chapter 9 - summarizing discussion

This final chapter summarizes the main findings of the work presented in this thesis and also provides some methodological considerations and background. Microvascular dysfunction is suggested to play a central role in the clustering of cardiometabolic risk factors such as obesity, hypertension and insulin-resistance. This thesis provides further insight in the regulation of microvascular vasoreactivity with specific emphasis on the roles of endothelin-1, VEGF and adiponectin. From these studies the following main conclusions could be drawn:

The main conclusions of this thesis are

a skin microvascular function is a proxy for systemic microvascular function (chapter 2, 3, 5, 6)

b neurogenic vasomotion and capillary recruitment are related (chapter 2)

c neurogenic vasomotion is impaired in obesity (chapter 7)

d changes in capillary density and blood pressure are directly related (chapter 4)

e AMPKa2 regulates insulin-mediated vasoreactivity and subsequent microvascular perfusion of skeletal muscle (chapter 8)

Chapter 1 serves as an introduction for the proposed pivotal role of the microcirculation in controlling both blood pressure and insulin sensitivity, explaining how these cardiometabolic parameters are intertwined in patho-physiology. Here, various features of the microvasculature, ways to study the microvasculature and key players influencing (insulin-induced) microvascular function are discussed.

Insulin has been shown to regulate its own delivery to skeletal muscle interstitium via actions on the microvasculature (1,2,3,4). In fact, these vascular effects are thought to be rate-limiting for insulin-stimulated glucose uptake by skeletal muscle (1). As discussed in chapter 1, insulin's influence on microvascular vasomotion (5) is one of the suggested levels of modulation. Insulin-induced changes in pre-capillary arteriolar vasomotion would result in downstream changes in functional capillary density i.e. capillary recruitment and subsequent changes in the microvascular exchange surface area (see also chapter 4a). Although inferred upon numerous times (2,6), a direct link between changes in vasomotion and changes in capillary recruitment had yet to be demonstrated. **In chapter 2**, we therefore addressed this very relationship within a group of healthy volunteers displaying a wide, continuous range in BMI and, as it turned out, insulin-sensitivity. Vasomotion and capillary recruitment were assessed in skin before and during a hyperinsulinemic, euglycemic clamp, the gold standard for the measurement of insulin sensitivity. Briefly, insulin was continuously infused at a high-physiological (postprandial in the obese) level, averaging 500 pmol/L. Meanwhile, glucose is kept at a fixed target of 5 mmol/L via a variable glucose infusion (7). The insulin-induced change in vasomotion and specifically the normalized neurogenic domain in the vasomotion spectrum as assessed via Laser Doppler flowmetry (LDF) was positively related with the change in capillary recruitment as assessed with capillary videomicroscopy. Furthermore, the positive association between the normalized neurogenic vasomotion domain and whole-body glucose uptake could largely be explained by capillary recruitment in a regression analysis, supporting the suggested physiological framework.
As a future step beyond the cross-sectional experimental setup, we would have to perform dedicated vasomotion studies using the hyperinsulinemic euglycemic clamp and a second intervention targeting insulin sensitivity and/or (neurogenic) vasomotion.

A second methodological prerequisite for our hypothesis, which states that impaired microvascular function is a generalized phenomenon leading to cardiometabolic sequelae, is addressed in **chapter 3a**. In the same

