

# **Adipose tissue as an active organ: blood flow regulation and tissue- specific glucocorticoid metabolism**

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*Dedicated to curious researchers and colleagues*



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# ABSTRACT

**Background:** Despite advances in the treatment of atherosclerosis, cardiovascular disease is the leading cause of death worldwide. With the population getting older and more obese, the burden of cardiovascular disease may further increase. Premenopausal women are relatively protected against cardiovascular disease compared to men, but the reasons for this sex difference are partly unknown. Redistribution of body fat from peripheral to central depots may be a contributing factor. Central fat is associated with hyperlipidemia, hyperglycemia, hypertension, and insulin resistance. Two possible mediators of these metabolic disturbances are tissue-specific production of the stress hormone cortisol and adipose tissue blood flow (ATBF). The aim of this thesis was to determine the adipose tissue production of cortisol by the enzyme  $11\beta$ -hydroxysteroid dehydrogenase type 1 ( $11\beta$ -HSD1) and to investigate the regulation of ATBF. **Materials and Methods:** Cortisol release was estimated by labeled cortisol infusions and tissue-specific catheterizations of subcutaneous and visceral adipose tissue (VAT) in men. We investigated ATBF by  $^{133}\text{Xe}$ -washout and its relation to autonomic activity, endothelial function, adipose tissue distribution, and adipokines in different groups of women. We further investigated the effect of two diets and of weight loss on ATBF in women. **Results:** We demonstrated significant cortisol release from subcutaneous adipose tissue in humans. Splanchnic cortisol release was accounted for entirely by the liver. Cortisol release from VAT (to the portal vein) was not detected. ATBF decreased according to increasing weight and postmenopausal status, and the level of blood flow was associated with nitric oxide (NO) activity and autonomic activity. ATBF was also highly associated with leptin levels and both subcutaneous adipose tissue and VAT areas. After 6 months of diet and weight reduction, a significant difference in ATBF was observed between diet groups. **Conclusions:** Our data for the first time demonstrate the contributions of cortisol generated from subcutaneous adipose tissue, visceral tissues, and liver by  $11\beta$ -HSD1. ATBF is linked to autonomic activity, NO activity, and the amount of adipose tissue (independent of fat depot). Postmenopausal overweight women exhibited a loss of ATBF flexibility, which may contribute to the metabolic dysfunction seen in this group. Weight loss in a diet program could not increase the ATBF, although there were ATBF differences between diet groups. The results will increase understanding of adipose tissue biology and contribute to the development of treatment strategies targeting obesity and obesity-related disorders.

**Key words:**  $11\beta$ -hydroxysteroid dehydrogenase type 1, adipose tissue, autonomic nervous system, blood flow, cortisol, nitric oxide, weight loss.

# LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by Roman numerals:

- I                   Stimson RH\*, **Andersson J\***, Andrew R, Redhead DN, Karpe F, Hayes PC, Olsson T, Walker BR. Cortisol release from adipose tissue by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in humans. *Diabetes* 2009;58:46-53. \*Joint first authorship
- II                   **Jonas Andersson**, Lars-Göran Sjöström, Marcus Karlsson, Urban Wiklund, Magnus Hultin, Fredrik Karpe, Tommy Olsson. Dysregulation of subcutaneous adipose tissue blood flow in overweight postmenopausal women. *Menopause* 2010;17:365-371.
- III                  **Jonas Andersson**, Fredrik Karpe, Lars-Göran Sjöström, Katrine Riklund Åhlström, Stefan Söderberg, Tommy Olsson. Association of adipose tissue blood flow with fat depot sizes and adipokines in women. *International Journal of Obesity*. Advance online publication, 26 July 2011.
- IV                  **Jonas Andersson**, Mats Ryberg, Lars-Göran Sjöström, Marcus Karlsson, Urban Wiklund, Fredrik Karpe, Bernt Lindahl, Tommy Olsson. Long term effects of a diet intervention on adipose tissue blood flow, heart rate variability and endothelial function. A randomized controlled trial. Manuscript.

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# ABBREVIATIONS

11 $\beta$ -HSD	11 $\beta$ -hydroxysteroid dehydrogenase
ADMA	Asymmetric dimethylarginine
ANS	Autonomic nervous system
ATBF	Adipose tissue blood flow
ATGL	Adipose triglyceride lipase
AUC	Area under the curve
BMI	Body mass index
CNS	Central nervous system
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular disease
eNOS	Endothelial nitric oxide synthase
FFA	Free fatty acid
FMD	Flow-mediated dilatation
GI	Glycemic index
GLP-1	Glucagon-like peptide-1
GR	Glucocorticoid receptor
HOMA	Homeostatic model assessment
HPA	Hypothalamic–pituitary–adrenal
HPLC	High-performance lipid chromatography

HRT	Hormone replacement therapy
HRV	Heart rate variability
hs-CRP	High-sensitivity CRP
HSL	Hormone-sensitive lipase
IL	Interleukin
LPL	Lipoprotein lipase
MCP-1	Monocyte chemotactic protein-1
MR	Mineralocorticoid receptor
MRI	Magnetic resonance imaging
NNR	Nordic nutritional recommendations
NO	Nitric oxide
NOS	Nitric oxide synthetase
PD	Paleolithic-type diet
PET	Positron emission tomography
PHF	Power of high frequency
PLF	Power of low frequency
PVAT	Perivascular adipose tissue
SAT	Subcutaneous adipose tissue
SNS	Sympathetic nervous system
TG	Triglyceride (triacylglycerol)
TIPSS	Transjugular intrahepatic portal-systemic shunt
TNF- $\alpha$	Tumor necrosis factor-alpha

t-PA	Tissue plasminogen activator
VAT	Visceral adipose tissue
VLDL	Very-low-density lipoprotein
WAT	White adipose tissue

# SAMMANFATTNING PÅ SVENSKA

Förekomsten av fetma i Sverige har fördubblats de två senaste årtiondena och fetma är starkt kopplat till diabetesutveckling och hjärt-kärlsjukdom. Hjärt-kärlsjukdom är idag den vanligaste dödsorsaken i världen. Yngre kvinnor har låg risk för hjärt-kärlsjukdom. Efter klimakteriet förändras kvinnors fettfördelning i kroppen till ökad fettansamling runt buken. Den förändrade fettfördelningen är starkt associerad till högt blodtryck, höga blodfetter och nedsatt insulinkänslighet, vilket kan vara en förklaring till den kraftigt ökade risken för hjärt-kärlsjukdom hos äldre kvinnor. Ökad förståelse för de bakomliggande mekanismerna till förändrad fettfördelning är därför av stort intresse och detta kan förhoppningsvis hjälpa oss att förebygga hjärt-kärlsjukdom hos både män och kvinnor.

Stresshormonet kortisol bildas i binjurarna och regleras via hypothalamus och hypofysen. Kortisol bildas också lokalt i fettväven via enzymet  $11\beta$ -hydroxysteroid dehydrogenas ( $11\beta$ -HSD) typ 1. Överproduktion av kortisol hos både försöksdjur och människa (t.ex. vid Cushing's syndrom) leder till fettansamling runt buken och en rad andra metabola förändringar liknande dem som ses vid vanlig fetma. Detta har lett fram till hypotesen om kortisol som betydelsefull aktör i utvecklingen av fetma och fetmarelaterade sjukdomar. Flera läkemedelsbolag utvecklar för närvarande hämmare av enzymet  $11\beta$ -HSD typ 1 i förhoppning om att kunna behandla fetmarelaterade sjukdomar.

Blodflödet i fettväv varierar flerfaldigt beroende på stress, fysisk aktivitet och födointag. Hos överviktiga personer ses ett kraftigt nedsatt blodflöde i fettväven efter måltid vilket kan bidra till förhöjda nivåer av cirkulerande lipider och glukos, vilket är viktiga komponenter i utvecklingen av fetmarelaterad sjuklighet. Variationerna i blodflödet har en oklar roll och regleringen av blodflödet i fettväv är endast delvis känd. Nyligen publicerade data visar att nedsatt upptag av triglycerider i fettväv och nedsatt frisättning av fria fettsyror från fettväven kan utgöra den bakomliggande mekanismen till inlagring av fett i andra organ (t.ex. lever och muskler), vilket i sin tur är grunden för nedsatt insulinkänslighet och diabetesutveckling. Dessa fynd ökar uppmärksamheten på blodflödets roll som transportör av olika substanser till och från fettväv.

I studie I undersöktes totalt 10 män med vävnadsspecifik provtagning av underhudsfett, lever och djupt liggande buk fett. För första gången hos människa visar vi att kortisol bildas i underhudsfettet via  $11\beta$ -HSD1 i sådana mängder att det sannolikt bidrar till den totala kortisolproduktionen och

kortisols effekter i övriga delar av kroppen. Aktiviteten av 11 $\beta$ -HSD1 i djupt liggande fettväv är otillräcklig för att öka kortisolkoncentrationen i vena porta och har sannolikt ej betydelse för kortisols effekter på levern.

I studie II studerades blodflödet i underhudsfett och dess reglering hos 43 kvinnor. Kvinnorna var indelade i fyra grupper utifrån ålder och BMI (unga/smala, unga/överviktiga, äldre/smala, äldre/överviktiga). Resultaten visar att basalt blodflöde är associerat till kväve-oxid (NO)-aktivitet och stimulerat blodflöde till aktiviteten i det icke viljestyrda nervsystemet (autonoma nervsystemet). Hos äldre/överviktiga kvinnor ses en störd blodflödesreglering, vilket kan bidra till metabola rubbningar hos denna grupp.

I studie III undersöktes fettfördelningens och fettvävshormoners effekter på blodflödet hos ovan beskrivna kvinnor (43 försökspersoner). Fettfördelningen (underhudsfett resp. djupt liggande bukfett) bestämdes med skiktröntgen och fettvävshormonerna leptin och adiponektin analyserades i blodprover. Resultaten visar att blodflödet i fettväv är relaterat till både underhudsfett och djupt liggande bukfett. Fettvävsblodflödet är dessutom relaterat till leptin, men inte till adiponektin.

I studie IV undersöktes blodflödet i underhudsfettet hos äldre/överviktiga kvinnor. Sjuttiofyra kvinnor lottades mellan kost enligt Nordiska näringsrekommendationer (NNR) och Modifierad stenålderskost (Paleolitisk kost, PD). Matlagningskurs följdes av undersökningar vid studiestart, 6, 12 och 24 månader. Individerna undersöktes med bl.a. fettvolymsbestämning (magnetrontgen), hjärtfrekvensvariabilitet och olika biokemiska markörer. Fettvävsblodflödet undersöktes på 14 av individerna. Resultaten vid 6 månader visar säkerställd viktnedgång och minskning av fettvolymerna i båda kostgrupperna. Förändringarna var mer uttalade i PD-gruppen. Blodflödet i fettväven minskade i NNR-gruppen, men förblev oförändrat i PD-gruppen.

Sammantaget visar denna avhandling att stresshormonet kortisol bildas i signifikanta mängder i underhudsfett via enzymet 11 $\beta$ -HSD1. Hos äldre/överviktiga kvinnor ses en störd blodflödesreglering i fettväv. Blodflödet i fettväv är relaterat till både mängden underhudsfett och djupt liggande bukfett. Paleolitisk kost kan leda till metabola fördelar jämfört med vanliga kostråd. Resultaten ökar förståelsen för fettvävens roll i utvecklingen av fetma och fetmarelaterade sjukdomar. Kunskaperna kan också bidra till utvecklingen av kostråd och läkemedel mot fetmarelaterade tillstånd.

Ytterligare studier krävs för att verifiera och öka kunskapen inom detta område.

# INTRODUCTION

In all animals, energy reserves are essential. The lack of such reserves leads rapidly to death, and animals have developed the capacity to store energy as fat that can be used to survive food shortages. However, both starvation and obesity are pathological, and the abundance of food in industrialized countries has led to an obesity endemic. The prevalence of obesity (BMI  $\geq 30$ ) in Sweden has doubled during the last two decades and now exceeds 10% in both men and women.<sup>1</sup> Increasing abdominal obesity in older women is particularly alarming.<sup>2</sup> Changes in food consumption behaviors and decreased energy expenditure resulting from a sedentary lifestyle are possible explanations for the persistently positive energy balance. There also has been the question of whether biological differences between individuals could contribute to the development of obesity. Despite a few positive trends in the prevalence of obesity,<sup>3</sup> it remains a global health problem associated with diabetes, cardiovascular disease (CVD), and shortened lifespan.<sup>4</sup> CVD is the leading cause of death in both men and women in Sweden.<sup>5</sup> A clinical observation is that postmenopausal women have increased visceral adiposity in comparison with premenopausal women<sup>6</sup> that may partly explain the postmenopausal increase in cardiovascular risk.

Adipose tissue functions include control of energy metabolism, such as storage of triglycerides (TGs) and free fatty acid (FFA) release. It also catabolizes TGs to release glycerol and FFAs that participate in glucose metabolism in liver and other tissues. Moreover, adipose tissue secretes hormones, cytokines, and proteins that exert specific biological functions within and outside adipose tissue. Knowledge of adipose tissue as a highly active metabolic organ with numerous endocrine functions and understanding the interaction between adipose tissue and other organs is critical for the development of treatment strategies targeting obesity and obesity-related disorders. This thesis focuses on adipose tissue-specific production of the hormone cortisol, regulation of adipose tissue blood flow (ATBF), and the effect of weight reduction and diet on ATBF.

## Physiology of adipose tissue

The main role of adipose tissue is storing energy in the form of TGs and releasing it in the form of non-esterified fatty acids when needed to other organs. Other basic functions are thermal insulation and mechanical cushioning. However, the last two decades have seen great strides in

recognizing the adipocyte as a secretory cell and the adipose tissue as a highly active metabolic and endocrine organ. Its functions are regulated by multiple influences, changing with time and nutritional state.

The adipose tissue consists of different cell types: adipocytes (cells that store fat), pre-adipocytes (that can differentiate into mature adipocytes), endothelial cells (lining blood vessels), and macrophages. Under the microscope, two types of adipose tissue can be distinguished, brown and white. Brown adipose tissue gets its color from a large number of mitochondria and has unique metabolic properties important to animals that need to generate heat, such as hibernating mammals. A previous report has also highlighted brown adipose tissue as a possible regulator of energy expenditure.<sup>7</sup> However, in humans, adipose tissue is almost all of the white type.

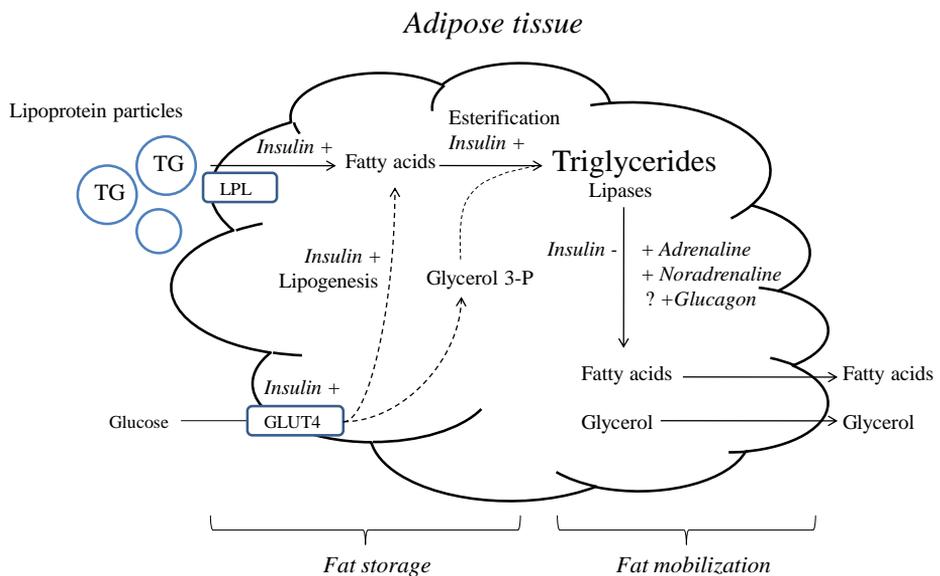
A large proportion of the total energy metabolism in the body consists of the flow of fatty acids in and out of the adipose tissue, and these transports require regulation on a minute-by-minute basis. The importance of intact regulation of adipose tissue is demonstrated in conditions with excessive concentrations of lipid fuels in plasma, leading to atherosclerosis, ectopic lipid deposition, diabetes, and in severe cases, ventricular fibrillation.<sup>8, 9</sup>

### **Fat storage and mobilization**

Fat mass depends on both adipocyte size and cell number. The number of adipocytes is determined during early childhood, and the adipocyte turnover rate in humans was recently established to be ~10% per year.<sup>10</sup> Parallel to increased body fat, fat cell mass increases to a maximum of 0.7–0.8  $\mu\text{g}$  lipids per cell, after which there is a more rapid increase in fat cell number.<sup>11</sup> Of note, obese people have a lower capacity for recruitment of new adipocytes<sup>12</sup> and an impaired differentiation of preadipocytes to adipocytes.<sup>13</sup> Therefore, most of adult-onset obesity appears to be related to adipocyte hypertrophy.

