Chapter 7

Main findings and general discussion
Due to the unfolding obesity epidemic there is a steep rise in the number of patients with the metabolic syndrome. The metabolic syndrome is a clustering of abdominal obesity, hypertension, glucose intolerance and dyslipidemia which increases risk of cardiovascular disease and type 2 diabetes [1]. Microvascular dysfunction provides a pathophysiological mechanism linking the components of the metabolic syndrome to end-organ damage and is proposed as a potential therapeutic target to combat the metabolic syndrome (2,3). The aims of this thesis were twofold:

1. To study the role of microvascular dysfunction in the development of hypertension and end-organ damage.
2. To study whether red wine polyphenols (RWPs), by improving microvascular function, could be beneficial to obesity associated insulin resistance, hypertension and/or dyslipidemia.

The main findings of these studies were the following:

In Chapter 2, we studied whether insulin regulates systemic vascular resistance (SVR) in physiological circumstances, as increased SVR plays a major role in the pathogenesis of hypertension. In this study, we showed that both (exogenous) hyperinsulinemia and meal ingestion (physiological endogenous hyperinsulinemia) equally decreased SVR. The insulin-induced decrease in SVR was related to metabolic insulin sensitivity, which means that SVR was more strongly decreased during hyperinsulinemia in insulin-sensitive individuals.

These data underscore the physiological relevance of insulin’s vascular effects on SVR and possibly provide a pathophysiological mechanism, (although we did not find direct effects on blood pressure, probably due to adaptive responses) how insulin’s vascular effects contribute to the development of hypertension in insulin resistance individuals.

In chapter 3 we report associations between proliferative retinopathy, microvascular dysfunction in skin and microbleeds in the brain. In this cohort study patients with type 1 diabetes with proliferative diabetic retinopathy, without proliferative diabetic retinopathy, and healthy controls underwent magnetic resonance imaging to assess cerebral microangiopathy (cerebral microbleeds) and ischemic damage (white matter hyperintensities and lacunes). In addition, all participants underwent peripheral microvascular function tests, i.e., determination of skin capillary density and capillary recruitment. We showed that only cerebral microbleeds, but not ischemic damage, were more prevalent in patients with proliferative diabetic retinopathy versus the other groups. Furthermore, in individuals with microbleeds, capillary recruitment was impaired compared with those without microbleeds.

These results suggest that both cerebral microbleeds and peripheral microvascular dysfunction are part of a generalized microvascular disorder in type 1 diabetes.
Chapter 4 describes the results of a systematic review addressing the effects of grape polyphenols on the metabolic syndrome components in humans. We included 39 studies in our analysis, of which the majority was rated as low- or medium quality. We found limited evidence to suggest that grape polyphenols may improve insulin sensitivity while no compelling evidence was found to conclude that grape polyphenols can positively influence glycemia, blood pressure or lipid levels.

In chapter 5 & 6 we describe a randomized, placebo-controlled, double-blind clinical trial in which we studied the metabolic and microvascular effects of red wine polyphenols (RWPs) in obese subjects. In this trial 29 obese volunteers were randomly allocated to either RWPs or matched placebo for 8 weeks. Participants were investigated at baseline and at the end of the study.

Chapter 5 describes the metabolic effects of RWPs. The most important finding was that that RWPs did not significantly alter insulin sensitivity, the primary outcome of the trial. An important strength is that we assessed insulin sensitivity using 3 different methods: a hyperinsulinaemic-euglycaemic clamp, a mixed-meal test and HOMA-IR. All methods showed similar results.

In Chapter 6 we focused on the effect of RWPs on vascular function in obesity. We assessed microvascular function in both skin and muscle using different techniques. In addition, we performed the microvascular function tests before and during hyperinsulinemia to study whether RWPs might affect the vascular effects of insulin. It has been suggested that RWPs stimulate the same intracellular signaling cascade in endothelial cells as insulin, hence a more favorable balance of insulin's vascular effects with predominant vasodilator effects would be expected. Finally, we determined blood pressure with a 24hr blood pressure devise.

In our study we did not find significant alterations after RWP treatment in any of our microvascular function tests, and blood pressure was not affected.

Taken together, we have demonstrated in the investigations resulting in this thesis that microvascular function, and in particular the effects of insulin on the vascular endothelium, are important for the regulation of systemic vascular resistance. This provides a better understanding of the pathophysiological mechanisms involved in the relationship between insulin sensitivity, microvascular function and blood pressure. Furthermore we have shown a relationship between microvascular dysfunction in the eyes, the brain and in the skin, supporting the hypothesis of a generalized microangiopathy associated with diabetes. Finally, in our studies we did not find evidence to support the hypothesis that RWPs improve cardiometabolic parameters in obese individuals.
Now, I will discuss these findings in the context of current knowledge of diabetes and the metabolic syndrome.

