

ADIPOCYTE-DERIVED HORMONES AND CARDIOVASCULAR DISEASE

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“We all live under the same heaven, but the horizon differs”

To everybody

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Abstract

Obesity is increasing globally and related to major changes in lifestyle. This increase is associated with an increased risk of cardiovascular disease (CVD). Knowledge about adipose tissue as a metabolic-endocrine organ has increased during the last few decades. Adipose tissue produces a number of proteins with increased body weight, many of which are important for food intake and satiety, insulin sensitivity, and vessel integrity, and aberrations have been related to atherosclerosis. Notably, the risk for developing CVD over the course of a lifetime differs between men and women. In Northern Sweden, men have a higher risk for myocardial infarction (MI). However, the incidence is declining in men but not in women. These sex differences could be due to functional and anatomical differences in the fat mass and its functions.

The primary aim of this thesis was to evaluate associations between the adipocyte-derived hormones leptin and adiponectin, and fibrinolysis and other variables associated with the metabolic syndrome, and particularly whether these associations differ between men and women. Another aim was to evaluate these associations during physical exercise and pharmacological intervention (i.e. enalapril). Finally, whether leptin and adiponectin predict a first MI or sudden cardiac death with putative sex differences was also investigated.

The first study used a cross-sectional design and included 72 men and women recruited from the WHO MONICA project. We found pronounced sex differences in the associations with fibrinolytic variables. Leptin was associated with fibrinolytic factors in men, whereas insulin resistance was strongly associated with all fibrinolytic factors in women. The second study was an experimental observational study with 20 men exposed to strenuous physical exercise. During exercise, leptin levels decreased and adiponectin levels increased, and both were strongly associated with an improved fibrinolytic capacity measured as decreased PAI-1 activity. Changes in insulin sensitivity were not associated with changing adiponectin levels. The third study was a randomised, double-blind, single centre clinical trial including 46 men and 37 women who had an earlier MI. The study duration was one year, and participating subjects were randomised to either placebo or ACE inhibitor (i.e. enalapril). Circulating leptin levels were not associated with enalapril treatment. During the one-year study, changes in leptin levels were associated with changes in circulating levels of tPA mass, PAI-1 mass, and tPA-PAI complex in men, but not vWF. These associations were found in all men and men on placebo treatment. In women on enalapril treatment there

was an association between changes in leptin and changes in vWF. In the fourth study, the impact of leptin, adiponectin, and their ratio on future MI risk or sudden cardiac death was tested in a prospective nested case-control study within the framework of the WHO MONICA, Västerbotten Intervention Project (VIP), and Västerbotten Mammary Screening Program (MSP). A total 564 cases (first-ever MI or sudden cardiac death) and 1082 matched controls were selected. High leptin, low adiponectin, and a high leptin/adiponectin ratio independently predicted a first-ever MI, possibly with higher risk in men in regards to leptin. The association was found for non-fatal cases with ST-elevation MI. Subjects with low adiponectin levels had their MI earlier than those with high levels.

In conclusion, the adipocyte-derived hormones leptin and adiponectin are related to the development of CVD with a sex difference, and fibrinolytic mechanisms could be possible contributors to CVD risk.

Keywords: leptin, adiponectin, fibrinolysis, vWF, myocardial infarction, sex differences, physical activity, risk factors

Summary in Swedish – Sammanfattning på svenska

Övervikt och fetma har ökat i hela världen och detta är relaterat till förändringar i livsstilen. Denna ökning är förknippad med ökad risk för hjärt-kärlsjuka. Kunskapen om fettväven som ett organ som har både metabola samt hormonella egenskaper har ökat. Vi vet nu att den producerar en mängd olika proteiner och att de flesta ökar i mängd med ökad fettmassa. Många av dessa är livsviktiga och medverkar bla i hungerskänsla och födointag samt känsligheten för insulin. Avvikelse i dessa proteiner har också starkt förknippats med försämring av kroppens blodkärl, ateroskleros. Risken för att utveckla hjärt-kärl sjuka skiljer sig mellan män och kvinnor. I norra Sverige har män en ökad risk för insjuknande i hjärtinfarkt. Dock minskar risken för detta hos männen men ej hos kvinnorna. Det spekuleras om vad denna skillnad beror på; skillnad i anatomi men även skillnad i fettmassa och dess funktion kan vara en förklaring. Det övergripande syftet med denna avhandling var att studera sambandet mellan fettvävshormonerna leptin och adiponektin och kroppens ”propplösande” (fibrinolytiska) förmåga och andra faktorer som är förknippade med det metabola syndromet. Sambanden är även studerade under hård fysisk aktivitet, efter genomgången hjärtinfarkt (med samtidig behandling med enalapril) samt för att utröna om det fanns ett samband mellan dessa hormoner och framtida insjuknande i hjärtinfarkt eller plötslig hjärtdöd. Speciellt viktigt var det se om sambanden skiljde sig mellan män och kvinnor.

