro lipolysis during Intralipid and heparin infusion [24,25]. We collected blood samples with and without THL during Intralipid and heparin infusion, and the control study. Measurement of plasma FFAs after 3 hours Intralipid and heparin infusion with addition of THL resulted in concentrations that were approximately half of the concentrations measured without addition of THL (Table 2). Also, with concomitant insulin infusion plasma FFA concentrations measured with addition of THL further decreased compared with plasma FFA concentrations measured without THL. During the control study plasma FFA concentrations were not different between measurements with and without THL. Furthermore, addition of THL to the test tubes reduced the amount of variation in FFA measurements as can be seen from the reduction in the standard deviation in Table 2. Given these results, it can be concluded that in vitro lipolysis considerably contributes to measured plasma FFA concentrations and variation therein during Intralipid and heparin infusion. Consequently, in studies applying Intralipid and heparin infusion adequate measures should be taken to limit in vitro lipolysis and its contribution to measurement of plasma FFAs.

Table 2. Plasma FFA concentrations in blood samples with and without THL before and during Intralipid and heparin infusion (FFA elevation) or saline infusion (control study)

<table>
<thead>
<tr>
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<th>FFA (mmol/l)</th>
<th>FFA + THL (mmol/l)</th>
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<tbody>
<tr>
<td><strong>FFA elevation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal state</td>
<td>0.55±0.16</td>
<td>0.52±0.17</td>
</tr>
<tr>
<td>Intralipid infusion</td>
<td>1.84±0.76</td>
<td>0.91±0.19*</td>
</tr>
<tr>
<td>Intralipid and insulin infusion</td>
<td>1.47±0.94</td>
<td>0.40±0.13*</td>
</tr>
<tr>
<td><strong>Control study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal state</td>
<td>0.55±0.16</td>
<td>0.55±0.19</td>
</tr>
<tr>
<td>Saline infusion</td>
<td>0.57±0.21</td>
<td>0.58±0.22</td>
</tr>
<tr>
<td>Saline and insulin infusion</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
</tr>
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*P<0.01 vs. FFA without THL

Measurement of cortisol
Urinary cortisol excretion in a 24-hour period is a highly reliable index of cortisol status and is considered the gold standard method for the diagnosis of Cushing’s disease [26,27]. It provides an integrated measure of cortisol through 24 hours and therefore is less susceptible to short-term fluctuations than single plasma or salivary measurements. In chapter 7, 24-hour urine collections were used to estimate of the amount of circulating biologically active cortisol. However, 24-hour urine collections are troublesome and can be inaccurate due to collecting errors [28,29]. In order to minimise sampling errors, in chapter 7, all participants were provided with collection bottles and clear instructions and were questioned afterwards about their compliance. Nevertheless, the mean of multiple 24-hour urine collections would probably have been a more precise measure of urinary cortisol excretion than a single measurement, because it allows correction for collection errors and daily variability [30]. Also, there is uncertainty whether urinary cortisol can best be expressed in term of cortisol/creatinine ratio or total cortisol excretion [31,32]. Correction
for the amount of creatinine excreted corrects for urine collection errors. However, it is
dependent on diet and muscle mass and therefore is likely to underestimate true urinary
cortisol excretion in men compared with women. The results in chapter 7 were not
influenced by correction for urinary creatinine excretion.

Measurement of insulin sensitivity
The hyperinsulinaemic clamp technique is generally accepted as the best available
method to assess insulin-mediated peripheral glucose uptake [33]. During a
hyperinsulinaemic clamp exogenous insulin is infused in a continuous manner with a
constant rate to reach and maintain a hyperinsulinaemic plateau. Simultaneously, the
sugar concentration is clamped by a variable exogenous glucose infusion at either the
fasting or a predetermined glucose concentration (5.0 mmol/l). In chapter 3, 6, 7 and 8,
the hyperinsulinaemic clamp method was used to assess peripheral glucose uptake. In
addition, in chapter 2, 3 and 6, the same method was applied to induce systemic
hyperinsulinaemia in order to examine effects of insulin on skin and muscle microvascular
function. We clamped at a glucose concentration of 5.0 mmol/l, which resulted in slightly
higher glucose levels during the hyperinsulinaemic clamp than during control studies. This
difference in glucose concentrations may have influenced our findings of insulin-mediated
microvascular effects in muscle and skin as described in chapter 2, 3 and 6. However,
because hyperglycaemia impairs microvascular function [34,35], insulin-mediated effects
on microvascular function, if anything, may have been somewhat underestimated.