**Part 1**

The role of microvascular dysfunction as a possible mechanism clustering the components of the metabolic syndrome has been the subject of several reviews and theses (3-6). In this discussion we will focus on two questions: 1) how microvascular dysfunction contributes to the development of hypertension and 2) whether microvascular dysfunction is organ-specific or part of a generalized microangiopathy.

**The role of microvascular dysfunction in the development of hypertension**

Hypertension is characterized by both functional and structural alterations in the microcirculation. The functional alterations include an imbalance in mechanisms regulating vasomotor tone, leading to enhanced vasoconstriction and/or reduced vasodilator responses. These functional changes probably lead to structural alterations which consist of changes in the density (rarefaction) and structure of precapillary resistance vessels and capillaries within a given vascular bed. Alterations in the microcirculation are probably not only the consequence of hypertension but have also been hypothesized to be involved in the etiology of hypertension (2,3). It is known that in most forms of experimental and clinical hypertension, systemic vascular resistance (SVR) is increased in proportion to the increase in blood pressure. This increase in systemic vascular resistance is likely to reflect changes in the microcirculation as it as at this level where a substantial drop in hydrostatic pressure occurs (5). Previously, it has been shown that microvascular rarefaction, similar in magnitude to the rarefaction observed in patients with established hypertension, can already be demonstrated in subjects with mild hypertension and in normotensive subjects with a genetic predisposition to high blood pressure (7,8). Furthermore, it has been shown that insulin's local vasodilator effects (in skin) relate to SVR, suggesting that the vascular effects of insulin are involved in SVR regulation (9). In chapter 1 we extended these findings by showing that systemic hyperinsulinemia (both during a hyperinsulinemic-euglycemic clamp and during a meal) decreased SVR and that the magnitude of this effect was associated with metabolic insulin sensitivity. Our study thus supports the hypothesis that insulin's vascular effects are indeed implicated in the regulation of SVR in humans. We did not observe an effect on blood pressure. However, one should bear in mind that disturbed effects of insulin on SVR alone may not lead to a increment of blood pressure as long as adaptive responses are intact. According to the Borst-Guyton principle chronic hypertension can occur only if renal function is abnormal with a shift in the renal pressure natriuresis relationship (10).
Microvascular dysfunction: organ specific or generalized?
Microvascular dysfunction has been proposed to be central to many forms of end-organ damage associated with the metabolic syndrome, including those involving the eye, kidneys, heart, and brain. It is currently unknown, whether microvascular dysfunction is organ-specific or part of a generalized microangiopathy. If the latter is true, microangiopathy should also be measurable in relatively easily accessible organs such as the skin. In chapter 2 we report on the relationship between microvascular function in skin and microvascular damage in the eye and brain of patients with type 1 diabetes. In this cohort study we showed that cerebral microbleeds, (representing hemosiderin leakage at mainly the capillary level and a direct measure of cerebral microangiopathy (11)), although rarely detected, were more prevalent in type 1 diabetes patients (T1DM) with proliferative retinopathy relative to T1DM without angiopathy and healthy controls. Furthermore, capillary function in skin was significantly more impaired in participants with cerebral microbleeds than participants without microbleeds, most notable in T1DM with proliferative retinopathy. This study thus supports the notion that microvascular dysfunction (at least in patients with type 1 diabetes) in skin is related to microvascular damage in the brain, suggesting both are part of a generalized microangiopathy. It is important to note that this study only shows that a relationship is present, but does not prove causality. Furthermore, this study does not address obesity associated microvascular dysfunction. It is likely, however, that similar relations will be present as it has been shown for example that the prevalence of cerebral microbleeds is related to retinal microvascular abnormalities in type 2 diabetes patients (12).

Conclusion
Microvascular dysfunction, and more specifically the disturbed vascular effects of insulin, contribute to increased SVR in insulin resistant individuals which contribute to the development of hypertension. Furthermore, the associations between cerebral microbleeds and peripheral microvascular function in skin and eye supports the hypothesis of a generalized microangiopathy.

Part 2
Red wine polyphenols have been proposed as a treatment option for the metabolic syndrome. Epidemiological studies have shown less cardiovascular morbidity and mortality in individuals drinking moderate amounts of red wine (13). Other epidemiologic studies have suggested beneficial metabolic and cardiovascular health effects for foods containing various polyphenols, implicating polyphenols are the active ingredients of red wine (14). This is supported by ex-vivo and animal studies which have suggested that RWPs ameliorate endothelial function (15-17) insulin sensitivity, blood pressure and lipid levels in models of obesity, hypertension and type 2 diabetes (18-20). In this thesis we focused on whether these presumed effects can
be reproduced in humans. We focused on the separate component criteria of the metabolic syndrome and (micro)vascular function as it has been hypothesized that the main protective mechanism of action of RWPs is depended on vascular function.