I det första arbetet (Paper I) studerades sambanden mellan leptin och fibrinolytiska faktorer hos 72 kvinnor och män som rekryterats i MONICA projektet. Dessa kvinnor och män var valda så att de skulle representera en stor spridning för hur känsliga de var för insulin. Hos männen var höga leptinnivåer starkt förknippade med försämring i den fibrinolytiska förmågan. Hos kvinnorna var försämrad fibrinolytisk förmåga mer förknippat med att ha en sämre känslighet för insulin.

I det andra arbetet (Paper II) så studerade vi 20 vältränade män som genomförde en fjälltur med hård fysisk prövning under två veckor. Under denna tur så förbättrades nivåerna av både leptin (sjunkande värden) samt adiponektin (stigande värden). Dessa förbättringar hade starka samband med ökad fibrinolytisk förmåga. Vi kunde även se att kroppens känslighet för insulin förbättrades. Dock sågs ingen förändring av vWF (=von Willebrand Faktor, ett sätt att värdera blodplättarnas förmåga att fästa till kärlväggen). Efter att fjällturen hade avslutats återvände samtliga värden till basnivåerna.

I tredje arbetet (Paper III) studerades sambandet mellan leptin, fibrinolys och vWF hos kvinnor och män som genomgått en akut hjärtinfarkt (83 personer). Studien varade under ett år i Skellefteå och de som deltog randomiserades till att få enalapril (ACE hämmare med indikation högt blodtryck eller hjärtsvikt) eller placebo. Vi kunde inte se något samband mellan leptin och behandling med enalapril. Dock fanns det ett samband hos alla män samt män som erhöll placebobehandling, mellan förändringarna i leptin och förändringar i fibrinolytisk förmåga. Hos kvinnorna som fått enalapril fanns det ett samband mellan förändring av leptin och förändring av vWF.

I arbete nummer fyra (Paper IV) studerades sambandet mellan nivåerna av leptin, adiponektin samt leptin/adiponektin ratio (förhållandet mellan leptin samt adiponektin) och framtida insjuknande i hjärtinfarkt och plötslig hjärtdöd. Vi jämförde 564 kvinnor och män som insjuknade med 1082 friska personer. Alla dessa hade tidigare undersökts när de deltog i antingen MONICA studien, Västerbottensprojektet eller Bröstcancerprojektet. Männerna som insjuknade hade vid tidigare provtagning högre nivåer av leptin och leptin/adiponektin ratio och både män och kvinnor hade lägre nivåer av adiponektin. De som hade lägre nivåer av adiponektin insjuknade även tidigare i sin hjärtinfarkt jämfört med de som hade högre nivåer.

Sammanfattningsvis visar avhandlingen att förändringar i fettvävshormonerna leptin (ökade nivåer) och adiponektin (sänkta nivåer) har ett samband med framtida insjuknande i hjärtinfarkt. Detta samband är inte lika hos kvinnor och män. Fibrinolytiska förändringar i samband med förändringar i nivåer hos dessa hormoner kan medverka till utvecklandet av ökad risk för hjärt-kärl sjuka.

Original papers

This thesis is based on the following papers, which will be referred to by their respective Roman numerals I-IV. Articles reprinted with permission.