During the hyperinsulinaemic clamp, normally, endogenous glucose production
(EGP) is effectively suppressed, so that the exogenous glucose infusion rate equals the
amount of glucose disposed of by all tissues in the body and thus provides a quantitation
of overall insulin sensitivity [33]. When the hyperinsulinaemic clamp is combined with lipid
and heparin infusion, the endogenous glucose production may not be completely
suppressed. Under euglycaemic, hyperinsulinaemic conditions, lipid and heparin infusion
either had no effect on EGP [36] or partially impaired the insulin-mediated decrease in
EGP [37-39]. In our study we did not measure effects of the lipid and heparin infusion on
EGP. Therefore, we cannot exclude that our calculation of insulin sensitivity from the
exogenous glucose infusion rate during lipid and heparin infusion lead to a slight
underestimation of true peripheral glucose uptake.

Overall interpretation of main findings and future perspectives

Intramuscular microvascular effects of insulin
In the past years, Clark et al. have elegantly demonstrated in rat studies that insulin
modifies microvascular perfusion redirecting blood flow from non-nutritive vessels to
nutritive capillary beds, thereby enhancing the access of insulin and glucose to peripheral
muscle cells [40-43]. Also, they recently demonstrated, in collaboration with the research
group of Barrett, a similar insulin-induced increase in capillary perfusion in humans with
the method of contrast enhanced ultrasound [4]. In parallel, we previously demonstrated in
human skin that systemic hyperinsulinaemia increases capillary recruitment after arterial
occlusion as visualised by videomicroscopy [20]. In addition, hyperinsulinaemia enhanced
the contribution of local endothelial activity to skin microvascular vasomotion [20]. In
chapter 2, we demonstrated that insulin augments the intramuscular microvascular response to reactive hyperaemia after arterial occlusion as measured directly with an intramuscular laser Doppler probe. In the same chapter, we showed that systemic hyperinsulinaemia augments intramuscular vasomotion in a way that is indicative of an increased contribution of both endothelial and neurogenic activity. These findings support an effect of systemic hyperinsulinaemia on microvascular perfusion measured directly in the human muscle. Whether these findings have direct implications for insulin-induced muscle glucose uptake was not examined in the present study. It is of interest to study whether these effects of insulin on muscle microvascular perfusion and vasomotion as measured with the intramuscular laser Doppler probe are impaired in insulin-resistant states such as hypertension and obesity. Indeed, if this were the case, this finding would support the hypothesis that these effects on muscle laser Doppler perfusion play a role in the availability of insulin and glucose to peripheral muscle cells and, thus, insulin-mediated glucose uptake. In addition, direct comparison of our skin and intramuscular methods to measure microvascular perfusion with other techniques, such as contrast enhanced ultrasound, is desirable to identify whether these different methods measure similar characteristics of the microvasculature.

In contrast to studies performed in (unconscious) rats with the laser Doppler technique [41] and in humans with contrast enhanced ultrasound [4], insulin did not influence the average human muscle microvascular perfusion at rest. Several explanations can be responsible for this difference. The study in chapter 2 was not designed, and thus may have been underpowered, to study effects of insulin on baseline microvascular perfusion. The limited power of the study may have resulted both of a restricted number of participants and of the considerable variation in laser Doppler perfusion at rest probably largely caused by movement artefacts. Also, the volume of muscle tissue measured by the intramuscular laser Doppler probe may have contained both nutritive and non-nutritive vessels and in that case, a shift of blood flow between these vessels may not have been detected. Improvement of the intramuscular laser Doppler technique by optimal immobilisation and fixation and a reduction in the measured muscle volume by the use of differently designed laser Doppler probes may resolve part of these issues.