**RWP and the metabolic syndrome**

*Glycaemia and insulin sensitivity*

In our review (chapter 4) we concluded that there was insufficient evidence to conclude that glycaemia can be ameliorated by RWPs, even though there is some evidence to suggest that insulin sensitivity can be improved. This was mainly based on 2 high-quality studies; one high-quality study suggested a trend towards improved HOMA-IR in 28 individuals with pre-hypertension (21) and another study showing that grape polyphenols (not specifically RWPs) prevented the 11% decrease in insulin sensitivity after a fructose challenge, while no effects after regular intake were demonstrated (22). In our randomised clinical trial (chapter 5) we did not find effects of RWPs on insulin sensitivity. Although the design of our trial was not identical (different type of mix and no fructose challenge) it was comparable to the previous high-quality studies regarding the number of (healthy) overweight/obese participants, the duration of the intervention, and the dosing interval. As such, the addition of our clinical trial to the previous (weak) evidence makes it unlikely that RWPs have relevant effects on insulin sensitivity in obese volunteers.

*Blood pressure*

The vast majority of clinical trials on the effects of grape polyphenols on blood pressure have shown neutral effects. In individuals with components of the metabolic syndrome only one of 6 high-quality studies showed significant lower blood pressure after treatment. In this study daytime SBP was reduced by 5% (approximately 7 mmHg) while DBP showed a nonsignificant decrease. Nighttime BP was not significantly changed and mean 24-hour BP was not reported in this trial (21). The volunteers in our trial were normotensive and we measured 24-hour BP. We did not observe significant reductions in either overall SBP or DBP, or when we made a distinction between day-time and nighttime BP. Our trial is thus in line with most of the evidence that RWPs are not effective to lower blood pressure in obese individuals with normal BP or grade 1 hypertension.

*Lipid levels*

In our review (chapter 4) we suggested that it is possible that triglyceride (TG) levels are affected in individuals without components of the metabolic syndrome although this was only based on one medium-quality study. In participants with component criteria of the metabolic syndrome we found a significant reduction in TC levels in two out of four high-quality studies, yet the relevance of these findings is not clear as the other lipoprotein parameters were unchanged. In our trial we measured TC, HDL-C, LDL-C and triglycerides in the fasting state and postprandially. We did not observe alterations in lipid levels after the supplementation of
RWPs. We conclude that it is unlikely that RWPs have clinically relevant effects on lipid levels in obese human subjects.

RWPs and vascular function
With regard to vascular function, a meta-analysis has shown improved flow-mediated dilation (FMD) at 30, 60 and 120 minutes after supplementation of grape polyphenols, while no significant effects were seen after 180 minutes. Subgroup analysis indicated that the increase in FMD could be more pronounced in individuals with more cardiovascular risk factors. The insufficient number of trials precluded meta-analyses of the chronic supplementation trials. These results, however, have to be interpreted with caution as only 9 (mostly not randomized and/or placebo-controlled) trials with small numbers of individuals were included (15). Chronic supplementation studies have predominantly been of limited quality and have shown inconsistent results. Some have suggested beneficial effects (23,24), yet others did not find relevant effects (25,26), also in higher-risk patients (21). Most studies measured vascular function in larger arteries, mostly FMD. In our clinical trial (chapter 6) we measured endothelial function at the microvascular level and studied the responses before and after insulin infusion. Previously it has been hypothesized that RWPs effects on the endothelium are dependent on stimulation of the intracellular phosphatidylinositol 3-kinase (PI3K) pathway (16). Since insulin also directly stimulates the same PI3K pathway, we hypothesized a more favorable balance of insulin's vasodilatory effects by relative amplification of insulin's activation of PI3K and nitric oxide synthase. In our trial, we found neutral effects of RWPs in all of our microvascular function tests. An important consideration is that the volunteers did not take their polyphenols on the examination days (to rule out acute effects). Whether polyphenols were taken on the examination days in the other trials is unclear as this is usually not mentioned. Some studies do indicate that the enhancing effect of RWP on vasodilation responses is greatest during peak concentrations of the polyphenols, which would occur 1–2 h after consumption (evidence from cacao studies (27)), this is also in accordance with the meta-analysis in which only the effects within 3 hours were significant. If however these effects are only present for a short period (less than 3 hours), it is questionable whether supplementation as it is currently done (1-2 times a day) is relevant for vascular health in the long term. But this still could be an explanation for the apparent conflict between acute and chronic studies. Therefore we conclude that it is unlikely but possible that RWPs are able to improve vascular function in human obesity, albeit only acutely.