I. Maria A. Eriksson, Eva Rask, Owe Johnson, Kjell Carlström, Bo Ahren, Mats Eliasson, Kurt Boman, and Stefan Söderberg. **Sex-related differences in the association between hyperleptinemia, insulin resistance, and dysfibrinolysis.** Blood Coagulation and Fibrinolysis 2008, 19:625-632

II. Maria Eriksson, Owe Johnson, Kurt Boman, Göran Hallmans, Gideon Hellsten, Torbjörn K. Nilsson, and Stefan Söderberg. **Improved fibrinolytic activity during exercise may be an effect of the adipocyte-derived hormones leptin and adiponectin.** Thrombosis Research 2008, 122:701-708

III. Maria A. Eriksson, Stefan Söderberg, Torbjörn K. Nilsson, Marie Eriksson, Kurt Boman, and Jan-Håkan Jansson. **Leptin levels are not associated with enalapril treatment after an uncomplicated myocardial infarction, but associate strongly with changes in fibrinolytic variables in men.** In manuscript.

IV. Maria A. Eriksson, Patrik Wennberg, Jan-Håkan Jansson, Göran Hallmans, Lars Weinehall, Tommy Olsson, and Stefan Söderberg. **Leptin and adiponectin predict independently a first-ever myocardial infarction with a sex difference: Data from a large prospective Swedish nested case-referent study.** In manuscript.

Abbreviations

ACE	angiotensin converting enzyme
AgRP	agouti gene-related protein
AGT	angiotensinogen
AHA/NHLBI	American Heart Association/National Heart, Lung and Blood Institute
AMI	acute myocardial infarction
AMPK	adenosinemonophosphate-activated protein kinase
APCE	antiplasmin-cleaving enzyme
Apo	apolipoprotein
AT II	angiotensin II
ATP III	third adult treatment panel
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CART	cocaine- and amphetamine-regulated transcript
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
CI	confidence interval
CNS	central nervous system
Con A	concanavalin
COX	cyclooxygenase
CRP	c-reactive protein
CV	coefficient of variation
CVD	cardiovascular disease
DBP	diastolic blood pressure
DM	diabetes mellitus
ELISA	enzyme-linked immunosorbent assay
ECG	electrocardiogram
FDP	fibrin degraded product
FFA	free fatty acids
GP1b	glucoprotein 1b
GPIIb/IIIa	glucoprotein IIb/IIIa
HDL	high density lipoprotein
HSL	hormone-sensitive lipase
ICAM	intercellular adhesion molecule
IFG	impaired fasting glucose
IGF	insulin-like growth factor
IGT	impaired glucose tolerance

IHD	ischemic heart disease
IL	interleukin
IR	insulin resistance
IS	insulin sensitivity
LDL	low density lipoprotein
LIPID	the long-term intervention with pravastatin in ischaemic disease study
LPL	lipoprotein lipase
MAPK	mitogen-activated protein kinase
MCP	monocyte chemoattractant protein
MI	myocardial infarction
MMP-2	matrix metalloproteinase-2
MONICA	MONIToring of trends and determinants in CARDiovascular disease
MS	the metabolic syndrome
MSP	the mammary screening project
NO	nitric oxide
NPY	neuropeptide Y
NSTEMI	non ST-elevated myocardial infarction
OGTT	oral glucose tolerance test
OR	odds ratio
PAI	plasminogen activator inhibitor
PHA	phytohemagglutinin
POMC	proopiomelanocortin
PON1	paraoxinase 1
PPAR	peroxisome proliferator activated receptor
PRIME	prospective epidemiological study of myocardial infarction
RIA	radioimmunoassays
RR	relative risk
SBP	systolic blood pressure
SD	standard deviation
SHBG	sex hormone-binding globulin
SHS	the Strong Heart Study
STEMI	ST-elevated myocardial infarction
STF	Svenska Turistföreningen
TAFI	thrombin-activatable fibrinolysis inhibitor
TG	triglycerides
Th 1	T helper cells
TNF- α	tumour necrosis factor-alpha
tPA	tissue type plasminogen activator
T2DM	type 2 diabetes mellitus
uPA	urokinase type plasminogen activator

VEGF	vascular endothelial growth factor
VIP	Västerbotten intervention program
VSMC	vascular smooth muscle cells
vWF	von Willebrands factor
WHO	world health organization
WHR	waist hip ratio
WOSCOP	the west of scotland coronary prevention study

Introduction

The epidemic of myocardial infarctions in Northern Sweden

The most frequent cause of death in Sweden during the 20th century has been cardiovascular disease (CVD), particularly in the county of Västerbotten [1, 2]. During the first half of the century, until the 1960s, the incidence of CVD increased, but the trend started to change in the 1980s, and the same pattern was seen in most industrialised countries [3].