There are two main pathways for fat deposition (Figure 1): (1) uptake of TGs from plasma and (2) de novo lipogenesis (synthesis of lipids from other sources, particularly glucose); of these, uptake from plasma is by far the most important.<sup>14</sup> TGs are transported in the plasma in the form of lipoprotein particles. Because of the size of the biggest particles, they cannot escape from the capillaries into the interstitial fluid. To overcome this difficulty, adipocytes produce the enzyme lipoprotein lipase (LPL). This

enzyme hydrolyzes TGs to release FFAs, which then can diffuse through the capillaries and reach the adipocytes. LPL is stimulated by insulin, and insulin itself is stimulated by elevation in blood glucose concentration. Thus, after a typical meal, the uptake of fat (and glucose) into adipose tissue will be stimulated.<sup>15</sup> The activation of LPL by insulin is rather complex and involves increased transcription of the enzyme; therefore, it is a slow process taking about 3–4 hours. The uptake of TGs into the adipocytes involves fatty acid translocase/CD36 (a member of the family of “scavenger receptors”). Within the adipocytes, the fatty acids are esterified to form TGs and stored as lipid droplets. Insulin also stimulates the other pathway of fat deposition, *de novo* lipogenesis, at multiple points. The amount of TGs stored within adipocytes then reflects the balance between energy intake and energy expenditure over a long period.



**Figure 1.** Overview of fatty acid and glucose metabolism in adipose tissue. Modified from Frayn 2010.<sup>16</sup> *Glut4*, glucose transporter 4; *Glycerol 3-P*, glycerol 3-Phosphate; *LPL*, lipoprotein lipase; *TG*, triglyceride.

In fasting or in times of stress, mobilization of fat results in liberation of fatty acids into the plasma, bound to albumin as non-esterified fatty acids. This process is called lipolysis. The enzymes catalyzing this process are hormone-

sensitive lipase (HSL) and adipose TG lipase (ATGL), situated within the adipocyte.<sup>17</sup> The activity of these lipases requires a fine-tuned and fast regulation. The better-understood HSL is inactivated by insulin and stimulated by catecholamines.<sup>17</sup> A further effect of insulin is increased production of glycerol 3-phosphate, resulting in increased re-esterification of intracellular fatty acids to form TGs. In obesity, catecholamine-induced lipolysis and HSL/ATGL expression are reduced, which has been proposed as a cause of excessive body fat accumulation.<sup>18</sup> On a hormonal level, there is also normally a balance between the effects of cortisol and insulin, promoting lipid accumulation, and sex steroids and growth hormone prevent such lipid accumulation. When this balance is disturbed either by elevated cortisol and insulin or low secretion of growth hormones or sex steroids, fat accumulation (especially visceral fat) will occur.<sup>19</sup>

## **Different fat depots**

Adipose tissue is distributed through the body in different depots. Some of them are small and seem to have a primary function other than energy depots (for example, the popliteal), while other fat depots are larger and have significant roles in fat storage and mobilization. Among the abdominal depots, the anterior subcutaneous depot is usually the largest and has the capacity to expand the most.<sup>20</sup> Since some early clinical observations<sup>21</sup> that upper-body obesity is associated with diabetes and atherosclerosis, much work has been carried out targeting an understanding of the physiological differences between fat depots. Visceral fat accounts for ~20% of total body fat in men, compared with only 6% in premenopausal women. Therefore, two separate phenotypes of fat distribution have been characterized, the female (or gynoid) fat distribution with accumulation of subcutaneous fat on hips, thighs, and buttocks, and the male (or android) distribution with particularly intraabdominal (central) fat. Of note, compared to men, women are more protected from CVD until their body fat distribution changes with menopause towards the android distribution.<sup>22, 23</sup> The general picture is that obesity with central fat accumulation is associated with increased blood pressure and plasma TG levels<sup>24, 25</sup> and various atherogenic and diabetogenic abnormalities,<sup>26, 27</sup> but it has been debated whether visceral fat is a causal factor or simply a marker of a dysmetabolic profile.<sup>28</sup> A possible link between fat distribution and metabolic profile could be that intraabdominal adipocytes have the highest metabolic activity (and thus the highest rates of lipolysis), followed by subcutaneous adipocytes and with the lowest response in lower body fat.<sup>29</sup> One reason for the attention to the visceral depot is that venous drainage from these depots is directed mostly into the portal vein,

and its metabolic products therefore reach the liver directly. Another possibility is that there seems to be a depot-specific secretion of adipose tissue-related proteins. Visceral adipose tissue (VAT) could contribute to a harmful adipokine profile by secreting less 'beneficial' adipokines and more pro-inflammatory molecules compared with peripheral fat. Moreover, obesity with peripheral fat accumulation seems to have a protective role.<sup>30</sup> The subcutaneous depot has also been proposed to act as a "metabolic sink," with the ability to accommodate excess TGs and thus prevent the flow of lipids to other organs such as the liver and skeletal muscle.<sup>31</sup> The protective properties of peripheral fat depots have been confirmed in many studies, showing not only an improved lipid profile but also direct effects on vascular health, with lower aortic calcification and reduced arterial stiffness. Unpublished data from our research group have even indicated that peripheral fat protects against total and cardiovascular death. In addition, a third abdominal adipose layer has recently been described, separating the subcutaneous adipose tissue (SAT) into a superficial and a deep compartment, which also may have metabolic significance.<sup>32</sup>

### **Ectopic fat deposition and lipotoxicity**

Inappropriately stored fat in non-adipose tissue, called ectopic fat deposition, has been proposed to underlie obesity-associated insulin resistance.<sup>33</sup> In obesity, poorly understood genetic and environmental factors in combination with a positive energy balance modify normal adipocyte biology leading to adipocyte hypertrophy, hypoxia, altered secretion of adipokines, activation of inflammatory pathways, and eventually adipocyte necrosis. These events lead to recruitment of macrophages, development of adipocyte insulin resistance, release of FFAs into the circulation, and excess lipids accumulated in distant tissues. Accordingly, the interaction between adipocytes and macrophages leads to lipotoxicity-induced metabolic dysfunction in the liver, pancreas, skeletal muscle, and heart.<sup>34-36</sup> Accumulation of lipids in muscle and liver is an early hallmark of type 2 diabetes, and in pancreas, lipid accumulation has been shown to precede changes in glucose-mediated insulin production.<sup>37</sup> The excess lipids in the heart muscle are suggested to induce insulin resistance, impaired glucose oxidation, and ultimately heart failure.<sup>38</sup> Moreover, reduced intra-myocellular lipid content by administration of peroxisome proliferator-activated receptor-gamma agonists or by weight reduction improves insulin sensitivity, supporting this association.<sup>39, 40</sup> Of note, deposition of ectopic fat differs between sexes and among ethnicities.<sup>41, 42</sup> Despite the close relationship between intra-myocellular lipid content and insulin resistance,

there are some contradictions. In studies including diets or physical activity, altered insulin resistance can be achieved independent of intra-myocellular lipid content.<sup>43</sup> Whether ectopic lipid deposition precedes or succeeds insulin resistance is unclear.

## **Blood flow**

Adipose tissue is highly vascularized. In obese people, the total perfusion through both abdominal subcutaneous and visceral depots can reach up to 900 mL/min,<sup>44</sup> which underscores ATBF as a potential powerful regulator of adipose tissue metabolism. The adipose tissue varies in its vascularity both between depots and within the tissue itself. For example, the tip of the epididymal fat pad contains a high vessel density compared to the rest of the depot.<sup>45</sup> Metabolically, the adipose vasculature serves to transport systemic lipids to their storage depot in the adipocytes, which means that expansion and reduction of the fat mass thus relies on the adipose tissue circulation. In addition to preventing hypoxia, the microvasculature is also a potential source of the adipocyte progenitors in adipose tissue.<sup>46</sup>

In the fasting state, the abdominal subcutaneous ATBF is around 3 mL blood per 100 g tissue per minute, compared to 1.5 mL in a resting skeletal muscle.<sup>47</sup> The ATBF is very labile, and the response to a meal varies significantly among individuals. In healthy people, the blood flow increases up to four-fold in response to a meal.<sup>48-50</sup> The physiological meaning of the postprandial increase in blood flow has been widely discussed. It has been speculated that ATBF alone could act as a modulator of insulin sensitivity by delivering FFAs at the right time either for the metabolic need or for the systemic and dynamic delivery of adipose tissue-derived hormones implicated in insulin sensitization, such as leptin and adiponectin.<sup>51</sup> Furthermore, ATBF may have importance in metabolic physiology in that the extraction of plasma TGs increases with increasing blood flow.<sup>52</sup>

The peak ATBF at about 30 minutes following a mixed meal<sup>50</sup> coincides with the suppression of circulating non-esterified fatty acids and increased plasma insulin concentration,<sup>53</sup> but insulin itself does not seem to be the actual stimulus.<sup>54</sup> Of note, ATBF is increased after glucose intake<sup>48</sup> and a mixed meal,<sup>53</sup> whereas fat intake alone does not evoke a blood flow response.<sup>55</sup> ATBF increases during the night, probably reflecting increased duration of fasting.<sup>56</sup> Extended fasting (14 h–22 h) causes no further change in flow,<sup>57</sup> but more extreme fasting (72 h) increases the blood flow further

about 1.5-fold.<sup>58</sup> An increase in ATBF is also seen during exercise,<sup>59, 60</sup> although it is not as marked except for very prolonged exercise. It is clearly shown that both fasting ATBF<sup>50, 61, 62</sup> and ATBF responsiveness to nutrients<sup>50, 62</sup> are reduced in obesity and that this impairment is associated with insulin resistance.<sup>49, 62</sup> A potential consequence of diminished or absent ATBF response to nutrient ingestion could be decreased tissue glucose and TG extraction, resulting in postprandial hyperglycemia and hyperlipidemia, conditions that predispose to CVD. Recent data from McQuaid and colleagues<sup>36</sup> demonstrated that obese individuals downregulate systemic FFA delivery from adipose tissue and exhibit an inability to store the chylomicron-TG-derived fatty acids after a meal, providing the possible pathophysiological basis for ectopic fat deposition and lipotoxicity. These findings in turn support the hypothesis of ATBF as a key player in the metabolic disturbances seen in obesity and related diseases.

**Table 1.** Factors found to regulate ATBF

	Type of regulation	References
Meals (carbohydrate and mixed meals)	↑	Bulow et al. <sup>48</sup> ; Karpe et al. <sup>49</sup> ; Summers et al. <sup>50</sup> ; Coppack et al. <sup>53</sup>
Fasting	↑	Hagstrom-Toft et al. <sup>56</sup> ; Klein et al. <sup>57</sup> ; Patel et al. <sup>58</sup>
Exercise	↑	Bulow et al. <sup>59</sup> ; Mulla et al. <sup>60</sup>
Obesity	↓	Blaak et al. <sup>61</sup> ; Jansson et al. <sup>62</sup> ; Summers et al. <sup>50</sup>
Mental stress	↑	Linde et al. <sup>63</sup>
Insulin resistance	↓	Jansson et al. <sup>62</sup> ; Karpe et al. <sup>49</sup>
α-adrenergic stimuli	↓	Flechtner-Mors et al. <sup>64</sup> ; Galitzky et al. <sup>65</sup> ; Ardilouze et al. <sup>66</sup>
β-adrenergic stimuli	↑	Barbe et al. <sup>67</sup> ; Millet et al. <sup>68</sup> ; Samra et al. <sup>52</sup> ; Simonsen et al. <sup>69</sup> ; Schiffelers et al. <sup>70</sup> ; Ardilouze et al. <sup>66</sup>
NO activity	(↑)	Ardilouze et al. <sup>66</sup>
Autonomous function		Funada et al. <sup>71</sup>
Angiotensin II	↓	Goossens et al. <sup>72</sup>

Regulation of ATBF has been studied extensively (Table 1). There is convincing evidence that  $\beta$ -adrenergic stimulation increases ATBF. This is shown, for instance, by adrenaline infusion<sup>52</sup> or by local delivery of  $\beta$ -adrenergic stimuli by microdialysis of the tissue using isoprenaline, dobutamine,<sup>67</sup> or isoproterenol.<sup>68</sup> In contrast, studies using  $\alpha$ -adrenergic stimuli such as norepinephrine<sup>64</sup> and clonidine<sup>65</sup> show an inhibitory effect on ATBF. An elegant study from Ardilouze et al.<sup>66</sup> demonstrated that fasting ATBF is primarily under nitric oxide (NO) regulation and to some extent under  $\alpha$ -adrenergic control and that the postprandial phase of ATBF is controlled principally by the  $\beta$ -adrenergic system. They also demonstrated that the postprandial enhancement of ATBF is independent of NO but that the NO activity determines the level at which this response takes place. A recent study using flow-mediated vasodilatation (FMD) of the brachial artery and heart-rate variability (HRV)<sup>71</sup> confirmed that endothelial function is related to fasting ATBF and that both fasting and stimulated ATBF have relationships with autonomous function.

A number of methods for determining ATBF are described in the literature. Laser Doppler flowmetry detects rapid perfusion changes but has disadvantages in the form of a propensity for movement artifacts and inability to yield absolute blood flow values.<sup>73</sup> Microdialysis of small molecules such as ethanol and urea has proved to be a valid indicator of small changes in ATBF, but rapid changes in blood flow are poorly reflected.<sup>74-75</sup> The use of radiowater and positron emission tomography (PET) also seems helpful for measuring ATBF.<sup>76</sup> Of all the techniques for determining ATBF, <sup>133</sup>Xe-washout has been the most widely applied since its introduction in 1966.<sup>77</sup> Other tracers (<sup>127</sup>Xe, <sup>81</sup>Kr, <sup>99</sup>Tc, <sup>131</sup>I-antipyrine) have been used for this purpose, but <sup>133</sup>Xe is one of the most lipid soluble and has a high distribution coefficient, allowing extended measurements on the same isotope depot.

## **Innervation**

Studies are lacking on the innervation of white adipose tissue (WAT) in humans, so most information is based on work in rodents. It has long been known that WAT is innervated by the sympathetic nervous system (SNS), and in recent studies, the presence of parasympathetic innervation has also been documented.<sup>78</sup> Activation of the SNS in the adipose tissue leads to release of the neurotransmitter noradrenaline, stimulating lipolysis.<sup>79</sup> Formerly, it was assumed that catecholamines from the adrenal medulla were the main source directing lipolysis in adipose tissue. However, several

studies have indicated that the control of lipolysis by the sympathetic innervation of adipose tissue is more important.

Surgical sympathectomy reduces lipolysis in the WAT depot. Conversely, electrical stimulation of sympathetic nerve endings stimulates lipolysis and the release of FFAs, and this response is reduced in obese women.<sup>80</sup> Parasympathetic activation also affects lipolysis.<sup>81</sup> Vagotomy reduces both insulin-dependent glucose uptake and FFA uptake by 30–40% compared to the contralateral fat pad. By contrast, the activity of the catabolic enzyme HSL increased by approximately 50% in the same experiment.<sup>78</sup> Transection of the vagal branch resulted in the decreased expression of messenger RNA for resistin and leptin.<sup>78</sup> Therefore, this work showed that both the metabolic and the endocrine functions of adipose tissue are modulated by parasympathetic innervation. Denervation of retroperitoneal adipose tissue in rats in another study resulted in increased fat pad weight<sup>82</sup>; histologically, there was proliferation of preadipocytes within a week and increased numbers of adipocytes within a month.