Studies in isolated rat arterioles have shown that effects of insulin on the microvasculature consist of both vasodilation and vasoconstriction mediated by nitric oxide (NO) and endothelin (ET), respectively [44]. In rats in vivo, blockade of endothelial NO production by concomitant infusion of N(G)-nitro-L-arginine-methyl-ester (L-NAME) completely blocked the effect of insulin on intramuscular microvascular recruitment as measured with contrast enhanced ultrasound [45]. In addition, the insulin-induced microvascular recruitment was completely abolished by ET infusion [46]. In humans, insulin has been shown to stimulate both NO and ET activity in the forearm vasculature [47]. Also, insulin-resistant obese individuals are characterised by impaired effects of insulin on endothelial NO production [48,49] and increased endogenous endothelin action as measured by changes in total limb blood flow [50-52]. To our knowledge, the contribution of NO and ET to (insulin-induced) intramuscular microvascular perfusion and vasomotion have hitherto not been investigated in humans. Studies applying intra-arterial infusion of NO-blockers, such as L-NAME, and ET-receptor blockers both with and without
insulin and concomitant microvascular measurements may clarify these issues. In addition, prostaglandins have been shown to function as an intermediate in insulin-induced vasodilation at the level of the resistance vessel [53]. Addition of cyclo-oxygenase inhibitors, such as aspirin or indomethacin, to the abovementioned experiments could lead to additional information regarding the role of prostaglandins in (insulin-induced) intramuscular microvascular perfusion. Elucidation of the pathways contributing to defects in insulin-induced microvascular recruitment may be an important step in the development of new targets in the prevention or treatment of microvascular dysfunction and its consequent reduction in insulin-induced glucose uptake.

**Obesity and microvascular dysfunction**

In chapter 3 of the present thesis, we showed that obesity is characterised by impaired microvascular function, i.e. postocclusive capillary recruitment and microvascular endothelium-dependent vasodilation. Also, both these measures of microvascular function were positively associated with insulin-mediated glucose uptake and negatively with blood pressure in lean and obese women together. These findings are in agreement with the hypothesis that obesity-related defects in microvascular function may contribute to obesity-associated insulin-resistance, hypertension and microangiopathy. In addition, the obesity-associated defects in microvascular function were present not only in the basal state but also during hyperinsulinaemia. Moreover, the direct and local vasodilatory effects of insulin on the microvasculature as demonstrated in lean individuals [20,54] were abolished in obesity. These impaired vasodilatory effects of insulin on the microvasculature may be called *microvascular* insulin resistance. This obesity-associated microvascular insulin resistance further supports a role for diminished insulin-induced microvascular vasodilatory effects in impaired insulin-mediated glucose uptake. Finally, total microvascular vasomotion was diminished in obesity in a way that is suggestive of a decreased contribution of endothelial and neurogenic activity. Remarkably, insulin was capable of stimulating microvascular vasomotion in the muscle of lean individuals specifically in those frequency-intervals that were impaired in obesity. Taken together, the data presented in this thesis demonstrate that obesity is associated with microvascular dysfunction and microvascular insulin resistance, which may contribute to, and thus be a potential therapeutic target in, impaired insulin-mediated glucose uptake. This hypothesis could be further strengthened by studies of insulin-mediated effects on microvascular function and vasomotion in other insulin-resistant states such as hypertension and the polycystic ovary syndrome. In addition, studies of the effects of insulin-sensitizing agents, such as metformin or peroxisome-proliferator-activated-receptors-agonists, on the microvasculature can support a role of microvascular defects in obesity-associated insulin resistance.

Several issues regarding this matter should be discussed. First, in our studies we included only healthy obese participants, i.e. non-smoking, non-diabetic and normotensive subjects without any vasoactive medication (except oral contraceptives). Nevertheless, these obese participants mirrored healthy obese individuals in the general population and thus were characterised by dyslipidaemia and slightly elevated blood pressure compared with the lean participants. It may be hypothesised that these differences in baseline characteristics between both populations
have contributed to differences in microvascular function. Multiple regression analyses in chapter 3 demonstrated that adjustment for dyslipidaemia did not materially affect the association between obesity and defects in postocclusive capillary recruitment or endothelium-dependent vasodilation. Additional multiple regression analyses of the data described in chapter 4 showed that differences in triglycerides do not explain the association between obesity and diminished microvascular vasomotion (change in beta less than 10% for all frequency intervals). Thus, dyslipidaemia is not likely to contribute to obesity-associated defects in the microvasculature. Also, analyses in chapter 3 showed that adjustment for systolic blood pressure did not change the association between obesity and impaired capillary recruitment, but reduced the association between obesity and impaired endothelium-dependent mediated vasodilation by 38%. In addition, systolic blood pressure could explain part of the association of obesity with reduced microvascular vasomotion. After adjustment for systolic blood pressure, the beta of the association between obesity and microvascular vasomotion decreased with 37%, 49% and 49% within the frequency intervals 0.01-0.02, 0.02-0.06 and 0.01-1.6 Hz, respectively. In order to specify the true contribution of systolic blood pressure to obesity-associated defects in microvascular function, microvascular characteristics should be measured in a group of lean and obese individuals matched for blood pressure. Nevertheless, our present data demonstrate that, statistically, a major part of the obesity-associated defects in microvascular function is not dependent on dyslipidaemia or blood pressure per se.