Alarmed by the high incidence and mortality of CVD, the World Health Organization (WHO) implemented a series of meetings in Geneva in 1979 with a primary aim of monitoring trends in CVD and developing standardized protocols in respect to myocardial infarction (MI) [4, 5]. Daily living habits, health care, or major socio-economic features have been measured at the same time in defined communities in different countries to evaluate whether these trends are related to changes in known risk factors. Two main null hypotheses were formulated for coronary heart disease (CHD): i) there are no relationships between the 10-year trends in serum cholesterol, blood pressure (BP), and cigarette use and the 10-year trend in CHD incidence, and ii) there are no relationships between the 10-year trend in 28-day case fatality rates and 10-year trends in acute coronary care [6]. These hypotheses were the background to the WHO initiated MONICA (Multinational MONitoring of Trends and Determinants in Cardiovascular disease) project, in which Västerbotten and Norrbotten participated as one centre. The official WHO project ended in 1995, but it has continued as a local project in Northern Sweden [7].

In Sweden during the 1970s and the beginning of the 1980s, the county of Västerbotten had the highest mortality from MI with 720 deaths per 100,000 inhabitants per year among those aged 16 to 74 years. Nearly 200 fewer deaths per year occurred in Halland, the county with the lowest MI mortality in Sweden [8].

In the municipality of Norsjö, a small community in Västerbotten, the mortality from CVD was even higher, and three-fold higher in men compared to women [9]. A collaborative project between the Västerbotten County Council, The Swedish Planning and Rationalization Institute for Health Services, and the Umeå University Department of Social Medicine was formulated after presenting these data to leading politicians and administrators, which was the start of the “Norsjöprojektet” in 1985, later renamed “Västerbottensprojektet” or VIP [9]. Significant differences in the gender-related incidence of MI events have been reported for

Northern Sweden. In men aged 25 to 64 years, the incidence decreased from 555 to 300/100,000 between 1985 and 2002, whereas the incidence did not decrease in women during the same time period. Furthermore, recurrent MI declined approximately 70% in men and 40% in women, age 55-64 years [7]. Furthermore, mortality due to MI was higher among men compared to women, but decreased faster for men than for women during the period studied [7]. Explanations could include different presentation in men with more diffuse symptoms in women, including less typical ECG changes with subsequent delay to treatment, and greater age at the first MI [10, 11].

Risk factors could also differ; though traditional risk factors such as cholesterol and BP have decreased in both men and women, smoking has decreased more among men, but there is still a higher prevalence in women compared to men [12]. Of great concern is that body mass index (BMI) and waist circumference (abdominal obesity) increased between 1986 and 2004 in Northern Sweden, particularly in younger men and elderly women [13].

Obesity

Obesity is an increasing problem in both the industrialized part of the world [14] and developing countries. For example, one-third of the population of the USA is overweight [15]. The prevalence of obesity is increasing in developing countries due to increasing urbanization and changing lifestyle, so called “coca-colonisation” [16, 17]. Twenty-five percent of adults in the industrialized world are estimated to be obese, making it a leading public health issue [18].

Several chronic diseases, including type 2 diabetes (T2DM) and CVD, are associated with obesity [19, 20], and the risk for CVD increases with increasing obesity [21-23]. Furthermore, people with severe obesity have an estimated reduced longevity of 5 to 20 years [24].

However, the definition of obesity has been debated, and different cut-offs are suggested for different ethnic groups [25-27]; the localisation of fat tissue is important, as visceral adipose tissue is associated more with inflammatory and oxidative stress than subcutaneous adipose tissue [28].

In women, a waist to hip ratio (WHR) equal to or greater than 0.76 or a waist circumference equal to or greater than 76.2 cm is associated with a two-fold greater risk of developing CHD compared to women with a WHR < 0.72 cm. The risk was three-fold higher with a WHR greater than 0.88 [29]. In line with this observation, a high BMI was found to be an independent predictor of MI in both men and women in the HOPE trial. Increased abdominal obesity was an independent predictor of all-cause mortality, CVD death, MI and congestive heart failure. In the

INTERHEART study, they concluded that WHR instead of BMI, increased the estimate of MI in most ethnic groups [30, 31].