Of interest, the sympathetic motor neurons in the spinal cord projecting to the intra-abdominal or subcutaneous fat depots appear to be separate sets of neurons in the spinal cord and brain stem, indicating differential autonomic innervation of intra-abdominal and subcutaneous WAT.<sup>78</sup> This feature suggests that differences in body fat distribution (visceral versus subcutaneous fat) may reflect differential activities of autonomic neuron sets in the central nervous system (CNS). A physiological model has been suggested in which determinants of body fat distribution may act via the CNS. In addition, a study from Bowers and colleagues<sup>83</sup> has reported indications for functional differences in sympathetic outflow to different WAT compartments *in vivo*, but it is currently unclear to what extent the SNS mediates functional differences between fat compartments or between different (patho)physiological conditions. To date, there are no available techniques for measuring specific *in vivo* inflow and outflow of autonomous activity in adipose tissue in humans.

Studies have shown associations between reduced parasympathetic nervous system function, increased plasma concentrations of FFAs, and insulin resistance in patients with obesity and type 2 diabetes mellitus.<sup>84</sup> A prospective study showed that autonomic nervous system (ANS) dysfunction is associated with the development of type 2 diabetes mellitus.<sup>85</sup> Moreover, a study from Kalsbeek et al.<sup>86</sup> identified that there is a functional interaction among the hypothalamus, the ANS, and the adipose tissue, at least with respect to leptin secretion.

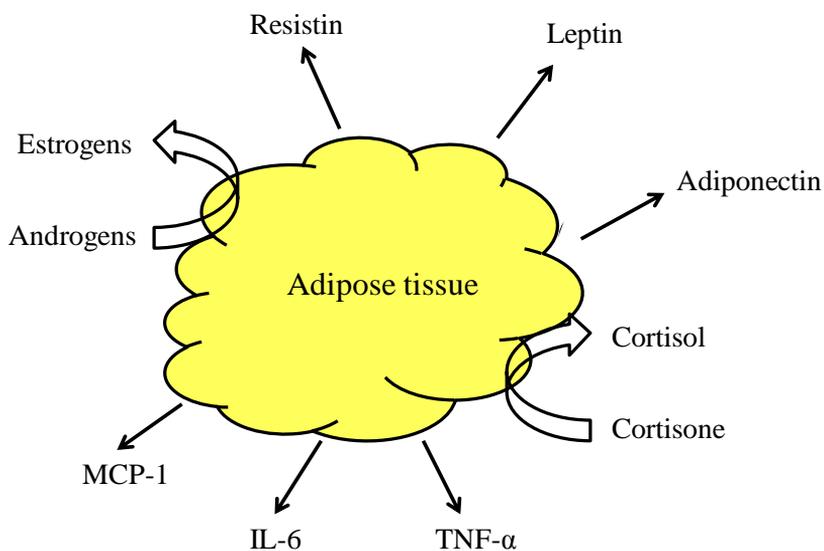
The implication of a dual innervation could be that the function of the SNS can be described in terms of catabolism, whereas the function of the parasympathetic system can be described as anabolic.<sup>87</sup> It is tempting to speculate on whether a shift in the balance between sympathetic and parasympathetic activity is of importance in human pathophysiology. However, it is unclear what the net effect would be of changes in the balance between autonomic nerve activity within or between adipose tissue compartments on whole-body glucose and fat metabolism. It is also unclear whether the ANS participates in the causes or consequences of the changes in body fat distribution seen in aging.

Three decades of studies in both animals and humans have shown a significant relationship between ANS activity and cardiovascular mortality, particularly in patients with congestive heart failure<sup>88</sup> and myocardial infarction.<sup>89</sup> The explanation is likely increased sympathetic or reduced vagal activity leading to modulated cardiac automaticity, conduction, ventricular tachyarrhythmias, and sudden death, which is one of the leading causes of cardiovascular-related mortality.<sup>90</sup> In 1965, Hon and Lee<sup>91</sup> discovered that fetal distress was preceded by alterations in interbeat intervals before any appreciable change occurred in the heart rate itself. During the 1970s, a number of simple bedside tests of short-term RR (the interval from the peak of one QRS complex to the peak of the next as shown on an electrocardiogram) differences were devised to detect autonomic neuropathy in diabetic patients.<sup>92</sup> The clinical significance of HRV became appreciated in the late 1980s, when it was confirmed that HRV was a strong and independent predictor of mortality after an acute myocardial infarction.<sup>93</sup> Moreover, the Framingham Study has found that reduced HRV in short-term recordings (2 h) predicts cardiac events, hypertension, and hyperglycemia.<sup>94-96</sup>

## **Metabolic and endocrine functions of adipose tissue**

Paradoxically, the absence of WAT, as in lipodystrophy, induces diabetes.<sup>97</sup> An adequate amount of adipose tissue and suitable physiological levels of adipokines seem to be required to maintain whole-body metabolic homeostasis. Adipose tissue was first suggested to have an endocrine function by Siitteri,<sup>98</sup> who showed the tissue's ability to interconvert steroid hormones. Cells within the adipose tissue thus express the enzymes to interconvert steroid hormones; for example, estrogens can be produced from androgens, leading to some important consequences,<sup>99</sup> as in obesity when increased estrogens may be produced. In postmenopausal women, estrogen levels drop because of loss of ovarian function, and estrogen production is

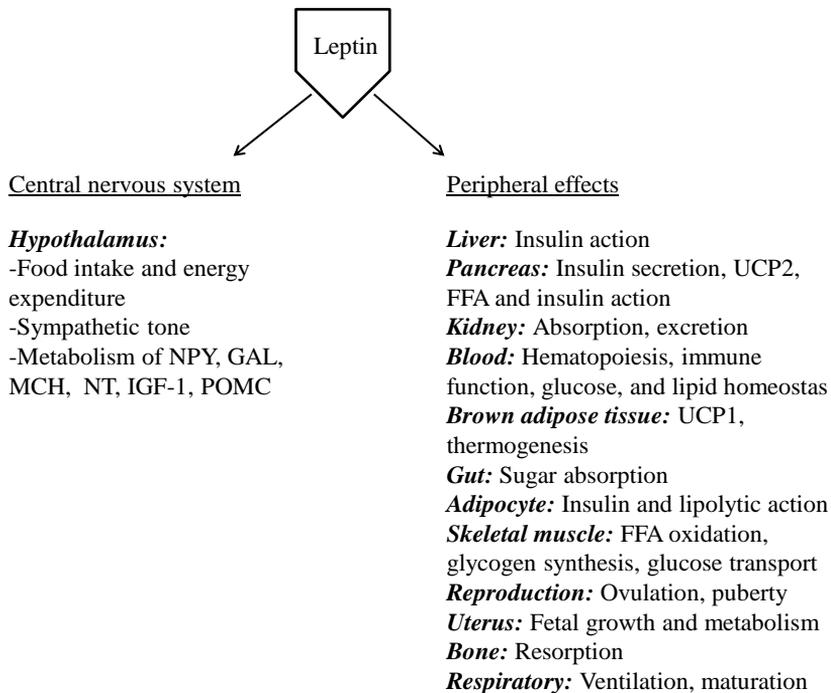
taken over by extragonadal tissues, mainly adipose tissue and liver. Increased attention has fallen on adipose tissue production and secretion of a wide range of proteins. Some of these proteins are classical cytokines and some are structurally related to cytokines, which has led to the term adipokines (adipocytokines) (Figure 2).



**Figure 2.** Endocrine functions of adipose tissue. MCP-1, monocyte chemotactic protein-1; IL-6, interleukin-6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

*Leptin* A paradigm shift came with the discovery of leptin,<sup>100</sup> the circulating peptide hormone produced by adipocytes, which regulates body weight by effects on food intake and metabolism (Figure 3).<sup>101, 102</sup> Leptin was identified in 1994 by cloning of the *ob* gene, which determines the development of obesity in *ob/ob* mice.<sup>100</sup> Because of the lack of leptin, these animals develop morbid obesity, insulin resistance, hyperinsulinemia, diabetes mellitus, stimulation of the hypothalamo–pituitary–adrenal (HPA) axis, and in the homozygous form, infertility. Chronic administration of leptin to these mice results in weight loss and maintenance of their weight loss.<sup>103</sup> Moreover, the administration of recombinant leptin to children with congenital leptin deficiency decreases fat mass, among other effects.<sup>104</sup> The circulating level of

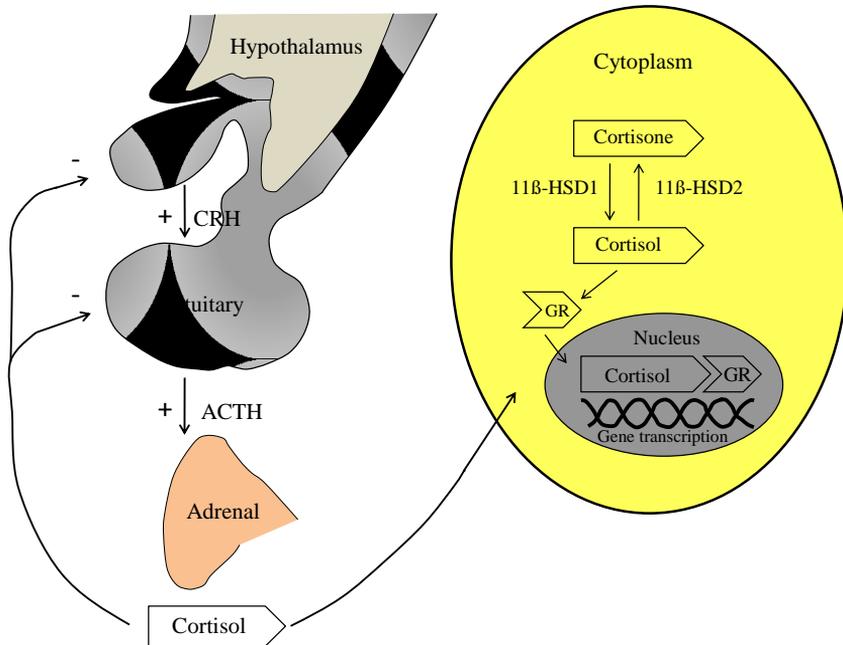
leptin is related to adiposity,<sup>105</sup> and recent evidence has implicated leptin as an independent risk factor for CVD.<sup>106-108</sup> Sex is an important factor determining plasma leptin, with women having higher leptin concentrations than men for any given degree of fat mass. Leptin secretion in vitro and higher levels of leptin mRNA are found in subcutaneous adipocytes compared with visceral fat.<sup>109, 110</sup> Several mechanisms have been proposed linking elevated leptin to vascular disease, including stimulation of platelet aggregation,<sup>111</sup> impairment of fibrinolysis,<sup>112</sup> and activation of the SNS.<sup>113</sup> Moreover, pro-inflammatory and immunostimulatory effects of leptin have also been described.<sup>114, 115</sup> There is evidence of leptin having regulatory effects on endothelial cells in rodents,<sup>116, 117</sup> but less is known about its effects on human endothelium.



**Figure 3:** Central and peripheral effects of leptin: NPY, neuropeptide Y; GAL, galanin; MCH, melanin-concentrating hormone; NT, neurotensin; POMC, proopiomelanocortin; IGF-1, insulin-like growth factor-1; UCP, uncoupling protein; FFA, free fatty acid.

## Glucocorticoids

Glucocorticoids are stress hormones with an important role in regulating metabolic and defense responses. These hormones are generated from cholesterol, arising within the adrenal cortex, and tightly regulated by the HPA axis, with glucocorticoids regulating their own generation by negative feedback inhibition on several levels of the axis (Figure 4). In healthy individuals, cortisol is released in a diurnal pattern with high levels in the early morning and low levels in the afternoon and night. Inactivation of glucocorticoids occurs predominantly in the liver, but also in the kidney, with inactive metabolites excreted in the urine.



**Figure 4.** Regulation of cortisol by the HPA axis and pre-receptor metabolism by 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ -HSDs) (modified from Strachan et al. 2011<sup>119</sup>). ACTH, adenocorticotropic hormone; CRH, corticotropin releasing hormone; GR, glucocorticoid receptor.

During acute stress, activation of the HPA axis results in elevated plasma cortisol levels, allowing liberation of fuel (glucose and FFAs), protection against shock, and activation of the immune response (anti-inflammatory effect). On the other hand, if high levels of cortisol are sustained, as in Cushing's syndrome, the result is maladaptive effects including hypertension, dyslipidemia, central obesity, and insulin resistance. The similarities between Cushing's syndrome and the features of obesity-related morbidity have led to the hypothesis that variations in cortisol secretion or action may contribute to obesity and obesity-related diseases.<sup>118</sup>

In the early 1980s, it was demonstrated that the cortisol secretion rate was elevated in obesity and later shown that the secretion was in proportion to lean body mass.<sup>118, 120</sup> Further, it was found that metabolic clearance of cortisol was increased in obesity, as measured from the sum of cortisol and cortisone metabolites or from urine excretion of free cortisol.<sup>120, 121</sup> Cohort studies further demonstrated that fasting cortisol levels were elevated in individuals with hypertension, insulin resistance, and hypertriglyceridemia.<sup>122-124</sup> Paradoxically, circulating cortisol concentrations were low to normal in patients with obesity, and secretion rates were higher, particularly in patients with visceral obesity, which made this issue more complicated.<sup>125, 126</sup> As further reports found no evidence for an enhanced central drive to cortisol secretion,<sup>127</sup> the focus moved towards tissue-specific responsiveness of cortisol by enzymes within target cells, which either limit or amplify the local intracellular concentrations.

The relevant enzymes are the  $11\beta$ -hydroxysteroid dehydrogenases ( $11\beta$ -HSDs).<sup>128</sup>  $11\beta$ -HSD1 is expressed in many tissues, including adipose tissue and liver, and this enzyme catalyzes the conversion of inactive cortisone to cortisol, thus potentially amplifying local cortisol concentrations and glucocorticoid receptor (GR) activation (Figure 4).<sup>129</sup>  $11\beta$ -HSD2 inactivates cortisol to cortisone, which protects the non-selective mineralocorticoid receptor (MR) mainly in the kidney and colon from cortisol excess.<sup>130</sup> Studies with transgenic mice show that overexpressing  $11\beta$ -HSD1 in adipocytes<sup>131, 132</sup> leads to central obesity with hypertension, hyperlipidemia, hyperglycemia, and hyperinsulinemia. Mice overexpressing  $11\beta$ -HSD1 in the liver develop insulin resistance, dyslipidemia, and hypertension without obesity.<sup>133</sup> Conversely, despite high-fat feeding,  $11\beta$ -HSD1 knockout mice are protected from obesity, hyperglycemia, and dyslipidemia.<sup>131, 134</sup>

These interesting observations in mice have raised a number of questions in humans, including the following: Is there a tissue-specific increase in glucocorticoid activity; how important is  $11\beta$ -HSD1 in determining intracellular cortisol concentrations; does increased  $11\beta$ -HSD1 contribute to

features of the metabolic syndrome; and could 11 $\beta$ -HSD1 inhibition be a potential therapy to reduce intracellular cortisol concentrations?