Second, the cross-sectional design of most of our studies does not allow any conclusions regarding causality. Therefore, it cannot be excluded that an (unmeasured) variable contributed to both obesity and defects in the microvasculature. Although the relation between foetal growth and obesity in later life is a complicated one, there is some evidence that intrauterine malnutrition particularly during the first trimester is related to central adiposity in adult life [55-58]. Several mechanisms have been suggested to play a role in this foetal programming such as dysregulation of appetite control and hypersecretion of corticosteroids [55]. In addition, impaired foetal growth measured as low birth weight is associated with impaired microvascular function [59]. Intrauterine malnutrition may contribute to alterations in the microvasculature either directly through influences on angiogenesis or indirectly through, for example, stimulation of the hypothalamic-pituitary-adrenal axis [60]. Another possible candidate to explain associations between obesity and defects in the microvasculature is physical fitness. Evidently, the amount of physical activity and physical fitness are inversely related to, in particular central, obesity [61]. In addition, physical fitness has been closely associated with skin microvascular endothelium-dependent vasodilation in heart transplant recipients [62]. Also, athletes are characterised by higher responsiveness of the microvascular endothelium than control individuals [63]. Moreover, in healthy sedentary subjects, endothelium-dependent dilation in skin microvasculature is enhanced by moderate exercise training and reversed to the pretraining state with detraining [64]. Beside this effect on microvascular endothelial function, aerobic training increases structural capillary density in muscle in lean elderly men [65] and in obese women even without a change in total body weight [66,67]. Taken together, impaired foetal growth and physical fitness are candidates to function as a common antecedent for obesity and defects in the microvasculature. Unfortunately, in the studies presented in this thesis information about
these variables was lacking and effects on obesity and microvascular function could not be examined.

Third, in previous studies, central adiposity was inversely associated with the amount of oxidative, well-capillarised type I muscle fibers [68,69] and within type 1 muscle fibers obesity was related to a lower capillary density and capillary-to-fiber area ratio [70]. Also, after surgical weight loss intervention, there was a positive relationship between the percentage of excess weight loss and the percentage of the well-capillarised type I fibers in morbidly obese patients [69]. Studies of weight loss through diet and behavioral programs show ambiguous effects on muscle fiber type and capillary density [71,72]. The obesity-associated decreases in muscle capillary density may be a generalised feature and thus may also be present in human skin, although this remains to be confirmed by direct comparisons between muscle and skin measurements. In the present thesis we measured the relative increase in skin capillary density after arterial occlusion. This measure may be used to detect functional recruitment of previously non-perfused capillaries and is likely to be dependent on both functional and structural factors [73]. In light of the obesity-associated alterations in muscle capillary density mentioned above, it would be interesting to study to what extent obesity-associated capillary defects in the skin are of a functional or structural nature.

**Body fat distribution and microvascular dysfunction**

It is nowadays well-known that not all adipose tissue compartments contribute equally to cardiovascular risk. Apart from overall obesity, detrimental effects of truncal adipose tissue and protective effects of peripheral adipose tissue on several cardiovascular risk factors, such as diabetes and hypertension, have been demonstrated [74,75]. In chapter 5 we showed that in healthy adults truncal adipose tissue, i.e. visceral adipose tissue measured with MRI and truncal subcutaneous adipose tissue measured with skinfolds, is related to impaired postocclusive capillary recruitment independently of overall body fat. These capillary defects may contribute to the development of insulin resistance and hypertension associated with central obesity. However, prospective trials have to study the true contribution of these (truncal) obesity-associated defects in capillary function to these cardiovascular risk factors. In addition, weight intervention programs specifically designed to alter body fat distribution and bariatric surgery can give us further information on effects of changes in body fat distribution on microvascular function.