experimental design as described in chapter 2, we investigated whether skin microvascular measurements correlate with microvascular measurements in skeletal muscle and as such could serve as a proxy for skeletal muscle when studying the relationship between insulin's vascular and metabolic effects. To address this long-standing debate in the microvascular community (8-27), we used two different state-of-the-art techniques to assess capillary recruitment. We used the capillary videomicroscope to assess capillary density during resting conditions and peak reactive hyperemia (PRH, ischemia induced hyperemia after 4 minutes of arterial occlusion) by counting capillary loops in nailfold skin. The difference in capillary density at baseline and during PRH is termed capillary recruitment. Contrast enhanced ultrasonography (CEU) was used to assess microvascular blood volume (MBV) within skeletal muscle (28). Both capillary recruitment and MBV were assessed before and during a hyperinsulinemic euglycemic clamp. The change in capillary recruitment in skin was positively related to the change in skeletal muscle MBV from baseline to steady-state hyperinsulinemia. Furthermore, both changes in microvascular surface area were positively related to whole body glucose uptake. Taken together, these data validate the use of skin (e.g. capillary videomicroscopy) as a proxy for skeletal muscle (e.g. CEU) when studying insulin and its vasculo-metabolic effects from the microvascular perspective.

Methodological considerations regarding the assessment of microvascular function

In chapter 2 (and chapter 7) we use **LDF** to measure skin perfusion (29). This non-invasive technique uses a beam of laser light from a fiber-optic probe to penetrate the skin. The light is then scattered (and partly absorbed) by tissue. Backscattered light is picked-up by the probe. When the laser light hits a moving object (usually blood cells moving within a blood vessel) the backscattered light undergoes a shift in wavelength according to the Doppler principle. Light hitting static tissue remains unchanged. The magnitude of the overall change in wavelength is directly related to the number of moving objects and their velocity within the sample volume. As the LDF measurement is non-vectorial and the exact sample volume is unknown, no absolute perfusion values can be derived. Measurements are expressed in arbitrary perfusion units (PU). The probes used for chapters 2 and 7 have an average measuring depth of 0.5-1.0 mm, depending on such factors as local tissue density and pigmentation. Most of the recorded signal will therefore come from the subpapillary plexus consisting of arterioles and venules, with only a small contribution coming from the capillary loops rising form the plexus towards the base of the epidermidis (29,30,31).

It is the precapillary arterioles within the subpapillary plexus which are responsible for local **vasomotion** (i.e. rhythmic contraction and dilatation of blood vessels) and subsequent flowmotion (variation in perfusion in the distal capillary bed) in response to local metabolic demand (32). The vasomotion frequency spectrum encompasses several domains. The 0.01-0.02 Hz domain has been suggested to be endothelium-dependant as the nitric oxide synthase (NOS) inhibitor N^G-monomethyl-L-arginine (l-NMMA) reduces vasomotion output within this range (33). Changes in the 0.02 - 0.06 Hz range after local or ganglionic nerve blockade and sympathectomy have attributed this domain to neurogenic activity and the sympathetic nervous system in particular (34,35). The 0.06–0.15 Hz interval is thought to be related to 'spontaneous' myogenic activity via alterations in vascular smooth muscle tension and/or cell stretch in response to changes in intraluminal pressure (36). Of note: the endothelial and neurogenic signals would also have to 'use' the vascular smooth muscle cells as effectors for their vasomotion contribution. The higher frequencies of the spectrum originate upstream, are conducted through the vascular tree, and registered in the periphery. 0.15–0.4 Hz corresponds to the respiratory cycle and the 0.4–1.6 Hz range reflects heart beat (37). The resulting flowmotion is the net sum of the different contributions of the vasomotion domains. The phenomenon of vasomotion can be viewed as an example of *metabolic autoregulation*.

The various frequency domains which make up the vasomotion spectrum are derived from the LDF signal using spectral analysis techniques such as Fast Fourier or, as used in this thesis, Wavelet transform (38). Put simply, these algorithms compare the signal under investigation to pre-selected sinusoids (in this case *Morlet*-wavelets)