Indeed, in obesity 11 $\beta$ -HSD1 mRNA and activity are increased in SAT biopsies<sup>135</sup> and either increased or unchanged in VAT.<sup>136</sup> The increased 11 $\beta$ -HSD1 activity in adipose tissue in obesity is balanced by decreased 11 $\beta$ -HSD1 activity in the liver so that there is no net change in whole-body cortisol generation by 11 $\beta$ -HSD1.<sup>127, 135</sup> In contrast, obese diabetic men have increased whole-body 11 $\beta$ -HSD1 activity, with sustained liver 11 $\beta$ -HSD1.<sup>137</sup> Moreover, it has been suggested that cortisol release into the portal vein from VAT contributes to hepatic insulin resistance associated with central obesity.<sup>138</sup> Overexpression of 11 $\beta$ -HSD1 in adipose tissue in mice results in a two- to three-fold increase in portal vein glucocorticoid concentrations without altering systemic levels.<sup>131</sup> However, in humans, the contribution of cortisol as generated by 11 $\beta$ -HSD1 from visceral or SAT, respectively, has been unknown.

## **Endothelial function**

Vascular endothelial cells play an important role in maintaining cardiovascular homeostasis in healthy people. In addition to providing a physical barrier between the vessel wall and lumen, the endothelium secretes a number of mediators that regulate coagulation, fibrinolysis, platelet aggregation, and vessel tone.<sup>139</sup> The primary vasodilator released by the endothelium is NO. Other relaxing factors include endothelium-derived hyperpolarizing factor, prostacyclin, C-type natriuretic factor, 5-hydroxytryptamine, adenosine triphosphate, substance P, and acetylcholine. The endothelium also releases contracting factors, such as endothelin-1, angiotensin II, and thromboxane A2. In addition, the endothelium releases tissue plasminogen activator (t-PA), playing a pivotal role in protecting against atherothrombotic events.<sup>140</sup> Endothelial dysfunction, broadly defined, occurs when there is an imbalance in the production of these mediators and when the endothelium fails to exert its normal physiologic and protective properties. This dysfunction can occur when the endothelium is damaged or missing, as in the case of arteries subjected to percutaneous coronary intervention, but in obesity, it is more likely to occur as a result of metabolic toxins.

The endothelium modulates the proliferation and injury response of the vascular smooth muscle layer. These roles of the endothelium parallel the pathology of atherogenesis,<sup>141</sup> which involves abnormalities in vascular

signaling, oxidative stress, inflammatory cells, and thrombosis. Normal endothelial function protects against these processes, and endothelial dysfunction is therefore probably central to the pathogenesis of atherosclerotic lesion development.

The pioneering experiments of Furchgott and Zawadzki first demonstrated an endothelium-derived relaxation factor that was shown to be NO.<sup>142</sup> The synthesis of NO from L-arginine is catalyzed by the endothelial nitric oxide synthase (eNOS), and NO then diffuses locally, leading to relaxation of vascular smooth muscle and inhibiting cell adhesion, platelet aggregation, and smooth muscle cell proliferation.<sup>143</sup>

Endothelial dysfunction has been closely linked to pathological findings such as obesity, hypertension, hyperlipidemia, coronary artery disease, peripheral artery disease, and subsequent manifestations of atherosclerosis.<sup>144</sup> Previous data also show that individuals with cardiovascular risk factors often have abnormalities in endothelial function before the onset of CVD.<sup>144</sup> The mechanisms by which obesity causes endothelial dysfunction are not well established but may be related to hyperglycemia, inflammatory cytokines (including interleukin [IL]-6 and tumor necrosis factor-alpha [TNF- $\alpha$ ]) or hormonal changes that occur as a result of increased subcutaneous and VAT. Hyperinsulinemia has been shown to impair endothelium-dependent vasodilatation,<sup>145</sup> and leptin, whose receptors are present in the arterial wall, has also been linked to vascular dysfunction via its effects on NO activity.<sup>146</sup> Of note, much data are lacking regarding the *in vivo* effects of leptin on endothelial function. Another candidate mechanism is elevated FFA levels. Serum FFA levels are known to be elevated in obesity and have been shown to reduce endothelium-derived relaxation in both animal models and humans.<sup>147</sup>

In the absence of CVD, advancing age is associated with a decline in endothelial function. With increasing age, there is a reduction in NO availability and an increase in the formation of reactive oxygen species. This alteration is linked to a reduction in the regenerative capacity of endothelial cells and an increase in endothelial cell apoptosis.<sup>148</sup> Women display a delayed onset and more rapid progression of endothelial dysfunction compared to men, suggesting a biologic effect of estrogen withdrawal and menopause.

Several clinical tests have been developed evaluating the functional properties of normal and dysfunctional endothelium. Today, there are two invasive (cardiac catheterization and venous occlusion plethysmography) and four non-invasive (ultrasound flow-mediated dilatation [FMD], pulse

wave analysis, pulse contour analysis, and pulse amplitude tonometry) methods described.<sup>149</sup> Because of clinical trial experience, validation, and association with cardiovascular events, the ultrasound FMD is currently the standard method for clinical non-invasive assessments. Another approach to investigating the functions of the endothelium is to study levels of molecules of endothelial origin in circulating blood. These include direct products of endothelial cells that change when the endothelium is activated, such as measures of NO biology, inflammatory cytokines, adhesion molecules, and regulators of thrombosis, as well as markers of endothelial damage and repair. One promising marker of endothelial function is asymmetric dimethylarginine (ADMA), an endogenously derived competitive antagonist of NO synthase.

The L-arginine analogue ADMA is an endogenous inhibitor of eNOS, inhibits NO formation, and thereby affects vascular function. Elevated ADMA concentrations are associated with impaired endothelium-dependent NO-mediated vasodilation, and has been used as a determinant of endothelial dysfunction.<sup>150</sup> Clinical and experimental evidence suggests that NO synthetase (NOS) activity is regulated by the ratio between the concentration of L-arginine (the natural substrate) and ADMA. Moreover, exogenous ADMA causes endothelium-dependent contraction of arteries.<sup>151</sup> Of note, ADMA level seems to be independent of traditional risk factors (age, smoking, lipid levels, etc.) and has demonstrated prognostic value in hemodialysis patients,<sup>152</sup> intensive care patients,<sup>153</sup> and patients undergoing percutaneous coronary intervention.<sup>154</sup>

## **Inflammation**

Obesity and obesity-related diseases are linked to dysfunctional adipose tissue with low-grade, chronic, and systemic inflammation. Large adipocytes release more inflammatory cytokines, such as monocyte chemoattractant protein-1 (MCP-1) and IL-6. Other consequences of adipocyte hypertrophy in both humans and mice are adipocyte cell death and local adipose hypoxia. This outcome has been demonstrated in diabetic mice showing a 30-fold increase in adipocyte death compared with control mice.<sup>155</sup> The death of the hypertrophic adipocytes facilitates infiltration of macrophages, which in turn release inflammatory proteins, causing further recruitment of macrophages and the release of inflammatory cytokines, especially TNF- $\alpha$ , which is likely synergistic with adipocytes to amplify local inflammation.<sup>156, 157</sup> Moreover, reduced tissue perfusion capacity, causing local adipose hypoxia, is thought to play a major role in macrophage accumulation. Of note, it was recently

observed that obese individuals with adipose tissue inflammation characterized by macrophage accumulation exhibit arterial dysfunction and insulin resistance compared with obese individuals with non-inflammatory adipose architecture.<sup>158</sup> Normally, the physiological role of infiltrating adipose tissue macrophages is thought to be debris clearing in nature. More than 90% of all macrophages in WAT in obese people are localized to dead adipocytes, forming multinucleate giant cells, which is a hallmark of chronic inflammation. The release of TNF- $\alpha$  and IL-6 is known to promote lipolysis, and the secretion of FFAs contributes to an increase in hepatic glucose production and insulin resistance.<sup>159</sup> Moreover, IL-6 promotes inflammation not only in adipose tissue but also in endothelial cells and liver cells.<sup>160</sup> IL-6 has also been shown to promote insulin resistance by interfering with the insulin signaling in adipose tissue.<sup>161</sup> Increased C-reactive protein (CRP) levels, which are at least partly mediated through IL-6, are found among obese individuals who are also insulin resistant.<sup>162</sup> As mentioned above, visceral fat secretes relatively higher levels of inflammatory markers.<sup>163</sup> A recent study in obese individuals showed a 50% increase in the secretion of IL-6 in the portal vein and a direct correlation between the concentration of IL-6 in the portal vein and systemic CRP levels, providing a potential link between visceral fat and systemic inflammation in individuals with abdominal obesity.<sup>163</sup>

## **Aspects of weight reduction and different diets**

The prevalence of obesity in Sweden in adults has doubled during the last two decades and now exceeds 10% among both men and women.<sup>1</sup> Although this rate is low from an international perspective,<sup>3</sup> this development during the last decades has been alarming. Improving diet and activity behaviors to reduce the prevalence of obesity and obesity-related diseases is a common goal for the EU Platform for Action on Diet, Physical Activity and Health,<sup>164</sup> and the World Health Organization global strategy on diet, physical activity and health.<sup>165</sup> Although aspects of diet have been linked to individual features of the metabolic syndrome,<sup>166</sup> the role of diet in the etiology of the syndrome is poorly understood and limited to only a few observational studies.<sup>167, 168</sup> Moreover, data are limited about the long-term effects of and adherence to different diets.

Six clinical trials<sup>169-174</sup> have examined the effects of energy-restricted diets together with increased physical activity in persons with impaired glucose tolerance, showing risk reductions in diabetes development between 30% and 70%. In two of these studies,<sup>172, 173</sup> lifestyle interventions were successful

in spite of no weight loss, and in the other four, diabetes rates decreased in relation to substantial reductions in body weight.<sup>169-171</sup> These studies provide convincing evidence that lifestyle modification reduces the incidence of diabetes among high-risk individuals. One systematic review<sup>175</sup> captured 40 intervention studies aiming at preventing weight gain, concluding that diet, alone and with the addition of exercise and/or behavior therapy, yielded significant weight loss and improvement in the metabolic syndrome and diabetes compared with no-treatment controls for at least 2 years. A reduced risk of breast cancer recurrence at 5 years and ovarian cancer in the final 4 years of an 8-year trial was observed, but no significant differences were seen between lifestyle interventions and control groups for deaths, stroke, or heart disease. Generally, the weight loss in diet studies is greatest at 6 to 12 months after initiation of the diet, with steady regain of weight subsequently.<sup>176</sup>

The mechanisms behind weight reduction and/or diets leading to improved metabolic profile are complex and largely unknown. It is known that weight reduction leads to decreased systolic and diastolic blood pressure,<sup>177</sup> decreased insulin levels,<sup>178</sup> increased insulin sensitivity,<sup>179</sup> improved lipid profile, decreased inflammatory activity (CRP, IL-6, IL-18),<sup>180</sup> and decreased levels of leptin.<sup>181</sup> Of note, adiponectin plasma levels are not as sensitive to the effect of diet interventions as are leptin levels.<sup>181</sup> Endothelial function also improves after weight reduction.<sup>182</sup> Many of these beneficial effects have a dose-dependent relationship with the amount of weight lost and appear with a weight loss of 5–10% of initial body weight.<sup>183</sup> In addition, physical activity leads to dramatic changes in leptin and adiponectin levels and improved fibrinolytic activity, contributing to an improved metabolic profile.<sup>184</sup> On a cellular level, as previously described, obese individuals have bigger cells, and because adipose cells enlarge only to a certain degree, the amount of cells increases in obese individuals. Of note, when an obese person loses weight, the number stays high but the cells shrink and are smaller than those of the non-obese controls.<sup>185</sup>

An independent risk factor of CVD is an increased ratio between the activity of the SNS and the parasympathetic nervous system. This imbalance is seen in obesity,<sup>186</sup> and weight loss seems to have a normalizing effect.<sup>187</sup>

Because chronic low-grade inflammation is a pathogenetic factor in obesity and diabetes, the anti-inflammatory properties of specific nutrients, such as of virgin olive oil<sup>188</sup> and nuts,<sup>189</sup> might also be relevant when discussing different diet regimes. Another observed aspect of nutrient composition is that a high-protein diet increases satiety, as shown in short-term studies.<sup>190</sup> Research on the glycemic index (GI) indicates that even when foods contain

the same amount of carbohydrate, there are up to five-fold differences in glycemic impact. Studies of low-GI diets have shown diverging results but yielded proven benefits in the control of diabetes.<sup>191</sup>

It has been postulated that foods that were regularly eaten during human evolution would have metabolic advantages. The Paleolithic diet, covering lean meat, fish, shellfish, fruits, vegetables, roots, eggs, and nuts but not grains, dairy products, salt, or refined fats and sugar,<sup>192</sup> became the main food pattern long after the appearance of fully modern humans. Two recent small, short-term studies have indicated that the Paleolithic diet provides health benefits by reducing blood pressure, decreasing postprandial insulin and glucose responses to an oral glucose tolerance test, and improving blood lipid profiles.<sup>193, 194</sup>

National dietary weight-loss guidelines<sup>195</sup> (energy-restricted, high in carbohydrate, low in fat) have been challenged in the last decade, particularly by proponents of low-carbohydrate diets. One meta-analysis pooled the results of early trials on low-carbohydrate diets, concluding that they were at least as effective as low-fat, high-carbohydrate diets in inducing weight loss for up to 1 year.<sup>196</sup> A study from Gardner et al.<sup>197</sup> compared four low-carbohydrate diets, showing that women assigned to follow the Atkins diet, which had the lowest carbohydrate intake, lost more weight and experienced more favorable overall metabolic effects at 12 months than women on the other three diets. Further support for low-carbohydrate diets was presented by the results of the OmniHeart trial<sup>198</sup> and Shai et al.,<sup>199</sup> demonstrating that the macronutrient composition of the diet could have effects on improving blood pressure, lipid levels, and other cardiovascular risk factors. In contrast, Sacks and colleagues showed that four diets with different contents of carbohydrates, fat, and proteins were equally successful in promoting weight loss and the maintenance of weight loss over the course of 2 years.<sup>200</sup>

# AIMS

The overall aim of the thesis was as follows:

- To investigate local cortisol production within the adipose tissue and to elucidate aspects of adipose tissue blood flow regulation.

Specific aims were as follows:

- To detect and quantify  $11\beta$ -HSD1 activity and local cortisol production within subcutaneous adipose tissue using an arteriovenous technique.
- To study adipose tissue blood flow in different groups of women and its relation to weight, menopausal status, endothelial function, and autonomic nervous system activity.
- To study the association between adipose tissue blood flow and different fat depots and adipokines.
- To study adipose tissue blood flow, endothelial function, and autonomic nervous system activity during diet and long-term weight reduction.

# SUBJECTS AND METHODS

This section briefly describes the participants and methods that were central to these studies. Detailed descriptions of the methods are presented in the respective papers. Local ethical approval and written informed consent were obtained from all individuals involved in the studies described in papers I–IV.

## Study design

### *Study I*

In study I, we aimed to detect adipose-specific production of cortisol and adipose-specific 11 $\beta$ -HSD1 activity using direct cannulation of veins draining adipose tissue depots during tracer cortisol infusion. This study included in total 10 men, ages 20–70 years, body mass index (BMI) 20–45 kg/m<sup>2</sup>, with normal full blood count and renal and thyroid function, and receiving no glucocorticoid therapy. To study the subcutaneous fat depot, we recruited six men (three had concurrent medical conditions and were on medications). The six participants were served breakfast (30 g cornflakes and 300 mL skim milk) at 0800–0830 h, and 5% dextrose (50 mL/h) was infused. This step was followed by tracer cortisol infusion, drawing parallel blood samples from an arterialized vein and the superficial epigastric vein (selectively draining the subcutaneous fat).

For the portal vein study, we recruited four men with transjugular intrahepatic portal-systemic shunts (TIPSS) in situ who underwent tracer infusion, drawing samples from an arterialized vein, the portal vein, and the hepatic vein. The TIPSS in each participant was in place because of portal hypertension and alcoholic liver cirrhosis, and the study was performed with individuals in a fasting state who were attending an annual check of TIPSS patency. Three of the TIPSS patients had no additional medical conditions, and three were on different medications. They had normal liver function tests and alcohol intake below 21 units/week.