Determination of characteristics in childhood that contribute to risk of cardiovascular disease in adulthood allows early identification of a population with increased cardiovascular risk and the early start of preventive strategies. Our findings in healthy children showed that the association between truncal adiposity and impaired capillary recruitment is present before puberty. This indicates that the capillary defects associated with central adiposity may already be present in childhood and that these associations may play a role in the development of obesity-associated cardiovascular risk factors, such as insulin resistance and hypertension, in later life. The study of microvascular function in children with an increased risk to develop obesity, insulin resistance or hypertension may further support this hypothesis. Children with a predisposition to develop these cardiovascular risk factors may be identified either by a
positive family history or by the presence of a low birth weight. Also, longitudinal studies are necessary to investigate the true consequences of microvascular defects in childhood.

**Possible mechanisms explaining obesity-associated microvascular dysfunction**

Previous animal studies have shown a potential mechanistic role for FFAs in obesity-associated microvascular dysfunction and insulin-resistance [76]. We demonstrated a similar role for FFAs in humans in chapter 6. Beside a detrimental effect of FFA elevation on microvascular function and insulin-mediated glucose uptake, FFA lowering in obese women concomitantly enhanced postocclusive capillary recruitment and insulin sensitivity. Furthermore, the FFA-induced effects on microvascular function in lean and obese women occurred both in the basal state and during hyperinsulinaemia and statistically explained 29% of changes in insulin sensitivity. Thus, these data indicate that elevated FFA levels in obesity may indeed contribute to microvascular dysfunction and, consequently, may play a role in the development of obesity-related insulin resistance. Thus, interventions directed at minimisation of FFA levels in obesity may have beneficial effects on microvascular function and its associated insulin resistance. Short-term administration of the antilipolytic agent acipimox we used in our studies is well-known to reduce plasma FFA concentrations and improve insulin-mediated glucose uptake in patients with diabetes mellitus [77-79]. However, administration for longer than 4 weeks did not influence FFA concentrations or even resulted in rebound lipolysis and had inconsistent effects on insulin resistance [79-82]. Therefore, other ways should be used to verify that the effects of an overnight FFA reduction persist or even increase during long-term FFA reduction.

In previous studies we and others have demonstrated that systemic hyperinsulinaemia induced capillary recruitment [4,20]. Hyperinsulinaemia inhibits the action of hormone-sensitive lipase and the release of fatty acids from adipose tissue cells into the circulation leading to suppressed systemic FFA concentrations. In the study described in chapter 6 we showed that lowering of systemic FFA concentrations by acipimox in obese women improved postocclusive capillary recruitment. Thus, it cannot be excluded that part of the effects of systemic hyperinsulinaemia on capillary recruitment is an effect of the simultaneously reduced FFA concentrations. However, it is likely that the systemic hyperinsulinaemia is responsible for, at least, part of the microvascular alterations, because insulin applied locally to the microvasculature is capable of inducing microvascular recruitment without inducing systemic effects ([20,54] and chapter 4).

The studies presented in this thesis were not designed to investigate the mechanisms underlying the relationships between obesity, FFA elevation, impairment of microvascular function and reduced insulin-mediated glucose uptake. Our data support the hypothesis that an FFA-mediated impairment of microvascular function reduces the availability of insulin and glucose to muscle cells and consequently limit insulin-mediated glucose uptake in obesity. Alternatively, a mechanism inducing simultaneous FFA-mediated effects on microvascular function and glucose uptake may lie in pathways of insulin signalling that are shared between vascular endothelial cells and skeletal muscle. Specifically, phosphotidylinositol (PI) 3-kinase activity is necessary for insulin-induced effects on both glucose transport in skeletal muscle and nitric oxide production in vascular endothelium [44,76,83,84]. Indeed, FFA elevation blunts insulin-induced PI 3-kinase
activation in human muscle [85]. In obese Zucker rats, insulin-induced PI 3-kinase activation is reduced in endothelial cells and isolated arterioles [83]. Further studies of effects of FFAs on insulin-signalling pathways specifically in the microvasculature could shed light on this issue.