General considerations about our polyphenol studies
There are multiple difficulties about studying functional foods, especially RWPs in contrast to conventional medication. Here we will sum up some of the limitations and difficulties we encountered during our studies.
**Which polyphenol(s) should be studied?**

Red wine contains more than 200 different forms of polyphenols and as every step in wine making (grape varieties, area of cultivation and vinification methods) has influence on the composition and concentration of polyphenols, every (type of) wine is different. A lot of different polyphenol mixes are on the market and the composition of the polyphenol mixes in trials differs hugely. In addition, the use of isolated polyphenols, e.g. Resveratrol or Quercetin is also frequently recommended. Currently, comparative studies between polyphenols are mostly lacking and it is uncertain what would be the most promising polyphenol (mix). In our trial we chose to study a mix of red wine polyphenols from the cabernet sauvignon grape. This mix corresponds most closely to the original epidemiological studies. Furthermore, the pre-clinical evidence that polyphenols act on the endothelium also stems from studies performed with mixed RWPs (16,28). In our review we chose to set up a rather broad search (grape polyphenols) to make sure no relevant literature was missing and have enough power, but decided to exclude all studies with individual polyphenols or combined interventions (e.g. combinations with other nutritional products). This combination of criteria let to a moderate amount of thirty-nine studies, but with such a large heterogeneity that was impossible to make a formal meta-analysis. In hindsight we could have limited our search criteria to more specific studies with RWP mixes more similar to ours (and thus original red wine) as one could argue that due to this large heterogeneity positive findings of this specific mix could be missed.

**How should RWPs be administered, and for how long?**

In humans polyphenols are extensively metabolized and their bioavailability is low. The absorption of polyphenols is influenced by differences in gut microbiota composition, dietary, environmental and host genetic variances. In addition, it has been suggested that dietary polyphenols also modulate the gut microbial population composition (29). Knowledge is limited about what are the most active metabolites and there is no good evidence base for what levels should be aimed at. Moreover, bioavailability analyses may not be comparable due to differences in analytical techniques. In most studies the dosage was based on in-vitro or animal studies which are frequently far higher as compared to the original observational studies. Some studies measured the plasma or urinary concentration of polyphenols, but found a large inter-individual variability and (frequently) failed to correlate this with the assumed effects (21). Furthermore, as highlighted in the section RWPs and vascular function, it is uncertain what the optimal dosing interval and study duration are. In our study we used a dosage of 600mg a day (corresponding to a polyphenol content of 6 glasses of red wine), divided over 2 intervals and supplemented for 8 weeks. Our dosage was in between the dosage which could be inferred from observational studies and animal studies. A previous study showed that a comparable (not similar) red wine extract in a similar dosage led to the bioavailability of several compounds or degradation products in urine after supplementation for 5 days in gelatin capsules (30). Yet, due to the arguments mentioned above the dosage, dosing interval, and/or study duration may have influenced the observed lack of effect.
Do polyphenols from other sources influence study results?
Polyphenols are widely distributed among food products, and it is almost impossible to make a reasonable diet without polyphenols in trials. In our trial participants were not restricted on the intake of polyphenols (except for red wine) but were asked to keep track on their intake. It turned out that the intake of polyphenols from other sources was substantial (approximately 1 gram per day), but not significantly different between the groups, therefore we think that it is unlikely that this has played an important role in our trial, but this is certainly not proven.

Conclusion
Red wine polyphenols have been proposed as candidates to ameliorate cardiometabolic complications of obesity. These assumptions are however mainly based on promising effects found in epidemiological, in-vitro and animal studies. In our well-controlled human studies we did not find relevant effects of RWPs on insulin sensitivity, glycaemia, blood pressure, lipid levels or vascular function in obese individuals. We feel that there is presently no evidence to advise supplementation of RWPs to improve cardiometabolic parameters in obese individuals.

Directions for future research
In the studies described in this thesis we did not find evidence to support the hypothesis that red wine polyphenols are beneficial to development of vascular complications of obesity. Yet, as mentioned in the discussion, there are several limitations to our studies and larger randomized double-blind placebo-controlled clinical trials are needed to draw definitive conclusions. These studies could clarify whether RWPs have acute effects, have effects in other populations, or that other polyphenols might be more effective than the ones used here.

Another interesting direction would be to investigate whether and to what extent new cardiovascular drugs (e.g. anti-inflammatory, lipid lowering and diabetes mellitus medication), which have shown to reduce cardiovascular morbidity and mortality, might affect microvascular function. Microvascular function potentially plays a central role in the etiology of metabolic and vascular complications and can easily be used as an intermediate end point in clinical trials.
Reference List