## ***Studies II and III***

In study II, we aimed to investigate subcutaneous ATBF and its relation to autonomic activity and endothelial function in different groups of women. In study III, the results of which are based on extended analyses of study II, we further investigated the role of adipose tissue depot sizes on ATBF. For these cross-sectional studies, we recruited 43 healthy women, ages 20–69 years, BMI 18–42 kg/m<sup>2</sup>, by advertisements in local newspapers. The participants were divided into four groups: normal-weight (n = 11) or overweight (n = 11) premenopausal women and normal-weight (n = 10) or overweight (n = 11) postmenopausal women. The premenopausal women were studied in the follicular phase of the menstrual cycle. The women were classified as postmenopausal when they had reported no menstrual periods for 12 consecutive months. Fertile women underwent a pregnancy test. The volunteers underwent blood flow measurement by <sup>133</sup>Xe-washout in a fasting state and during oral glucose load; HRV was assessed as a measurement of autonomic activity, and peripheral blood sampling was performed, including ADMA as a marker of endothelial function. With the exception of CT, the clinical investigations were performed with each participant at rest on one occasion between 8 AM and 1 PM following an overnight fast. The CT was performed within a month of the other investigations.

## ***Study IV***

This randomized, prospective study included 72 postmenopausal overweight women, recruited by advertisements in local newspapers to the planned 24-month investigation. The participants had to be overweight/obese (BMI of at least 27) and were classified as postmenopausal when there had been no menstrual periods for 12 consecutive months. The women were randomly assigned to group education led by dietitians (workshops, eight occasions), cooking according to either “Nordic nutrition recommendations” (NNR) or the “Paleolithic-type diet” (PD). To maintain an equal intensity of treatment, the workshop format and the quality of the materials were similar between the two diet groups, except for instructions and materials specific to each diet strategy. During the first study year, each month the participants maintained diet diaries for four days. All volunteers were recommended physical activity at least 30 minutes each day (walking, swimming, bicycling). Sixty-one completed a 6-month investigation. We performed ATBF measurements in a subgroup (14 individuals, eight from the NNR-group and six from the PD-group), with time points at the start and at the 6-month follow-up. This study includes data from baseline and 6-month

follow-up regarding anthropometry, oral glucose tolerance test, ATBF, adipose tissue volumes, HRV, and endothelial function. The magnetic resonance imaging (MRI) was performed on a separate day within a month from the other investigations. The clinical investigations were performed with the individual at rest between 8 AM and 1 PM after an overnight fast.

### **Anthropometric measurements**

Height, waist (at the umbilicus level), and hip (maximum circumference over the buttocks) circumference measurements were recorded to the nearest 0.5 cm and weight to the nearest 0.5 kg in studies I, II, and III, and to the nearest 0.1 kg in study IV. Blood pressure was measured with the participant in the supine position with a mercury sphygmomanometer to the nearest 5 mmHg in studies I, II, and III and with an automatic blood pressure meter to the nearest 1 mm in study IV. Total fat mass and percentage body fat were measured by bioimpedance (study I).

### **Measures of adipose tissue distribution**

In study III, we quantified abdominal adipose tissue depot areas using single-slice CT at level L4–L5. Areas of adipose tissue depots were measured using defined identical positions in all participants. The area determinations of adipose tissue were performed with an attenuation interval (given in Hounsfield units, HU) of -190 to -30 HU, and the area determinations were based on the number of pixels fulfilling given attenuation criteria. The method, validity, and reproducibility have previously been described.<sup>201, 202</sup>

In study IV, we determined abdominal adipose tissue volumes using MRI. The MRI was performed using a clinical 1.5 Tesla scanner. For abdominal adipose tissue imaging, an axial multislice T1-weighted gradient echo acquisition consisting of 16 slices 10 mm thick with 1-mm inter-slice gaps centered at the L4–L5 intervertebral disk level was performed during breath hold. The MRI data were analyzed using software developed in-house. The visceral and subcutaneous adipose depots were quantified using a modified version of previously described software.<sup>203</sup> Previous analysis of repeated measurements from 29 individuals (not included in this study) with varying BMIs ( $24.2 \pm 3.76$  kg/m<sup>2</sup>), including participant repositioning, gave average coefficients of variations (defined as standard deviation divided by mean) of 2.81% and 1.45% for VAT and SAT, respectively (unpublished data).

## **Blood chemistry**

In studies I, II, and III, we used arterialized blood by cannulating the cephalic vein in a retrograde direction and placed the hand in a heated chamber (60°C). To maintain flow, the catheter was infused with saline 50–100 mL/h, and the arterialization was checked regularly and was always greater than 90%. A 10-mL aliquot of arterialized venous blood was collected twice before the procedure and at 15, 30, 45, 60, 90, and 120 minutes after glucose intake. At each time point, 1.5 mL of blood was placed in an EDTA tube, immediately put on ice, and centrifuged at 4°C. The plasma was collected and stored at -80°C. The remaining blood volume was centrifuged, and the serum was stored at -80°C for later analyses.

In study IV, we used venous blood samples (not arterialized), handled as described above.

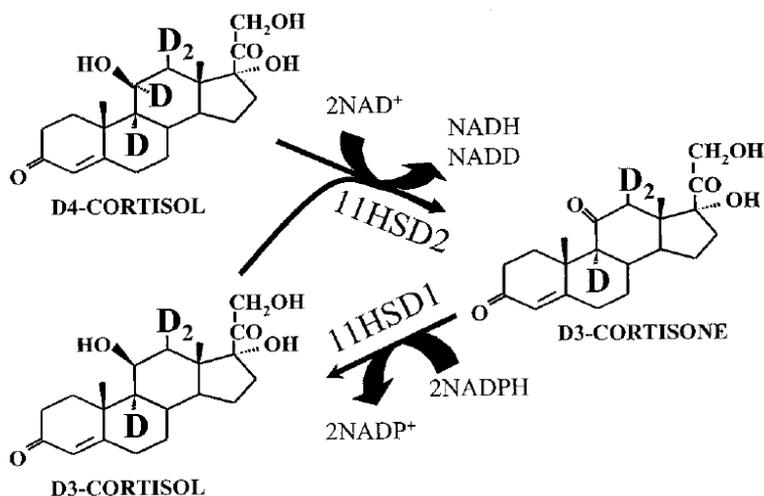
In study I, labeled and non-labeled cortisol and cortisone were measured by liquid chromatography–tandem mass spectrometry. In study II, ADMA was analyzed with a high-performance liquid chromatography (HPLC) system. In study III, high-sensitivity (hs)-CRP and IL-6 were measured by ELISA, and leptin and adiponectin levels were analyzed with double-antibody radioimmunoassays. In study IV, we determined levels of ADMA by using a commercial ADMA ELISA kit. All other blood samples were analyzed with standard laboratory methods in the clinical laboratories at the Umeå University Hospital, Umeå, Sweden, or at Uppsala University Hospital, Uppsala, Sweden.

## **Measuring cortisol release from subcutaneous adipose tissue (study I)**

In this study, we aimed to determine the release of 11 $\beta$ -HSD1–derived cortisol from adipose tissue by using a labeled cortisol molecule combined with adipose depot-specific catheterizations.

The tracer used in our study, (9,11,12,12-<sup>2</sup>H<sub>4</sub>)cortisol (called d<sub>4</sub>F) contained four deuteriums in the steroid skeleton, one of which was in the 11-position, analogous to that prepared by Ulick et al.<sup>204</sup> The tracer could be distinguished from endogenous cortisol by mass difference and was expected to be metabolized as shown in Figure 5. On metabolism by dehydrogenation, (9,11,12,12-<sup>2</sup>H<sub>4</sub>)cortisol loses 11-deuterium, forming a labeled cortisone molecule (trideuterated cortisone, called d<sub>3</sub>E) with a mass three times greater than that of the endogenous cortisone. The trideuterated cortisone is

then regenerated by reduction to trideuterated cortisol. The dilution of d4-cortisol by d3-cortisol therefore indicates 11 $\beta$ -HSD1 reductase activity and is independent of metabolism of both d4-cortisol and d3-cortisol by other enzymes. Using this technique, previous studies have shown substantial cortisol release into the hepatic vein by 11 $\beta$ -HSD1 in the splanchnic circulation.<sup>205, 206</sup> Because of the diurnal variation and response to stress of endogenous cortisol secretion, the participants were pre-treated with oral dexamethasone and infused with exogenous unlabeled cortisol to suppress the HPA axis. Another aim was to maintain circulating cortisol and cortisone concentrations at stable non-stressed physiological levels during steady-state measurements.



**Figure 5.** Metabolism of d4-cortisol to form d3-cortisone and d3-cortisol. D, deuterium = <sup>2</sup>H. <sup>205</sup>

## **Samplings from the subcutaneous adipose tissue**

By using a fiberoptic lamp and a 20-G 15-cm catheter, we identified and cannulated the superficial epigastric vein, as previously described.<sup>207</sup> Parallel blood samples were taken from the arterialized hand vein and superficial epigastric vein at intervals. The oxygen (O<sub>2</sub>) saturation was confirmed to be >85% to ensure that blood was not collected from deeper structures (the cava vein has a lower O<sub>2</sub> saturation).

## **Samplings from the portal and hepatic veins**

The right internal jugular vein was punctured under local anesthesia (5 mL 2% lidocaine), and a 5F pigtail catheter was passed into the TIPSS under X-ray guidance. The patency of the TIPSS was confirmed, and the catheter was then positioned in the portal vein for sampling, followed by separate cannulation of the hepatic vein with a 5F vertebral catheter for sampling. Simultaneous blood samples were taken from the portal, hepatic, and arterialized veins at intervals.

Kinetic analyses for each participant required knowledge of ATBF, infusion rates of unlabeled cortisol, and the venous and arterial concentrations of unlabeled cortisone and cortisol and labeled cortisone and cortisol. The calculations used the mean of measurements in steady state, between 180 and 210 minutes of d4-cortisol infusion. Where possible, kinetic calculations relying on tracer:tracee ratios rather than concentrations were favored to minimize variability. The equations derived from Wolfe and Chinkes<sup>208</sup> have been previously used in a number of similar studies.<sup>205, 206, 209</sup> The different fractions of labeled and unlabeled cortisone and cortisol were analyzed by mass spectrometry, a tool for measuring the molecular weight of a sample.

## **Measuring adipose tissue blood flow (studies I–IV)**

The <sup>133</sup>Xe-washout is based on the kinetic model of saturation–desaturation,<sup>210</sup> in which the blood flow is measured in the initial slope of the desaturation curve. Various adipose tissue depots exhibit different partition coefficients between blood and adipose tissue. In our SAT studies, the partition coefficient was taken as 10 mL/g.<sup>54</sup> A dose of 1–2 MBq of <sup>133</sup>Xe (gas) was injected paraumbilically (3–5 cm from the umbilicum) into the SAT (depth ~15 mm). The <sup>133</sup>Xe-activity was then monitored by collecting continuous 20-second readings from the portable gamma-counter probe

(Mediscint, Oakfield Instr, Oxfordshire, UK) placed over the exact site of injection and taped firmly in place.<sup>186</sup> Blood flow was calculated from a semilog plot of disappearance of counts versus time in 10-minute intervals according to the equation:  $ATBF \text{ (mL/min/100 g)} = \text{slope of semilog plot} \times 60000 \text{ (60 s} \times 10 \text{ partition coefficient} \times 100 \text{ g)}$ .<sup>77, 186</sup>

### **Heart rate variability (studies II, IV)**

The HRV is a non-invasive electrocardiographic marker of the sympathetic and vagal activity affecting the sinus node of the heart. Three main components are distinguished in a spectrum calculated from short-term recordings: very low-frequency band, low-frequency band, and high-frequency band components. The distribution of low frequency and high frequency is not fixed but may vary in relation to changes in autonomic modulations of heart period. Some authors consider low frequency as a measure of sympathetic modulations, but the consensus is that it reflects a mixture of both autonomic inputs while parasympathetic activity is the major contributor to the high-frequency component.<sup>211-214</sup> In a healthy heart, there will be continuous variations of sinus cycles reflecting a balanced sympathovagal state and normal HRV. Under resting conditions, both the sympathetic and parasympathetic systems are tonically active, with a predominant vagal effect. In a state of damaged heart or affected sympathovagal balance, the changes in afferent and efferent fibers will result in diminished HRV. Physiological conditions affecting HRV are age, sex, respiration, circadian rhythm, and body position.<sup>215</sup>

In study II, the participants performed a short-term recording followed by a 2-hour continuous recording during an oral glucose tolerance test. After 10 minutes of supine rest, blood pressure was measured with a sphygmomanometer and the continuous electrocardiogram, and respiration recording (using a nasal thermistor) was started. Free spontaneous breathing was continued for 6 minutes, and the participants were then instructed to perform controlled breathing at a rate of 12 breaths per minute for 2 minutes. After passive tilting to the 70-degree head-up position by a tilt table, the recording was continued for 3 minutes of breathing, after which blood pressure was measured again. The participants were then tilted back to the supine position, and the recording continued for 5 minutes. The following 2-hour recording was performed in the supine position with free spontaneous breathing.

The short-term recordings were analyzed by using 2-minute segments from each procedure, and the HRV results from the oral glucose tolerance test

were analyzed as the mean values of four different 6-minute intervals starting at 10, 30, 50, and 70 minutes after glucose load. Development of the recording and analysis software was performed at our hospital.<sup>216</sup> The validity and reproducibility of HRV have been described in previous articles.<sup>217, 218</sup> The HRV tests performed in study IV were exclusively short-term registrations.

### **Measuring endothelial function (studies II, IV)**

Levels of ADMA have been linked to preclinical atherosclerotic disease burden and an adverse outcome and are now considered to be a useful measure of endothelial status and a potential marker of risk in clinical practice.<sup>219</sup> In study II, we detected ADMA using HPLC. The method validity and reproducibility have previously been published by Teerlink.<sup>220</sup>

In study IV, we analyzed ADMA using ELISA (an assay based on the method of competitive enzyme-linked immunoassays). ELISA has been used in previous studies<sup>221</sup> and shown good correlation ( $r > 0.93$ ) with HPLC (unpublished data from Immundiagnostik®).

# STATISTICS

## Study I

Comparisons between artery and vein concentrations were made by paired t-test (subcutaneous study) or repeated measures ANOVA with post-hoc testing with Fisher's least significant differences test (visceral study). Differences from zero were determined using the one-sample t-test.

## Study II

Within groups, a pairwise t-test was used to test significant changes over time. Between groups, one-way ANOVA was used to test if group means differed, followed by post-hoc group comparisons using independent t-tests if significant. Pearson correlation coefficients were used for correlation analyses. Adjustment was performed by partial correlation.

## Study III

We used the non-parametric Kruskal–Wallis test to determine if group means differed, followed by the Mann–Whitney post-hoc test if results were significant. Area under the curve (AUC) was estimated according to the trapezoid rule. Partial correlation analysis was used to determine the relation of ATBF to other parameters allowing for adjustments. We calculated tertiles of adipose tissue areas using group-specific cut-off values for pre- and postmenopausal women separately.

## Study IV

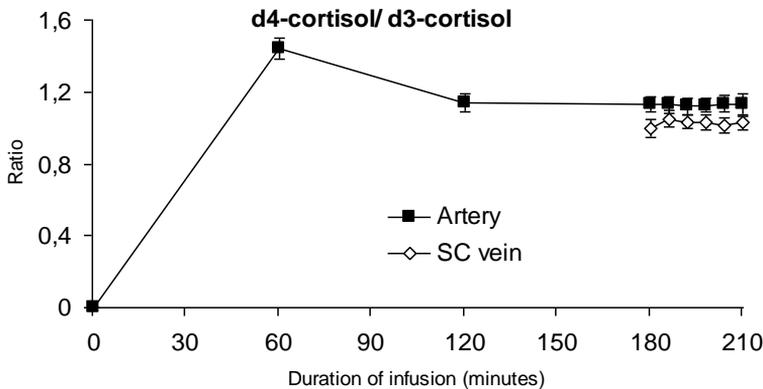
The Kruskal–Wallis non-parametric test was used to test differences between groups. To detect differences within groups over time, we used the Wilcoxon signed-ranks non-parametric test.