Beside FFAs, adipocytokines have been suggested to allow signalling between adipose tissue cells and the microvasculature. In Chapter 5, we showed that visceral adipose tissue is positively and capillary recruitment is negatively associated with circulating adipocytokines, such as TNF-α and IL-6, and that the association between visceral adipose tissue and impaired capillary recruitment can at least statistically be partly explained by concentrations of these circulating adipocytokines. At this point, it should be emphasised again that these data merely support but do not prove causal relationships between these variables. In order to gain more insight in the true contribution of each of these circulating cytokines to microvascular dysfunction, effects of stimulation or inhibition of these cytokines, such as by infusion of TNF-α, IL-6, or receptor blockers of these cytokines, should be examined. In addition, it is of interest to study effects of frequently used more general anti-inflammatory agents, such as aspirin and other cyclooxygenase inhibitors, on microvascular function. The mechanisms by which circulating adipocytokines may influence (micro)vascular function are not entirely clear. It has been suggested that defects in the cellular insulin-signalling pathways in the (micro)vasculature play a role. In macrovascular endothelial cells, TNF-α has been shown to impair insulin-mediated activation of endothelial NO synthase through inhibition of insulin-mediated activation of insulin receptor substrate-1, PI 3-kinase and Akt [86]. In isolated rat skeletal muscle resistance arteries, TNF-α impaired vasodilator but not vasoconstrictor effects of insulin through activation of intracellular enzyme c-Jun N-terminal kinase and impairment of insulin-mediated activation of Akt [87]. Further elucidation of mechanisms by which TNF-α and other adipocytokines influence insulin-mediated effects on microvascular function may eventually lead to new therapeutic targets.

The renin-angiotensin system (RAS) in the adipose tissue may constitute another pathway of signalling between adipose tissue and the microvasculature. All components of the RAS are expressed in human adipose tissue [88,89] and obesity is characterised by enhanced activity of the RAS both systemically and locally [90-92]. Effects of RAS activity on the microvasculature are complex and not entirely elucidated. In rat muscle, angiotensin II induces net vasoconstriction, but increases oxygen uptake indicating an increase in nutritive microvascular blood flow [93,94]. The capability of angiotensin II to redirect blood flow from non-nutritive to nutritive microvascular routes may contribute to the increase in insulin-mediated muscle glucose uptake. In humans, studies after the effect of angiotensin II on microvascular blood flow distribution have not been performed. Most studies using total limb blood flow as an estimate of muscle perfusion, have shown that systemic angiotensin II administration increases both muscle blood flow and insulin-mediated glucose uptake in healthy individuals despite a general vasoconstrictive effect measured as an increase in systemic pressure [95-98]. This seems in contrast with the general finding that blockade of the RAS by either angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) enhances insulin-mediated glucose uptake [99,100]. However, these studies are performed in populations characterised by the presence of diabetes or with a predisposition to develop diabetes. As compared with
angiotensin II effects in healthy men, in insulin-resistant states, such as hypertension and obesity, angiotensin II administration reduces insulin-mediated glucose uptake and decreases total limb blood flow [101,102]. These data suggest that RAS activation may have opposite effects in insulin-resistant states compared with healthy individuals. In order to shed light on these issues and, in particular, on the role of the microvasculature therein, it is of particular interest to investigate effects of angiotensin II administration, ACE inhibitors and ARBs, with and without concomitant insulin infusion on microvascular blood flow distribution both in healthy individuals and in insulin-resistant states.

Although the possible mechanisms contributing to obesity-associated microvascular defects mentioned above and in chapter 1 are described separately, a complex interplay between these mechanisms is more likely to play a role. Animal studies have shown that both FFAs and TNF-α induce an activation of the sympathetic nervous system [103,104]. In humans, elevation of FFAs enhances vascular reactivity to phenylephrine administered either systemically [105] or locally in dorsal hand veins [106]. In addition, elevation of FFA levels by lipid and heparin infusion caused endothelial dysfunction via RAS activation [107]. Also, angiotensin II has been recognised to induce a variety of pro-inflammatory events [108] and blockage of the RAS has potential beneficial effects on systemic inflammation [109]. Thus, FFA and cytokine concentrations, and activation of sympathetic nervous system and the RAS are interrelated and a combination of these mechanisms probably contributes to obesity-associated microvascular defects.