Obesity, central obesity in particular, is a strong predictor for T2DM and insulin resistance [32-36]. Obesity expressed as BMI was the dominant predictor of T2DM in men in the Health Professionals Study [37], whereas BMI, WHR, and waist circumference predicted T2DM in women, with waist circumference as the strongest predictor [38, 39]. Even a small increase in BMI increases the risk of T2DM. For women, a BMI between 23 and 25 kg/m² increases the risk of T2DM three-fold compared to a BMI <23 kg/m². A BMI of ≥35 kg/m² is associated with a relative risk (RR) of 20 [40]. However, differences are found due to ethnicity [41-43], and BMI-based national and international recommendations should reflect this.

Obesity is associated with metabolic disturbance, known as metabolic syndrome (MS), and MS is associated with an increased risk of CVD [44-47]. The definition of MS has been debated, which is reflected in the many definitions of the syndrome. In 2004, the International Diabetes Federation convened a workshop with participants from all five continents and the WHO and National Cholesterol Education Program-Third Adult Treatment Panel (ATP III), launching the so-called IDF definition [48]. Notably, expressing central obesity as a waist circumference above an ethnic specified cut-off was mandatory. In 2010, Eckel et al. presented a revised version of the definition of MS that included three or more of the following five risk factors: triglycerides (TG) ≥150 mg/dL (1.7 mmol/L) or treatment, high density lipoprotein cholesterol (HDL) <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women or treatment, hypertension with systolic BP ≥ 130 mmHg and/or diastolic BP ≥85 mmHg or treatment, increased fasting glucose >100 mg/dL (5.5 mmol/L) or treatment, and increased waist circumference with different cut-off points for different ethnicities. However, these cut-offs also differ between different organisations; for example, according to the IDF the cut-offs for people of European origin are 94 cm for men and 80 cm for women, and according to the American Heart Association/ National Heart, Lung and Blood Institute (AHA/NHLBI) the cut-offs are 102 cm for men and 88 cm for women [49, 50].

Adipose tissue and adipocytes

Knowledge about adipocytes and their functions and interactions has increased dramatically over the last few decades. Pre-adipocytes originate from a multipotent stem cell of mesodermal origin and has the potential to generate new fat cells that persist during an individual's entire life [51]. Previously assessed as being the body's storage of free fatty acids (FFAs)

after a meal, releasing the FFAs during the fasting state, adipocyte tissue is now known as an advanced endocrine and paracrine organ, secreting adipokines (hormones and cytokines) that participate in diverse metabolic processes [52], and to be dysfunctional in relation to T2DM and CVD. More macrophages infiltrate the adipose tissue with increasing obesity, and more infiltrate the visceral fat tissue compared to subcutaneous fat tissue [53]. Thus, obesity is a chronic low-grade inflammatory state with subsequent up- and down-regulation of adipocytokine secretion [54, 55]. After weight loss, the number of macrophages decreases [56]. Several adipokines both affect metabolism and integrate into different organ functions, including food intake (e.g., leptin, adiponectin), insulin resistance (e.g., adiponectin, resistin, visfatin, omentin, vaspin), inflammation (e.g., adiponectin, resistin, tumor necrosis factor [TNF]- α , interleukin [IL]-6, adipsin, C-reactive protein [CRP]), vasodilatation (e.g., apelin), lipid metabolism (e.g., cholesterol ester transfer protein [CETP], lipoprotein lipase [LPL], hormone-sensitive lipase [HSL]), BP (e.g., angiotensin converting enzyme [ACE], angiotensinogen [AGT], angiotensin II [AT II]), fibrinolysis (e.g., plasminogen activator inhibitor-1 [PAI-1]), and macrophage activation (e.g., monocyte chemoattractant protein-1 [MCP-1], intercellular adhesion molecule-1 [ICAM-1]) [57]. Most of these adipokines have increased plasma levels with increasing amounts of adipose tissue and adipocyte volume, except for adiponectin, which shows an inverse relationship [58, 59]. Neuroendocrine feedback is also possible due to autonomous nervous system innervation of the adipose tissue, both visceral and subcutaneous [60].

Leptin is an important regulator of fat mass [61, 62] through effects on central signalling and neuroendocrine responses [63]. The lipid content and corresponding size of an adipocyte cell correlates to its expression of leptin [64], and the expression of the leptin gene and circulating leptin levels correlate with the adipose tissue mass [65].