# RESULTS AND DISCUSSION

## Paper I

### *Superficial epigastric vein cannulation study*

Plasma concentrations of cortisone, cortisol, d3-cortisol, d4-cortisol, and d4-cortisone were not different between artery and superficial epigastric vein. However, there was a trend for increased d3-cortisol levels in the vein ( $P = 0.06$ ). We found significant release across the subcutaneous bed of both cortisol (15 pmol/min/100 g) and d3-cortisol (8.7 pmol/min/100 g) (both  $P < 0.05$ ) (Figure 6).



**Figure 6.** *d4-cortisol:d3-cortisol ratio for the subcutaneous study. SC vein, subcutaneous vein.*

### *Hepatic and portal vein cannulation study*

In the hepatic vein, we found an increase in d3-cortisol and a decrease in both cortisone and d3-cortisone resulting from intrahepatic steroid extraction and  $11\beta$ -HSD1 activity. In the portal vein, we detected increased d3-cortisone concentrations and a trend for increased cortisone concentrations ( $P = 0.051$ ), consistent with visceral  $11\beta$ -HSD2 activity. Splanchnic production of cortisol was estimated at 13.5 nmol/min and production of d3-cortisol at 8.0 nmol/min. Hepatic cortisol production was

13.3 nmol/min and d<sub>3</sub>-cortisol production was 7.7 nmol/min (not significantly different from splanchnic production), entirely accounted for the splanchnic production. No visceral cortisol or d<sub>3</sub>-cortisol release into the portal vein was detected.

## ***Discussion***

Because the site(s) of cortisol production can have important theoretical and therapeutic implications, especially concerning development of selective 11 $\beta$ -HSD1-inhibitors,<sup>222</sup> it has been important to try to determine where significant cortisol production takes place. Our data, for the first time in humans, show the contributions of SAT, visceral tissues, and liver to whole-body cortisol production. We found significant cortisol release into veins draining SAT, but we could not detect any cortisol release from the visceral tissues. All splanchnic cortisol production seemed to be attributed to the liver. Although previous studies have shown that 11 $\beta$ -HSD1 gene expression is present in visceral fat,<sup>223</sup> albeit at levels far lower than in the liver, the viscera do not appear to release cortisol into the portal vein or convert d<sub>4</sub>-cortisol to d<sub>3</sub>-cortisol. The data confirm earlier studies performed in dogs<sup>224</sup> and humans<sup>225</sup> but seem to contradict earlier studies by Andrew and colleagues<sup>205</sup> that suggested that up to two thirds of splanchnic cortisol production might originate from the viscera.

The absolute values of secreted cortisol from adipose tissue differ markedly from study to study, and some results seen are incompatible with normal daily cortisol secretion rates. For example, we report a hepatic cortisol production rate of 18 mg/d and Basu et al.<sup>225</sup> report 33 mg/d, both greatly in excess of the established daily cortisol production rate of ~10 mg/d.<sup>226</sup> There are several explanations for the diverging results. Studies including portal vein cannulation are not ethically permitted in healthy individuals. Patients in these studies had either severe obesity and were undergoing bariatric surgery or had liver disease and were undergoing portal venous shunting; both scenarios have been shown to significantly alter cortisol metabolism. It is also possible that anesthesia could affect visceral 11 $\beta$ -HSD1 activity. Furthermore, the catheterization of the portal vein does not specifically sample omental fat but reflects drainage from other visceral tissues (especially the gut), which may cause some dilution. One can argue that patients with liver cirrhosis have an abnormal portal blood flow,<sup>227, 228</sup> but modeling the portal flow from 10–80% of the total hepatic blood flow did not change the results. The presence of liver disease in the four patients could be further involved, creating uncertainty regarding liver cortisol release and limiting the ability to extrapolate data to healthy individuals, although it is

less likely to alter the visceral cortisol release. The low number of participants in both the subcutaneous and visceral parts of the study could be questioned, and the explanations are related to the technical difficulties involved in the protocol. In the visceral part of the study, we performed a power calculation based on a previously published model,<sup>205</sup> suggesting visceral cortisol production to be 30 nmol/min; given the low variance of our steady-state kinetic measurements, with four participants, we were able to power the study with >80% confidence to detect cortisol production as low as 10 nmol/min.

Previous methods determining the activities of 11 $\beta$ -HSDs *in vivo*<sup>229-231</sup> have relied only on the balance between cortisol and cortisone or the concentrations of their major metabolites in urine. These approaches do not measure turnover and cannot distinguish between the two reactions. Of note, this feature is important particularly because disorders involving either isozyme are suspected often to occur together (e.g., obesity and hypertension), so that it is not possible to determine which isozyme is responsible for a change in equilibrium. This difficulty underscores the advantage of using labeled cortisol.

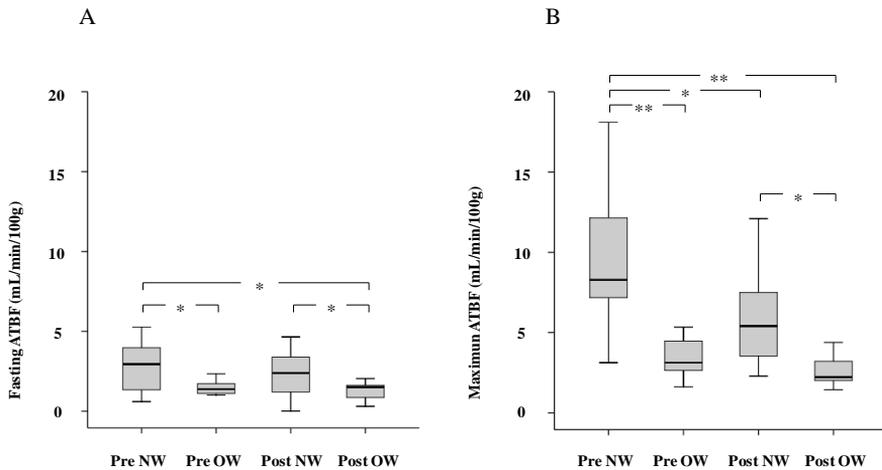
All equations in this study are based on a compartment analysis described by Wolfe and Chinkes.<sup>208</sup> The goal of compartment analysis is to use the pattern of change in enrichment over time to quantify the exchange between pools and pool sizes, and the rate of appearance and the rate of irreversible loss.<sup>208</sup> A tracer is a compound that is chemically and functionally identical to the naturally occurring compound but distinct in some way that enables detection. Stable isotopes (like the deuterium used in this study) have the advantage that they are non-radioactive and present little or no risk to humans. In these and other tracer studies, the tracer:tracee ratio is more appropriate than concentration to minimize variability. The technique using a priming dose followed by a constant infusion of the tracer was first described in 1954<sup>232</sup> and has previously been used for measuring kinetics of lactate,<sup>233</sup> FFAs,<sup>234</sup> and other substances. There are a number of assumptions (and limitations) associated with the compartment analysis. One is that the rate of change of the amount of tracer in a pool is equal to the rate at which that tracer enters the pool minus the rate at which that tracer exits the pool. Another is that the rate at which a tracer exits a pool is proportional to the amount of tracer in the pool.

In conclusion, we found significant cortisol release from SAT and that all splanchnic cortisol production seems to be attributed to the liver.

## Paper II

### *ATBF in fasting and during the oral glucose tolerance test*

The main findings were that fasting ATBF was significantly decreased in both overweight groups compared to normal-weight premenopausal women. Normal-weight and overweight postmenopausal women had significantly lower maximum ATBF compared with normal-weight premenopausal women. Moreover, overweight postmenopausal women exhibited lower maximum ATBF compared with normal-weight postmenopausal women (Figure 7).



**Figure 7.** Fasting (A) and maximum (B) ATBF in the different study groups. The bars in the box plots represent median, quartiles, and extreme values. \* $P < 0.05$ ; \*\* $P < 0.01$ . Pre NW, premenopausal normal-weight; Pre OW, premenopausal overweight; Post NW, postmenopausal normal-weight; Post OW, postmenopausal overweight.

### *ADMA and ATBF*

ADMA levels did not differ significantly among groups, but a significant negative association was seen between ADMA and fasting ATBF. In contrast, no association was seen between ADMA and postprandial ATBF.

## ***HRV and ATBF***

In the fasting state, postmenopausal overweight women had a significantly higher power of low frequency (PLF):power of high frequency (PHF) ratio, indicating relatively higher sympathetic activity versus the other groups. Postprandially, all groups except the postmenopausal overweight group increased their PLF:PHF ratio. A significant negative association was found between the PLF:PHF ratio and maximum ATBF, whereas no association was found between the PLF:PHF ratio and fasting ATBF.

## ***Discussion***

The striking influences of age and BMI on ATBF in women highlight the potential role of ATBF as a powerful regulator of adipose tissue metabolism. ATBF exhibits the highest degree of response to food intake, illustrated by either mixed-meal ingestion or glucose, but the actual stimulus has not been fully understood. The obese postmenopausal women seemed to have both a decreased basal and stimulated ATBF. Diminished or absent ATBF response to nutrient ingestion may lead to decreased extraction of adipose tissue glucose and TGs with subsequent postprandial hyperlipidemia and hyperglycemia,<sup>49</sup> conditions that predispose to the metabolic syndrome and CVD. Furthermore, decreased ATBF could affect signaling between adipose tissue and other tissues to regulate metabolism.<sup>51</sup> Our statement that loss of ATBF may predispose individuals to metabolic dysfunction (in postmenopausal overweight women) is based on findings that alterations in ATBF seem to be related to the metabolic activity in the adipose tissue.<sup>59, 60</sup> With increasing ATBF in healthy people (by adrenaline infusion), TG extraction increases exactly in parallel with increased blood flow, implying that TG extraction is normally limited by substrate delivery.<sup>52</sup> Based on these findings, we cannot believe that poor ATBF has no bearing on metabolic function. Our conclusion also fits with the findings that lipid mobilization is disturbed in conditions related to the metabolic syndrome,<sup>56</sup> and our results are in line with previous studies<sup>50, 62</sup> showing that ATBF responsiveness to nutrients is reduced in obesity. The impairment of ATBF in obesity has been confirmed in a recent study, suggesting parallels with endothelial function, but also relationships with autonomic cardiovascular control as reflected in HRV.<sup>71</sup>

The associations of ADMA and ATBF are in agreement with studies using an NO synthase inhibitor, NG-monomethyl-L-arginine (also known as L-

NMMA), showing that the postprandial enhancement in ATBF is independent of NO but that NO activity determines the level at which this response takes place.<sup>66</sup> Another study using *NG*-monomethyl-L-arginine did not seem to alter local ATBF,<sup>235</sup> but this study used the ethanol outflow:inflow ratio, which has an inherently low sensitivity.

Our data suggest a loss of ANS flexibility in the overweight postmenopausal group. Physiologically, in healthy people, at least two mechanisms are thought to contribute to the postprandial activation of SNS: a postprandial increase in plasma insulin and the entrance of a nutrient into the gastrointestinal tract.<sup>236</sup> The postprandial SNS stimulation is considered to be physiologically important in the regulation of thermogenesis and the rise in energy expenditure after dietary intake.<sup>237</sup> Our results in postmenopausal women are in line with previous studies showing that HRV declines with age in both sexes, which has previously been explained by a reduction in parasympathetic activity with advancing age.<sup>238</sup> Studies from Ribeiro et al.<sup>239</sup> and Brockbank et al.<sup>240</sup> both showed a decrease in parasympathetic modulation in postmenopausal women compared to young women, but as in our study, they could not distinguish the separate effects of age and hormonal factors. It has also been proposed that the relatively higher HRV variability reported in premenopausal women may be responsible for the protection of women compared with men against coronary heart disease. A possible explanation could be reduction of arrhythmias related to enhanced vagal activity.<sup>241</sup> Of note, HRV is associated with CVD, type 2 diabetes, and insulin resistance and has previously been shown to be reduced in postmenopausal women as compared with premenopausal women.<sup>240</sup>

There are several reasons for using the <sup>133</sup>Xe-washout technique for monitoring blood flow. One is that it is more discriminating for monitoring physiological changes in ATBF compared to microdialysis.<sup>75</sup> However, the technique has some possible limitations. One study comparing the <sup>133</sup>Xe-washout and laser Doppler techniques showed that <sup>133</sup>Xe-washout was more closely related to cutaneous than to subcutaneous perfusion. This finding may indicate that a subcutaneous xenon depot is not drained solely through the local adipose tissue microcirculation but also through the capillaries in the epidermal layer.<sup>73</sup> Rapid ATBF changes (within 30 s) are also unlikely to be detected at all.<sup>73</sup> Moreover, the probes detecting the radiation are sensitive to movement artifacts, caused by changes in the distance between the isotope depot and the detector.

The choice of short-term HRV assessment was based on the fact that this time interval is sufficient to allow a full stabilization of R-R interval

variability, as reported in previous studies.<sup>211</sup> The literature has reported the use of a great variety of different HRV indexes. However, no single index has been demonstrably proven to be superior to another.<sup>211</sup>

The study has some limitations. By the design, we could not dissect out the effect of age per se. The postmenopausal women were included at least one year after cessation of menstruation and had a mean age of 60 years (range 50–70 years). This group therefore seems reasonable for group of women within a postmenopausal stage, but we did not have estradiol levels available. It has also been questioned why ATBF was obtained at the umbilicus. ATBF changes in the abdominal fat depot have been well studied in different settings; however, little is known about ATBF regulation in other fat depots. Gluteal fat was shown to have lower basal ATBF,<sup>242</sup> and in lean women, femoral ATBF showed an attenuated increase during systemic adrenaline stimulation compared to abdominal fat.<sup>243</sup> There is only one study describing postprandial ATBF in different depots. This study found increases in both the abdominal and femoral depots in women, but only in the abdominal depot in men.<sup>244</sup> In this study, we therefore decided to use the most significant one, at least in terms of the main function of adipose tissue: delivery of non-esterified fatty acids to the systemic circulation.<sup>245</sup>

In conclusion, we demonstrated striking influences of age and BMI on ATBF in women. Endothelial dysfunction and ANS imbalance may contribute to the absolute and maximum levels of ATBF, respectively.

### **Paper III**

#### ***ATBF associations with measures of obesity, biomarkers, and adipokines***

ATBF was highly associated with all measures of obesity. Moreover, ATBF was associated with hs-CRP and the homeostatic model assessment (HOMA) index, and the associations remained significant after adjustment for age and menopausal status but not after adjustment for BMI. Leptin was strongly associated with ATBF, and the association remained after adjustments for age and menopausal status but not after adjustment for BMI. No association was seen between blood flow and adiponectin.

## ***ATBF relation to total fat, SAT, and VAT***

Independent of fat depot, we found highly significant differences in ATBF between the lowest and medium tertiles and between the lowest and highest tertiles of adipose tissue areas. Significant differences in ATBF were found between the medium and highest tertiles of subcutaneous and intraabdominal AT.

## ***Discussion***

Visceral and subcutaneous adipose tissue have constitutive biological effects. Even though both deposits serve as energy depots, they differ in levels of lipid mobilization, adipokine production, and adipocyte differentiation in ways that can be important in response to diet and exercise. On one hand, the subcutaneous fat depot in the obese is probably the main source of increased circulating FFA levels.<sup>246</sup> On the other hand, the physiological impact of VAT is demonstrated by data showing a 50% increase of IL-6 in the portal vein (compared to artery) in the obese,<sup>163</sup> providing a potential link between visceral fat and systemic inflammation in individuals with abdominal obesity. These data are supported by the fact that visceral fat expresses higher levels of inflammatory cytokines<sup>247</sup> compared to subcutaneous fat. Both visceral and subcutaneous fat are correlated with insulin sensitivity in women, which is one of the important mediators of ATBF.<sup>49</sup> In our study, the strong correlation between ATBF and SAT as well as VAT areas suggests that total adipose tissue mass is linked to dysregulation of ATBF, rather than to specific sites of fat accumulation. Previous data have demonstrated that obese individuals show both downregulation of lipolysis and a reduced ability to store fat in adipose tissue after meals,<sup>36, 248</sup> underscoring the importance of our results. The lowering of processes involved in fatty acid trafficking in obesity has previously been explained by altered activity of LPL, HSL, and ATGL.<sup>18, 249</sup> Moreover, ectopic fat deposition has been suggested to underlie obesity-associated insulin resistance.<sup>33</sup> Therefore, an efficient regulation of fat storage in adipose tissue, including ATBF flexibility, seems important to protect other organs from lipotoxic effects.