**Cortisol and microvascular dysfunction**

Cardiovascular disease is the major cause of morbidity and mortality in situations of cortisol excess [110]. The cardiovascular consequences of cortisol excess are multiple and include, among others, elevation of blood pressure, truncal obesity, insulin resistance and dyslipidaemia. Increases in total or regional peripheral resistance have been suggested as a primary mechanism in the cortisol-induced rise in blood pressure [111,112]. A major part of peripheral vascular resistance is determined at the level of the microvasculature [113]. In chapter 7, we demonstrated that urinary cortisol excretion is inversely associated with capillary recruitment in women, but not in men. Also, capillary recruitment after arterial occlusion statistically explained part of the association between urinary cortisol excretion and blood pressure in women. This is in agreement with, but no direct evidence for, a pathophysiological role of these defects in the microvasculature in cortisol-induced hypertension in women. Nevertheless, it warrants longitudinal studies of relationships between defects in capillary perfusion and blood pressure. It would be of interest to examine these relationships before, during and after therapy with different dosages of corticosteroids and during and after situations of endogenous cortisol excess such as Cushing’s syndrome.

An explanation for the apparent gender difference in these results is not clear-cut, but may be sought in gender differences in 11β-hydroxysteroid dehydrogenase (11β-HSD) activity. The net interconversion of inactive cortisone to active cortisol by 11β-HSD type 2 may determine local tissue exposure to glucocorticoid action. The vascular wall is a target tissue for glucocorticoids [114]. 11β-HSD type 2 expression and enzyme activity have been demonstrated in vascular endothelial and smooth muscle cells [114-117]. Gender differences in local activity have been demonstrated in other tissues and, thus,
may also play a role in local microvascular activity [118,119]. Therefore, it is of interest to examine whether 11β-HSD type 2 is expressed in the microvasculature and whether there are gender differences in this expression.

The origin of the relationship between cortisol and characteristics of the microvasculature, which we found in women, may lie in prenatal life. Increased activity of the hypothalamic-pituitary-adrenal axis and consequent overexposure to endogenous corticosteroids during foetal life has been hypothesised to link low birth weight with elevated blood pressure and insulin resistance in later life [60]. In a variety of animal models, prenatal exogenous glucocorticoid exposure or an impairment of the placental barrier to maternal glucocorticoids, reduces birth weight and induces permanent elevated blood pressure, hyperglycaemia, and increased hypothalamic-pituitary-adrenal axis activity [120]. In humans, increased foetal exposure to cortisol due to reduced placental 11β-HSD type 2 activity or 11β-HSD type 2 deficiency is related to low birth weight [121]. In addition, low birth weight is associated with higher plasma cortisol concentrations in adulthood and these, in turn, are associated with elevated blood pressure [122]. Also, low birth weight is related to impaired microvascular function and elevated blood pressure in later life [59]. Previous studies in isolated cells and vessels have shown that corticosteroids may impair angiogenesis [123-125] and that corticosteroids are able to disrupt microvascular architecture, leading to loss of functional capillaries [124]. Through these mechanisms, foetal glucocorticoid exposure may link low birth weight with alterations of microvascular structure and function. Obviously, more research is necessary to disentangle the relationships among microvascular characteristics, birth weight, cortisol, blood pressure and insulin sensitivity.

Salt sensitivity and microvascular function
Lately, there has been a renewed interest in the hypothesis that subtle renal microvascular defects initiate renal arteriolar vasoconstriction and interstitial inflammation and, consequently, lead to increased salt sensitivity of blood pressure [126,127]. Previously, salt sensitivity of blood pressure has been shown to be closely associated with the presence of hypertension and insulin resistance [128-134]. We demonstrated in chapter 8, in both hypertensive and normotensive individuals, that salt sensitivity of blood pressure is inversely and closely associated with microvascular function in the skin. Furthermore, skin microvascular function, particularly postocclusive capillary recruitment, statistically largely explained relationships between salt sensitivity on the one hand, and elevated blood pressure and insulin resistance on the other hand. These data are in agreement with a potential central role for general microvascular dysfunction as a link between salt sensitivity, insulin resistance and hypertension. Prospective studies are required to further explore the role of microvascular dysfunction as a link between these cardiovascular risk factors. It should be kept in mind that even the presence of temporal relationships does not proof causality. Although animal studies have supplied supportive evidence for a causal relationship between renal microvascular defects and the development of salt sensitivity [126,135,136], this evidence is hard to obtain in humans partly due to the invasive nature of kidney biopsies. This problem can be largely avoided by measuring microvascular function in other, more easily accessible, organs, such as
skin or muscle, although direct comparisons of microvascular characteristics between the kidney and these other organs remain to be done.