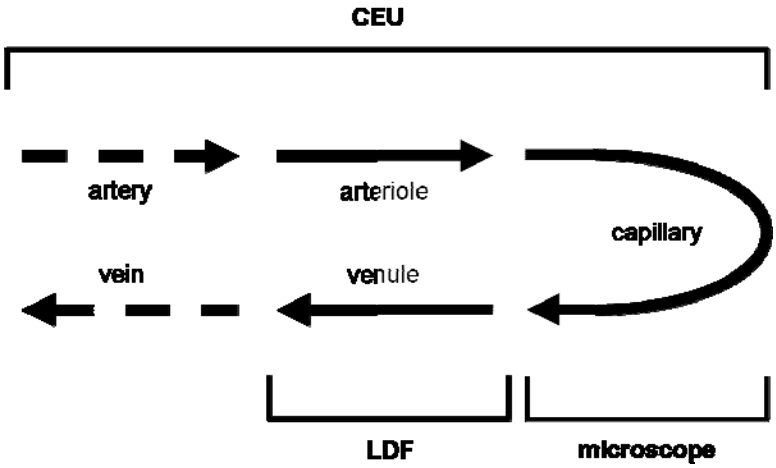
with varying frequencies. The more a selected wavelet 'matches' the source signal, the higher the resulting output for that specific frequency domain. A factor influencing vasomotion output is the amplitude of the LDF source signal (for typical examples see chapter 7 supplemental figure 1). In order to take into account this variation between/within individuals (for example because of different placements of the LDF probe on a heterogeneous vascular bed), we divided the average amplitude of a frequency domain by the average amplitude of the entire frequency spectrum, thus normalizing the output for better comparison (37,39). In chapter 2 we refer to a general rule-of-thumb for vasomotion analyses requiring a minimum recording of 10 successive arteriolar contractions in a frequency domain in order to perform robust spectral analyses for that particular domain. In this case the low-end of the frequency domain, 0.01 Hz, is the bottle neck, corresponding to at least 16.7 min of recording to 'register' 10 'endothelium-derived' alterations in flowmotion.
One must realize that the spectral analysis output is a mathematical derivative of a semi-quantitative measurement (LDF) and as such, should be interpreted conservatively. In fact, vasomotion is best used as a complementary parameter alongside other (quantitative) measures such as functional capillary density and whole-body glucose uptake, to provide additional (patho)physiological context.

In chapters 2, 3, 4 and 6 we use the **capillary videomicroscope** to assess (functional) capillary density in skin. This is the only available non-invasive technique that can directly visualize individual capillaries for quantification. In finger skin, just proximal of the nailfolds, capillary loops are located perpendicular to the surface (the loops rising up from the subpapillary plexus). The apex of these loops can be visualized using a standard light microscope with a final magnification of ~100x. Using a specific combination of light-characteristics, filters (resulting in a green light) and paraffin oil on the skin surface to reduce reflections and improve transparency, red blood cells stand out from the surrounding tissue. This technique depends on the presence of erythrocytes in capillaries for their identification. During resting conditions one can observe differences in perfusion between individual capillaries within a single field of view (~ 1 mm^2) (see online figures x or x). Some capillaries are continuously filled while others are only intermittently perfused. This temporal heterogeneity of capillary perfusion is thought to be the downstream flowmotion-effect of vasomotion in proximal arterioles (in the subpapillary plexus) (example video). From a metabolic perspective, the fraction of intermittently perfused capillaries can act as a 'reserve' to be *recruited* when the tissue-needs so demand. To expose this reserve, we have used venous congestion (VC) (chapter 4) and post-occlusive reactive hyperemia (PRH) (chapters 2, 3 and 6). Where peak capillary density during VC is thought to primarily depend on the anatomically available number of capillaries (40,41), peak capillary density after PRH reflects the functional reserve (42). The increase in the number of continuously perfused capillaries at baseline to total capillary density after PRH is defined as *capillary recruitment*.
In chapters 2, 3 and 6 we performed a hyperinsulinemic euglycemic clamp to determine whole body glucose uptake or metabolic insulin sensitivity. The increase in capillary recruitment during the hyperinsulinemic intervention from baseline was defined as *insulin-augmented capillary recruitment* or (a form of) vascular insulin-sensitivity. This method and the definition of capillary recruitment have received critique from colleagues in the field. Poole et al have suggested that the results (reported in chapter 3) merely reflect (a change in) red blood cell (RBC) distribution rather than 'actual capillary recruitment' (43). Although the overall critique is more semantic in nature, the remark that non-RBC-perfused capillaries are probably still being perfused by plasma is justified. Indeed, it is the flow of plasma with its hormones (e.g. insulin) and nutrients (e.g. glucose) and their delivery to target tissues in which we are interested. As a mere RBC redistribution does not directly explain the relationship between capillary recruitment and whole body glucose uptake (44,45), the amount of (insulin-augmented) capillary recruitment indirectly assessed via RBC-density is likely to be, to a certain extent, proportional to the increased flow of plasma. Interestingly, the concept of insulin-induced changes in vasomotion can easily be reconciled with the concept of already plasma-perfused capillaries becoming more perfused to encompass