The increase in visceral fat depot after menopause seen in our and other studies<sup>6</sup> highlights the concern for the development of associated adipokine changes leading to an adverse metabolic profile in postmenopausal women. A previous longitudinal study of the menopausal transition showed an

increase in t-PA, MCP-1, and serum amyloid A.<sup>250</sup> Levels of leptin and adiponectin across the menopausal transition have shown diverging results, likely because of the effects of age and fat mass.<sup>250</sup>

Previous data indicate that leptin can be involved in vascular tone control. Leptin receptors are expressed in endothelial cells,<sup>251</sup> and in a vascular ring model, leptin induces vasorelaxation in a dose-dependent manner, an effect abolished by eNOS inhibitors.<sup>117, 252</sup> A previous study in healthy males demonstrated no correlation between leptin and blood flow following administration of NO synthase inhibitor.<sup>253</sup> Similarly, no relationship was observed between plasma leptin and flow-mediated dilatation of the brachial artery (which is mainly NO mediated) in normolipidemic healthy obese women<sup>254</sup> and in healthy adolescents.<sup>146</sup> The non-significant results between leptin and ATBF (after adjustments for BMI) in this study should be interpreted with caution because leptin and all measures of obesity are strongly associated. It has also been debated whether models exploring the effect of leptin should be adjusted for obesity. Thus, the role of leptin in regulating endothelial function in humans remains controversial.

The highly significant correlation between ATBF and hs-CRP highlights the role of inflammatory cytokines produced by both adipocytes and adipose tissue macrophages. It also highlights the potential role of the adipose tissue surrounding the blood vessels. As previously described, obesity is associated with an increase in plasma levels of CRP, IL-6, and TNF- $\alpha$ . Notably, increased levels of IL-6 and TNF- $\alpha$  may affect NO activity.<sup>255</sup> Although these cytokines have shown strong associations with the risk for type 2 diabetes and CVD,<sup>256</sup> prospective studies will be necessary to determine causality. Another possibility is that increased perivascular fat may lead to overproduction of vasoactive substances generating both inflammation and altered vascular function and thereby affecting blood flow and predisposing an individual to atherosclerosis.<sup>257</sup>

The study has some limitations. The number of participants in each group was limited, and the study design was cross-sectional, making it impossible to explore causality. In line with other studies,<sup>20</sup> we found a high correlation between the subcutaneous and intra-abdominal depots, leading to difficulties separating the effect of each depot.

In summary, we have demonstrated that ATBF in women is closely linked to subcutaneous and visceral adipose tissue size. We also found a strong inverse association between circulating levels of leptin and ATBF. Further

mechanistic studies are needed to identify the putative role of leptin as a modulator of blood flow.

## **Paper IV**

### ***Adipose tissue volumes and anthropometrics***

At baseline, AUC insulin levels were significantly higher in the NNR group compared with the PD group. The other baseline data did not differ between groups. At six months follow-up, weight, waist, BMI, subcutaneous and visceral fat volumes, and blood pressure were significantly reduced in both diet groups. Insulin, HOMA index, and TGs were significantly reduced in the PD group but not in the NNR group. The levels of TGs and subcutaneous fat volumes were significantly lower in the PD group compared with the NNR group ( $P = 0.005$  and  $P = 0.04$ , respectively). There was a trend towards larger changes for the other metabolic parameters in the PD group compared to the NNR group.

### ***ATBF at baseline and 6 months***

Baseline AUC blood flow values did not differ between groups. At 6 months, AUC ATBF was significantly reduced in the NNR group but preserved in the PD group. Focusing on the individual blood flow response, an increased blood flow was seen in three of six individuals in the PD group compared with only one of eight individuals in the NNR group.

### ***Heart rate variability***

We found a borderline significant reduction of sympathetic activity (PLF) between baseline and 6 months in the total cohort of women ( $n = 61$ ) and a significant reduction of heart rate in the PD group. No significant changes in parasympathetic activity (PHF) or sympathovagal balance (PLF/PHF) were seen over time or between groups.

## ***Endothelial function***

Levels of ADMA were equal between groups at baseline, and no significant changes were observed over time, within or between groups. There were no significant associations between ADMA levels and HOMA-indices at baseline or at 6 months follow-up.

## ***Discussion***

The effect of weight loss and/or different diets on ATBF has previously been investigated in only a few studies. A 30-day intervention using the DASH diet did not alter ATBF or endothelial function, and these individuals only lost less than 1 kg.<sup>258</sup> A 6-week study with a very low calorie diet using PET showed a reduction of total perfusion in both the subcutaneous and visceral compartments, but the ATBF was not changed when expressed per gram of mass (as in our study).<sup>44</sup> Although the investigated groups in our study were small, we observed a significant difference in ATBF between groups at 6 months follow-up. A possible explanation is the fact that reduction of weight and fat volumes was more pronounced in the PD group, and potentially there may be a threshold beyond which increased ATBF could be seen (a trend towards increased blood flow in the PD group). An alternative explanation is that PD markedly improves insulin sensitivity, which in turn affects the ATBF.<sup>49</sup>

This study was the first to evaluate the effect of a Paleolithic diet on ATBF. Previous studies have, however, measured the effect on other cardiovascular risk factors. A randomized study in men with ischemic heart disease and impaired glucose tolerance or type 2 diabetes showed improved glucose tolerance independent of weight loss after 12 weeks of a Paleolithic diet compared to a Mediterranean-like diet.<sup>259</sup> O'Dea and colleagues showed that reversion to a hunter-gatherer lifestyle in Aborigines with diabetes during 7 weeks led to 10% weight loss and reductions in fasting and 2-hour glucose and fasting insulin.<sup>260</sup> In another study, 10 days of a Paleolithic diet improved diastolic blood pressure, glucose tolerance, insulin sensitivity, and lipid profiles.<sup>193</sup> The Paleolithic diet has also shown effects on lowering CRP<sup>261</sup> and plasminogen activator inhibitor-1.<sup>194</sup> In addition, Shai and colleagues<sup>199</sup> showed increasing improvement in levels of some biomarkers over time up to the 24-month point, despite the achievement of maximum weight loss by 6 months, suggesting that low-carbohydrate diets have benefits beyond weight reduction. In contrast, a recent prospective study

from Fung and colleagues showed that a low-carbohydrate diet based on animal sources was associated with higher all-cause mortality in both men and women, whereas a vegetable-based low-carbohydrate diet was associated with lower all-cause and CVD mortality rates.<sup>262</sup>

A rather surprising finding from our study was the lack of changes in ADMA levels with time and between groups. A previous study from McLaughlin and colleagues<sup>263</sup> showed that ADMA levels were higher in insulin-resistant women compared to insulin-sensitive women and that decreased ADMA resulting from weight loss was associated with enhancement of insulin sensitivity. In contrast, a recent review including 12 trials in the area of weight loss and vascular function showed that improvements in vascular dysfunction did not correlate with the amount of weight loss within the vast majority of the individual trials.<sup>264</sup> A clear relationship between the degree of weight loss and improvement in vascular function was in fact reported only in 2 of the 12 trials. This result suggests that aspects other than weight loss per se are important in mediating any improvement in the vascular health of overweight patients. Another observation from vascular function studies<sup>264</sup> is that the benefits are more robust in initially less healthy individuals and in those who undergo concomitant exercise, which may have affected the lack of results in our quite healthy group of women.

Both diet groups together demonstrated a borderline significant reduction in sympathetic activity, but no significant differences could be seen within each group or between groups. Nor did we observe any changes in the ratio between the two components of the ANS, the sympathetic and the parasympathetic nervous systems. In the literature, there are limited data on the independent effects on HRV of different weight loss approaches. A 6-month diet study including men and women showed a general decrease in the sympathetic component while the parasympathetic component increased, indicating an improvement in the sympathetic/parasympathetic balance.<sup>265</sup> As a comparison, previous studies using measurements of plasma norepinephrine concentration, regional norepinephrine spillover, and from direct microneurographic recordings of muscle sympathetic nerve activity have shown that weight loss causes sympatico-inhibition.<sup>266</sup>

The study had some limitations. Information about physical activity was not included; however, preliminary results from automatic registration of both heart rate and movements indicate no difference in physical activity between the two diet groups. The physical activity data will be published in a separate paper. The HOMA index is not the optimal method for assessing insulin resistance, and ideally, we would have used a euglycemic hyperinsulinemic

clamp. Moreover, ADMA is not the ideal choice for measuring endothelial function, but the method is non-invasive and has been shown to be a strong predictor of cardiovascular events.<sup>152</sup>

In conclusion, this randomized diet study including overweight postmenopausal women demonstrated reductions in measures of adipose tissue after 6 months. In a subgroup, the ATBF was reduced in the NNR group but preserved in the PD group. The degree of weight loss and changes in insulin sensitivity could possibly mediate the ATBF differences between groups. The metabolic changes were most pronounced in the PD group. In contrast to previous studies, we did not find significant changes in ADMA levels with time or between diets.

# GENERAL DISCUSSION

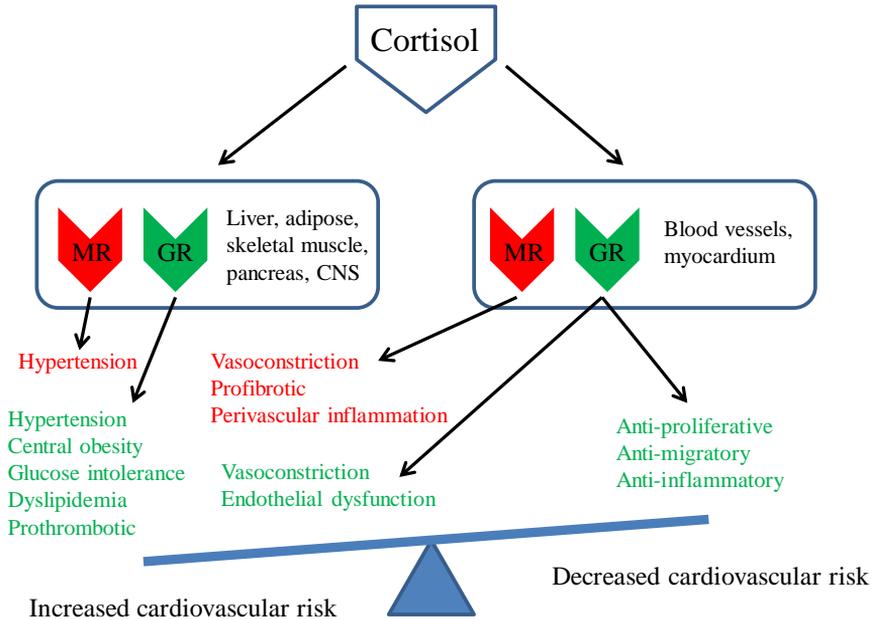
The main findings of this thesis are the quantification of cortisol production from SAT, VAT, and liver by  $11\beta$ -HSD<sub>1</sub> in humans. We confirmed that cortisol is released from SAT and that splanchnic cortisol production is attributed entirely to the liver. We have demonstrated loss of ATBF flexibility in postmenopausal overweight women and associations between ATBF and autonomic activity, NO activity, leptin, and the amount of adipose tissue (independent of fat depot). Moreover, weight loss in a diet program including postmenopausal overweight women demonstrated reduced ATBF in NNR group, but preserved in the PD group, indicating that Paleolithic diet may have metabolic advantages. Mechanistic studies are needed to evaluate the association between leptin and ATBF. The results will contribute to the knowledge of adipose tissue biology and may provide important information for the development of treatment strategies against obesity and obesity-related disorders.

The role of adipose tissue as an endocrine organ critically depends on its circulation for metabolic function and transport. It is tempting to suggest that metabolic derangements associated with obesity might be related to variations in vascularization and alterations in tissue perfusion.<sup>267</sup> Impaired vascular reactivity is not seen only in adipose tissue. Studies of muscle arterioles in obese and insulin-resistant individuals show endothelial dysfunction and resistance to the effect of insulin on enhancement of endothelium-dependent vasodilation.<sup>268</sup> In study II, we investigated ATBF in women of different ages. The effect of age on endothelial function and blood flow is controversial. Functionally, vascular aging typically has been associated with endothelial dysfunction, impairment of the NO pathway, and elevated oxidative stress,<sup>269</sup> but the results diverge markedly depending on method, vessel of interest, and conditions (rest, exercise, etc.). Whether ATBF is related to measures of endothelial function in other vessels was recently assessed by Funada and colleagues, who found a significant correlation between ATBF and FMD ( $R = 0.32$ ,  $P = 0.008$ ) of the brachial artery and a significant negative correlation between ATBF and carotid intima-media thickness ( $R = 0.51$ ,  $P = 0.02$ ).<sup>71</sup> Whether these results are transferable to postmenopausal women is unknown. In the post-menopausal group, it was also difficult to separate the effect of vascular aging from the effect of estrogen withdrawal because biologic age and time since menopause are intrinsically linked. Of note, studies of post-menopausal women without cardiovascular risk factors have shown that women not using hormone replacement therapy (HRT) have impaired microvascular reactivity, while postmenopausal women on HRT have endothelium-dependent and

endothelium-independent vasoreactivity similar to that of premenopausal women.<sup>270</sup> Regarding different methods of measuring endothelial function, we must consider that vascular function in different arteries is not uniform. For example, there is evidence of a larger blood flow response to physiological vasodilators in arm arteries compared to leg arteries,<sup>271</sup> whereas a greater sympathetically mediated vasoconstrictor response has been recognized in the legs.

Modulation of vascular function and blood flow by perivascular adipose tissue (PVAT) could possibly interact with our ATBF results. Several studies have shown that PVAT modulates vascular tone and lowers the response to a broad spectrum of vasoconstricting agonists by releasing transferable substances with direct vasodilator properties. PVAT seems to exert anticontractile effects through two distinct mechanisms: (i) by releasing factors that induce endothelium-dependent relaxation through NO release, and (ii) by endothelium-independent mechanisms involving hydrogen peroxide.<sup>272</sup> The role of PVAT-derived NO has been demonstrated in saphenous veins harvested traumatically and used as grafts in patients undergoing coronary artery bypass surgery.<sup>273</sup> Of note, in obese individuals, local inflammation and hypoxia abolish the protective anticontractile effects of perivascular fat.<sup>274</sup>

The link between glucocorticoids and CVD has been evident since the 1950s, when Adlersberg and colleagues<sup>275</sup> discovered that cortisone treatment affected serum lipids and that these changes might cause premature atherosclerosis. It is evident that glucocorticoids have the potential to regulate the cardiovascular system both indirectly, by systemic modulation of risk factors,<sup>123</sup> and directly by interaction with cells in the cardiovascular system. Glucocorticoids stimulate LPL activity, predominantly in the visceral depot, leading to central obesity,<sup>276</sup> and may also affect the release of adipokines from adipose tissue. For example, leptin release is upregulated and IL-6 is downregulated from visceral adipocytes by glucocorticoid treatment.<sup>277, 278</sup> Glucocorticoids have complex and contradictory influences on CVD. Systemic excess of glucocorticoids is associated with increased cardiovascular risk and linked to the prognosis of increased cardiovascular events, while local effects of glucocorticoids on cells of the cardiovascular system (mediated by GRs and/or MRs) could oppose lesion development and have a protective effect<sup>136</sup> (Figure 8).