Hyperuricaemia has been proposed as an initiator of renal microvascular injury and the consequent development of salt sensitivity of blood pressure and hypertension [127]. Experimental hyperuricaemia in rats results in blood-pressure-independent renal arteriolar disease and the development of hypertension. Both these effects could be prevented by concomitant allopurinol administration [137,138]. In human epidemiological studies, one of the best predictors for the development of hypertension is hyperuricaemia [139-142]. Also, an open label pilot study demonstrated potential blood pressure lowering effects of treatment with allopurinol in adolescents [143]. In view of these data, it is of interest to investigate in humans whether uric acid levels are associated with generalised microvascular defects and whether this association explains part of the relationship between uric acid and blood pressure elevation. In addition, in search for preventative strategies, a double-blind, placebo controlled study of the effects of uric acid lowering agents on microvascular characteristics and the development of elevated blood pressure is of importance. Uric acid levels have also been hypothesised to link low birth weight with the development of hypertension in later life [143,144]. In children, low birth weight is associated with increased uric acid levels and these, in turn, are related to elevated blood pressure [143,144]. These associations may be caused by a reduced number of nephrons leading to a relative hyperfiltration of the glomeruli and a consequently increased tubular uric acid and sodium reabsorption [145]. It also seems likely that genetic and/or environmental mechanisms contribute, because mothers of low birth weight infants are often characterised by elevated uric acid levels themselves [146,147]. Alternatively, uric acid is able to modulate endothelium-dependent vasodilation in resistance vessels [148-150] and, therefore, a direct effect on (micro)vascular function may also explain part of relationship between low birth weight and elevated blood pressure. Obviously, more work is needed to determine whether characteristics of the microvasculature play a pathophysiological role in the associations of low birth weight and uric acid with hypertension.

Other possible candidates in the initiation of renal (and generalised) microvascular injury are overactivity of both the sympathetic nervous system (SNS) or the renin-angiotensin system (RAS). Indeed, in rats administration of catecholamines induced permanent renal microvascular injury and the development of salt sensitivity of blood pressure [151]. In humans, there is also evidence that activity of the SNS contributes to the development of salt sensitivity of blood pressure [152]. RAS activation influences salt sensitivity of blood pressure directly through decreased pressure natriuresis and increased tubular sodium reabsorption [153]. In addition, angiotensin II infusion in rats is capable of inducing focal microvascular and interstitial injury to the kidney resulting in permanent salt sensitivity of blood pressure [154,155]. Human studies have suggested that sympathetic nerve activity is increased in adults with low birth weight, particularly in those without catch-up growth [156-158]. In addition, animal studies provide support that an adverse environment in utero may be linked to inappropriate activation of RAS leading to enhanced sodium reabsorption in adulthood [159]. Thus, both the RAS and SNS are likely to play a role in linking low birth weight with (salt sensitivity of) blood pressure.
Whether microvascular defects constitute a pathophysiological pathway in these relationships remains to be established.

**Final conclusions**

The studies described in the present thesis demonstrated a close association between obesity and microvascular dysfunction. In addition, we showed that the vasodilatory effects of insulin on the microvasculature are impaired in obesity, so-called microvascular insulin resistance. Furthermore, our data suggest a role for microvascular function as a link between obesity and several other components of the metabolic syndrome, namely insulin resistance and hypertension, as depicted in Figure 1. In addition, our studies showed associations of microvascular dysfunction with two other cardiovascular risk factors, i.e. salt sensitivity of blood pressure and elevated urinary cortisol excretion rates, although the latter finding occurs in women only. Moreover, microvascular dysfunction also explained part of associations of these cardiovascular risk factors with hypertension and insulin resistance. These data are in line, but do not prove, a pathophysiological role for microvascular defects in the development of salt sensitivity, hypertension and insulin resistance. The abovementioned relationships are complex and other factors than microvascular function are also likely to contribute. Nevertheless, microvascular dysfunction may identify populations characterised by or at risk for the development of these cardiovascular risk factors. Moreover, interventions targeted at amelioration or prevention of microvascular defects may prevent the development of these cardiovascular risk factors.

**Figure 1.** The relationships between microvascular dysfunction and several cardiovascular risk factors. The solid arrows represent the relationships investigated in the present thesis and the striped arrows are postulated relationships which, among others, may be interesting to study in future research. RAS, renin-angiotensin system; SNS, sympathetic nervous system.
References


General discussion


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