RBC's. It suggests temporal heterogeneity in microvascular perfusion which can be influenced by insulin. Indeed, in support of this hypothesis, capillary recruitment explained almost 90% of the positive association between the normalized neurogenic vasomotion domain and whole-body glucose uptake in a regression analysis (chapter 2).

A third technique used for this thesis is **CEU** (chapter 3 and 8). Gas filled lipid microbubbles are infused intravenously before an ultrasonographic measurement. These echogenic microspheres are a fraction smaller than, and are thought to display rheological properties similar to, RBC's (46). When subjected to ultrasound the bubbles will start to oscillate, creating a reflective signal which can be picked up by the ultrasound probe. As the bubbles remain intravascular, the distinct echo-reflection will contrast the vascular bed from its surrounding tissue within a region of interest (ROI). Oscillating bubbles will burst when signal intensity reaches a threshold. The deliberate destruction of bubbles in a field of view during steady state infusion enables the registration of inflow-curves based on video-intensity (see also chapter 1, figure 1). Similar to LDF, CEU is a semi-quantitative measurement as the exact volume of the ROI is not known. To take into account possible differences in bubble concentration between/within individuals (for example due to differences in bubble preparation or probe placement on a heterogeneous vascular bed) CEU output is normalized to the video intensity of an artery within the field of view (outside the ROI). Where video microscopy solely visualizes RBC's in capillaries and LDF registers blood cells in arterioles and venules, CEU detects microbubbles in all three parts of the microvascular bed (figure 1). Microbubbles can damage cells or tissue when they burst in close proximity (47). This property is being studied as a deliberate intervention in several fields of research including cardiology (thrombus destruction) (48) and oncology (precise delivery and subsequent release of anti-cancer drugs in bubbles within a tumor) (49). However, damaged endothelium could affect microvascular vasoreactivity.

figure 1 - levels of microvascular measurement



A major potential (for future investigations) of CEU is the simultaneous measurement of microbubbles within different vascular beds (skeletal muscle, heart, kidney etc.) (50). In summary, when keeping in mind the methodological considerations, these validated techniques have shown a strong potential for future research. LDF/vasomotion and videomicroscopy are easy in use and non-invasive and have proven to be applicable in large epidemiological cohorts (51,52,53). The more laborious and semi-invasive CEU has established itself as the primary technique in pre-clinical, mechanistic in-vivo studies, both in animal and human research (54,55).
As a bridge to the next section of the thesis, microvascular function and blood pressure control, we have performed a small study exploring the potential of CEU in blood pressure research. In **chapter 3b** we performed a post-hoc analysis on the studied population of chapter 3a. As parameters determining cardiac output (CO), blood pressure and (systemic) vascular resistance are closely related. In these healthy, normotensive volunteers,

skeletal muscle perfusion as measured with CEU in the forearm was indeed inversely related to blood pressure. This proved the case for systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP). These results illustrate the possibility for more dedicated studies using CEU in blood pressure research. Future validation-studies could, for example, focus on the relationship between CEU-parameters and the gold standard for CO measurement thermo-dilution or pulse dye densitometry (56).