**Figure 8.** Systemic versus local effects of glucocorticoids on the cardiovascular system, mediated by GR and MR. Modified from Hadoke et al.<sup>222</sup>

The pioneering results with the  $11\beta$ -HSD1 global knockout mice, leading to enhanced hepatic insulin sensitivity, reduced gluconeogenesis, and glycogenolysis, suggest that inhibiting  $11\beta$ -HSD1 may be a therapeutic target in the metabolic syndrome and type 2 diabetes.<sup>279</sup> These mice also exhibit low serum TGs, increased HDL cholesterol, and apo-lipoprotein A1 levels, suggesting that  $11\beta$ -HSD1 inhibition may prevent atherosclerosis.<sup>280</sup> Moreover, the mice seem protected against age-related cognitive impairment, suggesting that inhibitors may also be useful in the treatment of diseases like Alzheimer's disease.<sup>281</sup>

Outside the area of this thesis, there are other possible explanations for the development of climacteric obesity, such as reduced levels of progesterone and estradiol that lead to excessive stimulation of GRs by cortisol, and increased testosterone levels, in turn leading to visceral fat accumulation.<sup>282</sup> Other potential mechanisms causing increased visceral fat mass could be disturbances in neuroendocrine control of appetite and satiety, a decreased number of insulin receptors, and decreased levels of growth hormone.

One of the key questions is why insulin resistance arises in obesity. The answer is not entirely clear. Changes in insulin action have been shown, with a decrease in the number of insulin receptors on the cell surface and a decreased activity of insulin receptor tyrosine kinase and metabolic changes within cells that render them less sensitive to insulin. The close relationship between insulin resistance and fat deposition in muscle and liver is one potential explanatory model. Ectopic fat may interfere with insulin signaling, perhaps via protein kinase C activation. Another belief is that secreted adipokines from the enlarged adipose tissue cause adverse effects in other tissues. One of the candidates is adiponectin, which is thought to regulate insulin resistance. Another candidate is the low-grade inflammation seen in obesity, though adipose tissue in obesity is infiltrated with macrophages secreting pro-inflammatory cytokines and potentially inducing insulin resistance in other tissues. It is also important to remember that the increased circulating level of glucose resulting from insulin resistance is often aggravated by decreased insulin secretion arising from  $\beta$ -cell dysfunction.

Different patterns of fat distribution display different associations with insulin resistance. When comparing individuals with similar abdominal subcutaneous depots but different visceral depots, those with larger VAT depots had higher plasma glucose values during oral glucose tolerance testing and lower glucose disposal rates. In addition, individuals with matched VAT depots and different abdominal SAT depots displayed no differences for these values.<sup>283, 284</sup> In contrast, others have questioned the importance of visceral fat.<sup>285, 286</sup> It may be that a reason not to overlook the adverse and contributing effects of abdominal subcutaneous fat is that it can compose up to 80% of total adipose tissue.<sup>287</sup>

It has been suggested that adipose tissue acts to “buffer” the influx of dietary fat into the circulation. Inappropriately stored fat in muscle, liver, and other organs (ectopic fat deposition) has been proposed to underlie obesity-associated insulin resistance.<sup>33</sup> Therefore dietary fat should be stored in adipose tissue and not “overflow” to other organs. Between obese and lean

individuals, the adipose tissue masses may vary over a 10-fold range, while fasting FFA concentrations typically vary only within a two-fold range. This difference highlights mechanisms for restriction in release of FFAs from adipose tissue. In addition to dysfunctional ATBF, possible mediators could be lower functional LPL activity or an absence of postprandial upregulation of adipose tissue LPL, as seen in obesity.<sup>288</sup>

Currently prescribed anti-diabetic drug therapies present differential effects on adipose vasculature, despite the positive effects on insulin sensitivity. The drug metformin, for example, reduces adipose tissue angiogenesis,<sup>289</sup> whereas the thiazolidinedione class of drugs results in more vascularized adipose tissue with increased adiponectin secretion.<sup>290</sup> At present, only one drug is licensed for treatment of obesity in Sweden. Orlistat (Xenical®) is an inhibitor of gastrointestinal lipases, leading to excretion of dietary fat in feces and therefore not absorbed by the body. A recently withdrawn drug, sibutramine, acts to raise the concentration of serotonin in the brain, and which tends to reduce appetite. Another previous drug, rimonabant, an antagonist of the cannabinoid receptor-1, showed promising weight-loss properties but has been withdrawn because of adverse psychological effects. Several pharmaceutical companies are trying to use the enormous expansion of knowledge in the area of appetite regulation for drug development, for instance focusing on MC4R antagonists. In addition, there are attempts to increase energy expenditure. None of these anti-obesity drugs has yet shown any effects on adipose tissue perfusion (but studies are lacking). We think that further studies targeting modulation of components of adipose tissue metabolism and adipose tissue expansion will provide a strong basis for future research into this complex tissue.

Pre-menopausal women are relatively protected against CVD compared with men,<sup>291</sup> but the reasons for this sex difference are not completely understood. An understanding of the mechanisms underlying this difference may help us prevent worsening of cardiovascular risk factors in men and women in later life. There is evidence for some sex differences in the plasma metabolic profile in young adults. Short-term fasting studies reveal sex differences in people who are young and lean; after fasting, women have lower plasma glucose and higher plasma FFA concentrations than men.<sup>292</sup> However, plasma very-low-density lipoprotein (VLDL)-TG concentrations were lower in women<sup>293</sup> in response to the extended fasting. The results from that study suggested that the liver in women secretes fewer but more TG-rich VLDL particles than the liver in men, and that clearance is faster in women.

Significant differences in hepatic metabolism have been demonstrated in healthy, lean young men and women.<sup>294</sup> The greater ability of women to oxidize plasma FFA into ketone bodies may partly explain the fact that premenopausal women have a better metabolic profile than men and may help protect women against the accumulation of liver fat. The protective role of estrogen is controversial because postmenopausal women who use HRT soon after menopause for 10 or more years show a 40–50% reduction in cardiovascular mortality,<sup>295</sup> while HRT in older postmenopausal women is associated with an increase in cardiovascular events.<sup>296</sup>

There are significant sex differences in the control of vascular function and blood flow. It is evident that women have a smaller heart than men and that this translates into a smaller stroke volume and thus a lower cardiac output. Less evident is that women have a higher heart rate than men.<sup>297</sup> Under conditions of cardiovascular stress (exercise, loud noises, or psychological stress), men respond by increasing mainly vascular resistance, which is manifested as an increase in blood pressure, whereas women predominantly increase heart rate.<sup>298</sup> Given these different mechanisms, the long-term effects of stress in men will lead to remodeling of peripheral arteries and sustained hypertension with attenuated tissue perfusion. In women, this physiological response ultimately leads to heart disease. At all ages, women have reduced sympathetic activity and enhanced parasympathetic activity relative to men.<sup>299</sup> Similarly, women are found to have lower plasma norepinephrine levels than men.<sup>300</sup> Interesting studies of ANS activity have identified different aging patterns for HRV in men and women, resulting in an earlier and more marked age-related decline of sympathetic:parasympathetic ratio in men than in women, consistent with the greater life expectancy and lower risk for CVD of women.<sup>301</sup> Endothelial function (measured by endothelial dependent vasodilation) is impaired by age in a sex-specific pattern. In men, the derangement starts in the third decade and is gradual and progressive until old age. In women, no impairment is seen until menopause, and after menopause the decline is steeper than in men.<sup>302</sup> Moreover, consistent data show that men have higher blood pressures than premenopausal, age-matched women.<sup>303</sup> After menopause, the sex difference in hypertension is lost.<sup>304</sup> A technical aspect worth mentioning is that we have experienced difficulties cannulating the superficial abdominal veins in women, with a very low success rate (due to spasm and small veins), which has restricted our arteriovenous studies to men. Recognition of the differences as well as the similarities in vascular function and adipose tissue metabolism between sexes will help in designing future studies and hopefully contribute to the development of appropriate treatments for obesity in both men and women.

The burgeoning growth of obesity worldwide may lead to a considerable reduction in life expectancy.<sup>305</sup> With this in mind, it is important to understand the mechanisms by which obesity promotes CVD as well as the effect that weight loss has on clinical outcomes and in altering cardiovascular physiology. One of the crucial questions is whether overweight people have a better response in the long term to diets that emphasize a specific macronutrient composition. So far, there have been some concerns in diet studies: These include the novelty of the diet, media attention, the enthusiasm of the researchers, small samples, limited generalizability, a lack of blinded ascertainment of the outcome, a lack of data on adherence to assigned diets, and a large loss to follow-up, all of which have limited the interpretation of many weight-loss trials.<sup>306</sup> There are contradictory conclusions about whether carbohydrate reduction or limiting fat intake are superior in achieving weight loss. It seems important to distinguish between situations of acute weight reduction, controlled by a specific diet plan, and long-term effects to control or reduce body weight. The conflicting impressions, often promoted by much publicity, have caused confusion in the public. However, the conflicting data regarding the optimal diet can possibly be explained by realizing that severe restriction of any major macronutrient reduces the selection of desirable foods. This limitation will reduce the stimuli to overeat.<sup>307</sup> Another possibility that cannot be ruled out is direct metabolic effects of different food compositions, supported by diet studies demonstrating positive effects independent of weight loss.<sup>172, 173</sup>

In study IV, we chose a diet from an evolutionary perspective. It has been suggested that the food eaten during primate and human evolution, in particular during the Paleolithic (the 'Old Stone Age,' 2.5–0.01 million years ago), may be optimal to prevent insulin resistance and glucose intolerance. This diet was highlighted when it was noted that traditional Pacific Islanders of Kitava, Papua New Guinea, had no signs of CVD or markers of the metabolic syndrome, possibly because of their traditional lifestyle.<sup>308</sup> Candidate mechanisms and explanations could incorporate the fact that the Paleolithic diet contains relatively high levels of protein and a high content of water (due to fruits and vegetables), which are thought to be satiating and to facilitate a reduced caloric intake.<sup>259</sup> Alternative explanations for satiation, such as dietary effects on leptin resistance, have also been suggested.<sup>309</sup> The relevance of GI and glycemic load is presently being discussed.<sup>310</sup> Some studies show beneficial effects of a low GI/glycemic load diet on cardiovascular risk factors, while other studies do not.<sup>311, 312</sup>

To illustrate the difficulties in studying the development of obesity, we have to realize that most people who are overweight have become so over a period of many years. Here is an example of an obese man.<sup>16</sup> His body weight is 100

kg (height 1.8 m), and he has increased in weight from 70 kg over a period of 10 years. After calculations, this means a total mismatch between energy intake and expenditure of 90 MJ/year, or 250 kJ/day, which is only 2.5% outside the normal daily energy intake. To study this, we would have to be able to measure both energy intake and energy expenditure to a degree of precision of 2.5% outside the normal values, which is almost an impossible task.

This section ends with a few words about people being more or less susceptible to the development of the various components of the metabolic syndrome. Some individuals are obese but never develop the metabolic syndrome (called “metabolically normal obese”), or its serious consequences, but they are in the extreme minority. A recent report shows that among men who were obese but did not meet the criteria for the metabolic syndrome, the risk for CVD over the next 30 years was, in fact, doubled<sup>313</sup> relative to normal weight men. This finding suggests that although there are some obese people who do not have a phenotype that meets the criteria for diagnosis of the metabolic syndrome, it would be foolish to describe them as metabolically normal. The risks of obesity itself are serious, often under-estimated, and not restricted to metabolic disturbances. In line with this, epidemiological data demonstrate that central obesity precedes future metabolic changes.<sup>314</sup>

### **Therapeutic implications/future views**

Two principal therapeutic strategies have been identified in efforts to decrease glucocorticoid activity in adipose tissue and liver: antagonism of the GR or its signaling pathway and reduction of ligand availability.<sup>315</sup> Treatment with the GR antagonist RU38486 in leptin receptor-deficient mice or patients with Cushing’s syndrome leads to decreased glucose levels.<sup>316, 317</sup> The non-specific 11 $\beta$ -HSD1 inhibitors carbenoxolone and glycyrrhetic acid have resulted in an improved lipid profile and improved hepatic insulin sensitivity.<sup>318, 319</sup> This outcome has prompted a drive by pharmaceutical companies to produce selective inhibitors of 11 $\beta$ -HSD1.<sup>320</sup> A few promising inhibitors have been studied in mice and are now being investigated under phase 2B clinical trials in humans, but the results have so far been inconsistent, probably because of differences in tissue specificity.<sup>321</sup> However, the candidate inhibitor INCB 13739 has recently shown improved glucose control in patients with type 2 diabetes,<sup>322</sup> and it has been suggested that diabetic patients may be the group most susceptible to inhibition of 11 $\beta$ -HSD1.<sup>137</sup>

In the near future, it would be of interest to investigate the effects of glucagon-like peptide-1 (GLP-1) receptor agonists on ATBF, endothelial function, and adipokines. GLP-1 is secreted from intestinal cells within minutes of nutrient ingestion and has shown favorable effects on insulin secretion, appetite, satiety, blood pressure, and weight.<sup>323</sup> Of interest, GLP-1 also exerts an anti-inflammatory effect on vascular endothelial cells by increasing NO production,<sup>324</sup> but the effect on adipose tissue metabolism and ATBF is partly unclear. The GLP-1 receptor agonists provide new mechanisms of action and potential treatments for type 2 diabetes and obesity-related diseases.

Another important question following our ATBF results is, Can a direct vascular effect of leptin on endothelial function explain our findings? To answer this question requires mechanistic studies. It would also be of certain interest to investigate whether there is a critical threshold of weight loss (or degree of reduction in adiposity) at which reversal of ATBF could be seen, or if the differences between diet groups can be explained by the compositions of the different diets.

Our conclusion that decreased ATBF in overweight women during fasting is linked to endothelial dysfunction is based on a single parameter. In future studies, we believe that the endothelial function data would be strengthened if we had another reliable indicator of endothelial function, for example, brachial plethysmography. The HRV data in study IV were limited to basal conditions. In light of current knowledge,<sup>187</sup> it would be good to include measurements of sympathetic neural responsiveness to glucose ingestion in such a diet study. The growing evidence indicating the selectivity of autonomic regulation and the highly differentiated sympathetic responses of peripheral vascular beds also has to be considered in future studies of the ANS.<sup>325</sup>

Previous data indicate a concomitant impairment in endothelial and ANS functioning in the children of people with type 2 diabetes.<sup>326</sup> Because physical activity has been demonstrated to improve both autonomic cardiovascular regulation and endothelial function, it would be of major interest to study these parameters in relation to ATBF during a physical training program. What are the effects of exercise on adipose function?

Regarding adipose tissue function as a whole, there are several key questions to be answered in the future: (1) What is the root cause of insulin resistance? (2) Does the secretion of peptides and hormones from adipose tissue regulate metabolic processes in other tissues to achieve appropriate fat

balance? (3) Are there single or multiple signals from other organs that regulate adipocyte fat storage pathways, and what is the nature of such signal(s)? Further clinical studies are essential to clarify these issues.

Many of the environmental factors and behaviors associated with obesity do indeed contribute to weight gain, but there is a possibility that trying to reduce obesity by changing behavior may not have relevance to the central issue. Hypothetically, there may be a central biological defect that makes obesity partially or totally unrelated to the societal environment. One strong argument for this supposition could be the simple fact that the current modes of therapy are less than satisfying.

Finally, irrespective of diet, weight loss, or medical treatment of obesity, we need to know the duration of metabolic changes and whether the improvement actually translates into a better long-term cardiovascular prognosis. The answer remains to be determined in large-scale prospective studies.

# CONCLUSIONS

- Significant cortisol release attributed to  $11\beta$ -HSD1 activity is found in the veins draining the SAT. Splanchnic cortisol production by  $11\beta$ -HSD1 is substantial and accounted for entirely by the liver. Cortisol release by  $11\beta$ -HSD1 into the portal vein was not detected.
- The ATBF is highly influenced by age and BMI in women. Postmenopausal overweight women display a loss of ATBF flexibility that may contribute to the metabolic dysfunction seen in this group. Endothelial dysfunction and ANS activity are associated with the ATBF disturbances.
- A strong association was seen between ATBF and SAT, as well as VAT areas, suggesting that total fat mass rather than specific sites of fat accumulation is linked to dysregulation of ATBF. Mechanistic studies are needed to clarify the highly significant association between circulating levels of leptin and ATBF.
- Weight loss in a diet program demonstrated that ATBF was reduced after 6 months in NNR group, but preserved in the PD group. In line with other metabolic outcomes in this study, we suggest that PD may have a more favorable metabolic effect than the NNR.

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