There is a large body of evidence suggesting that microvascular dysfunction not only results from, but can also precede elevated blood pressure, resulting in a vicious cycle (57). Most of these results however are derived from mathematical models (58) and epidemiological, association based studies (59). Intervention models directly investigating the relationship between (changes in) blood pressure and microvascular function e.g. capillary density are sparse, mainly because of the burden of such interventions. However, some patients have a strong indication to use a drug with hypertension as a known side effect for another reason. An example of such a category of drugs are inhibitors of vascular endothelial growth factor and/or its receptors (VEGF/VEGF-R), anti-angiogenic compounds used in oncology and ophthalmology (60,61). The receptor tyrosine kinase inhibitors such as telatinib (62) and the anti-VEGF monoclonal antibody bevacuzimab (63), have also been linked to a reduction in capillary density in patients during on-drug periods. This reduction in capillary density induced by bevacuzimab was shown to be reversible as measured 3 months after discontinuation of the drug (64).

In **chapter 4a** we were able to investigate the relationship between acute changes in capillary density and blood pressure in a group of 16 patients with metastatic renal cell cancer (mRCC) treated with sunitinib. The drug was administered orally at a dose of 50 mg daily for four weeks followed by a 2 week rest period in cycles of 6 weeks. 24 hour blood pressure measurements and capillary video microscopy were performed at baseline, 2 weeks and 4 weeks. Already at 2 weeks, SBP, DBP and MAP were increased and capillary densities at baseline and after venous congestion were decreased. These changes in blood pressure and capillary density during 2 weeks of sunitinib were inversely related. Moreover, in this small group of patients, sunitinib-induced capillary rarefaction (decrease in capillary density) turned out predictive of progression free survival as assessed via computed tomography and overall survival.
After our study, Kappers et al. have found sunitinib induced elevations in blood pressure to be accompanied by an increased peripheral resistance as early as 4 hours after administration of sunitinib in swine. These hemodynamic changes were fully abolished when pretreated with tezosentan, a dual endothelin-1 receptor blocker (65).
In **chapter 4b** we found these effects to be reversible during the off-drug period. The relatively fast and reversible changes in blood pressure and capillary density suggest *functional* rather than structural rarefaction. Notwithstanding the pleiotropic effects of sunitinib, it is the VEGF-receptor and its downstream MEK/ET-1-signaling cascade which seems to be predominantly involved in microvascular vasoreactivity and resulting blood pressure. The similarities between the insulin and VEGF signaling cascades strongly suggest shared intracellular mechanics, conceivably resulting in shared (patho)physiology ~ two knobs tuning the same radio (66). Future research could focus on such interplay combining for (an ambitious) example the hyperinsulinemic euglycemic clamp with acute VEGF/VEGF-R blockade as a second intervention in 5'AMP-activated protein kinase (AMPK) knock-out mouse models with or without prior dual endothelin-1 receptor blockade.

Chapter 4 demonstrates that the relationship between microvascular function and blood pressure is direct and dynamic. A reduction in capillary density, conceivably resulting from on increased peripheral vascular resistance, is associated with an increase in blood pressure within days. However, according to the Borst-Guyton concept, chronic hypertension can only occur if renal function is abnormal with a shift in the renal pressure–natriuresis relationship (67,68). In the absence of the latter, increased peripheral resistance only temporarily raises blood

pressure, to be followed by an increase in renal sodium excretion restoring blood pressure towards normal. Subtle renal microvascular disease (69,70) may reconcile the Borst-Guyton concept with the putative role of vessel rarefaction in the etiology of high blood pressure. De Jongh et al. have shown skin capillary recruitment to be inversely related with salt sensitivity of blood pressure, suggested to be a proxy for renal microvascular injury (71,72). This construct suggests a temporal relationship where renal microvascular dysfunction with a subsequent shift in the pressure-natriuresis relationship precedes manifest elevations in blood pressure. Ideally, one would find a cohort of healthy individuals, identify the ones with increased salt sensitivity and prospectively keep track of any changes in blood pressure. Alternatively, although less ideal, one could retrospectively assess the relationship between salt sensitivity of blood pressure and an established proxy for early developmental changes in both the kidney and the (micro)vasculature (69,73). One such proxy is a low weight at birth, which epidemiological studies consistently have demonstrated to be associated with raised blood pressure later in life (74,75,76).

In **chapter 5** we investigated the relationship between birth weight and salt-sensitivity of blood-pressure during adulthood. Indeed, normotensive subjects with lower birth weights were more likely to have a salt sensitive blood pressure. We could also confirm the inverse association between capillary recruitment in skin and salt sensitivity of blood pressure. These findings underline the concept of microvascular dysfunction as a generalized phenomenon (see also chapter 3) and suggest that is starts in-utero.

As discussed throughout this thesis, we propose that microvascular dysfunction (i.e. vascular insulin resistance) explains an important part of the clustering of obesity, hypertension and metabolic insulin resistance. Although often found together, obesity and hypertension are both regarded to be insulin-resistant states in their own right. It is presently unclear to what extend vascular insulin resistance is independent of adiposity in hypertensive subjects.

In **chapter 6**, we addressed this question in a four-corner model, using four distinct phenotypic groups of subjects: non-obese normotensive, non-obese hypertensive, obese normotensive, and obese hypertensive.. Capillary video microscopy was performed before and during steady state hyperinsulinemia of a hyperinsulinemic euglycemic clamp in order to assess insulin-augmented capillary recruitment i.e. vascular insulin resistance in skin. Both vascular and metabolic insulin sensitivity showed a trend in decreasing from the normotensive/non-obese end to the hypertensive/obese end of the spectrum. When the subjects were dichotomized depending on the presence of either hypertension or obesity, obesity seems to be of greater impact than hypertension on metabolic insulin-sensitivity, whereas both have a similar impact on vascular insulin sensitivity. This suggests that besides an impaired delivery of glucose to the target tissue, there are additional rate-limiting factors at play in the presence of obesity with regards to metabolic insulin-sensitivity. Examples of such additional factors are diminished levels and activity of GLUT4 at the cellular level (3)

The data in this chapter suggests that obesity and hypertension have compounding detrimental effects on metabolic and vascular insulin sensitivity.

The last two chapters address the modulating potential of adiponectin and its downstream effector AMPK in vasoreactivity.

In **chapter 7**, we performed wavelet analyses in a healthy population-based cohort, The Amsterdam Growth and Health Longitudinal study (AGHLS). The normalized neurogenic vasomotion domain was inversely associated with BMI (throughout the normal range) and positively associated with adiponectin. In a multivariable analysis, adiponectin could explain part of the relationship between BMI and the normalized neurogenic vasomotion domain. At the time of writing, this was the first study to investigate the probable relationship between adipokines and vasomotion, a form of vasoreactivity. These results are in line with data from Greenstein et al. who

demonstrated that norepinephrine can induce the release of adiponectin from perivascular adipose tissue resulting in vasodilation of resistance arteries ex-vivo (77). Recently, Zhao et al. demonstrated an increase in microvascular recruitment and whole body glucose uptake in Sprague-Dawley rats after the administration of the globular domain of adiponectin (gAdn) (78). As discussed in chapter 1 and above, this microvascular recruitment by gAdn might (at least partly) be explained by the upstream effects on arteriolar vasomotion and the neurogenic domain in particular. The results in chapters 2 and 7 consistently show the neurogenic domain to be the key domain in the vasomotion spectrum to be associated with factors such as BMI, adiponectin, capillary recruitment and whole body glucose uptake. As discussed earlier in the chapter, neurogenic vasomotion is assumed to be a proxy for the sympathetic nervous system. It is suggested that insulin resistant states such as obesity are characterized by an increase in sympathetic output (79). Therefore, we would have expected the normalized neurogenic vasomotion domain to show a positive relationship with BMI. However, studies by Andersen and Agapitov have demonstrated that the relationship between increased sympathetic activity and vascular reactivity may not always be straight forward (80,81). Andersen reported insulin to increase SNS activity, as assessed through microneurographic sympathetic nerve activity to skeletal muscle (MSNA), yet also to induce vasodilation in healthy subjects (80). Later, Agapitov et al. demonstrated dissociation between elevated SNS activity and normal sympathetic vascular tone, as assessed via adrenergic receptor blockade by phentolamine, in normotensive obese (81).

These epidemiological observations prompted us to look deeper into adiponectin's suggested modulating effect on vasoreactivity. Such modulation is likely to be exerted via adiponectin's downstream substrate AMPK. The AMPKα2 isoform in particular has been shown to regulate whole-body insulin sensitivity (82). Although AMPKα2 -/- mice in-toto are insulin resistant, AMPKα2-deficient myocytes show normal insulin sensitivity (82,83). Here, vascular impairment seems likely as the rate-limiting factor for insulin-stimulated glucose uptake by skeletal muscle.

In **chapter 8**, we used Wistar rats and AMPKα2 wildtype/knockout mice to mechanistically unravel the effect of gAdn and its downstream substrate AMPKα2 on insulin-mediated vasoreactivity. Ex-vivo, gAdn revealed nitric oxide-dependent, insulin-mediated vasodilation of resistance arteries from both species in a pressure myograph. This vasodilator response is dependent on AMPKα2, which, when present, impairs endothelin-1 mediated vasoconstriction. In-vivo, using CEU, insulin resistant AMPK-/- mice and not their wildtype littermates showed impaired perfusion of skeletal muscle during the hyperinsulinemic euglycemic clamp. In these - proof of principle - experiments, the absence of AMPKα2 can be regarded as a proxy for the obese phenotype were hypoadiponectinemia leads to a downregulation of AMPKα2. As a next step, a study aiming to corroborate the pivotal role of AMPKα2 in the microvasculature using a vascular specific AMPKα2-/- model is underway (E. Van Poelgeest, thesis). Recent studies addressing blood vessels in the context of their local milieu (e.g. perivascular adipose tissue) have shed more light on the experimental path towards approximation of in-vivo complexity (R.I. Meijer, thesis). Another, more clinical approach (aiming to counter obesity's detrimental effects) would be to focus research on AMPK-stimulation, both pharmacologically (e.g. metformin, Adiporon (84)) and through lifestyle. Examples of the latter could be polyphenols (J. Woerdeman, thesis) and exercise.

Conclusion

The data presented in this thesis give extra support for the concept of the microvasculature as a nexus in cardiometabolic risk. Microvascular dysfunction (be it through low-grade inflammation or obesity-driven) is a generalized phenomenon (chapters 2, 3 and 5) the seeds for which might already be sown during early development (chapter 5). Because of this generalized character where no tissues or organs are spared, readily accessible vascular beds can provide a window to the less accessible parts of the body (chapters 2, 3, 4 and 7)

when studying (insulin-mediated) vasoreactivity. Using a variety of techniques, the interplay between vascular and metabolic insulin sensitivity proved a reproducible theme across several phenotypes and genotypes (chapters 2, 3, 5, 6 and 8).

'With an excess of fat diabetes begins and from an excess of fat diabetics die'. These prophetic words were written by Elliott Joslin, a diabetes pioneer, almost 87 years ago, long before the current diabesity pandemic (85). Echoing Joslin's words, the different angles of investigation presented in this thesis are coming together and are found to be more and more intertwined, rather than distinct players in the development of obesity related cardiometabolic sequelae.

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