According to animal studies, local hypoxia has been suggested to occur when the adipocyte hypertrophies, inducing hypoperfusion. As a result of hypoxia and cell death, transcription factors that trigger the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, and PAI-1, are expressed. These factors contribute to the inhibition of adiponectin gene transcription with subsequently low circulating levels [66-68].

Atherosclerotic plaques

Over a lifetime, men have a higher risk of developing CVD compared to women, and they experience plaque rupture more often [69, 70]. In contrast, women with T2DM have a higher mortality rate due to CHD

[71]. In an autopsy study, 1460 coronary thrombi were characterised in relation to their surface characteristics and the underlying plaque. Even though the clinical presentation was MI or sudden death, plaque rupture was only identified as the cause of coronary thrombosis in 76% of thrombi, with a higher frequency in men (81%) compared to women (59%) [72]. The most common cause of coronary artery disease (CAD) is atherosclerosis, which can manifest as acute thrombosis on a ruptured and/or eroded atherosclerotic plaque [72, 73]. A vulnerable plaque develops in seven steps according to Libby [74]. A lesion initially starts within endothelial cells activated by risk factors (e.g., high cholesterol levels). The subsequent expression of adhesion and chemoattractant molecules recruits monocytes and T lymphocytes. The monocytes invade the artery wall and become macrophages, expressing scavenger receptors that bind lipoproteins. The macrophages become foam cells and secrete inflammatory cytokines and growth factors, initiating the recruitment of leukocytes and the migration and proliferation of smooth muscle cells. During this lesion progression, inflammatory mediators cause the expression of tissue factor and matrix-degrading proteinases, causing a pro-coagulant stage and weakening of the fibrous cap of the plaque.

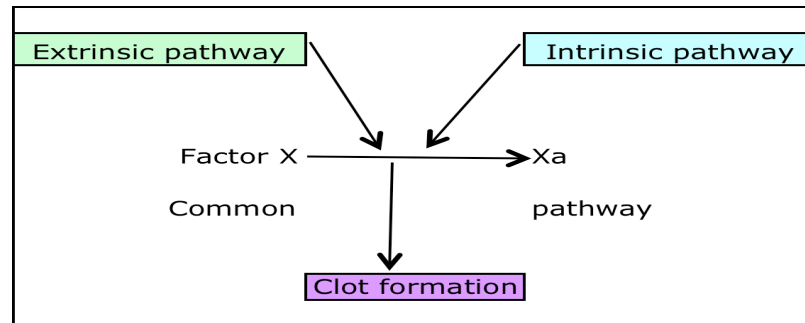
If a plaque ruptures, the flow obstruction caused by platelet aggregation with the blood proximal and distal to the occlusion stagnates with subsequent coagulation. However, this process is dynamic with thrombosis and thrombolysis, with a risk for vasospasm and distal microembolisation [72], sometimes resulting in a dramatic picture with a prompt reduction in oxygen and nourishment to distal myocardial cells, which manifests as myocardial cell death. Thus, the aetiology of thrombosis on an eroded plaque is heterogenic and probably influenced by systemic factors, such as platelet hyperaggregability, hypercoagulability, circulating tissue factor, and/or dysfibrinolysis, together with tissue factor delivered locally by leukocytes [75, 76]. Calcified plaques are less inflamed and less responsible for the acute coronary syndrome (ACS) and more related to stable angina pectoris [77]. Due to vascular remodelling and an intact lumen, many rupture-prone plaques are not visible angiographically, and these plaques are very thrombogenic after rupture due to a high amount of tissue factor [76].

Thrombosis and fibrinolysis

Blood circulation is essential for life, and the integrity of this process must be maintained. Leaks can occur in this system with subsequent blood lost. On the other hand, clot formation may lead to the obstruction of vessels, which results in the cessation of blood flow. Haemostatic equilibrium consists of the coagulation system in balance with the fibrinolytic system.

Once a blood clot has formed, it can either be dissolved by fibrinolysis or invaded by fibroblasts [78]. Fibrin formation results from the classical common pathway of coagulation, which can be activated by either the extrinsic or intrinsic pathway [79, 80]. The activation of these pathways results in the formation of an enzyme complex that activates factor X (Fig 1).

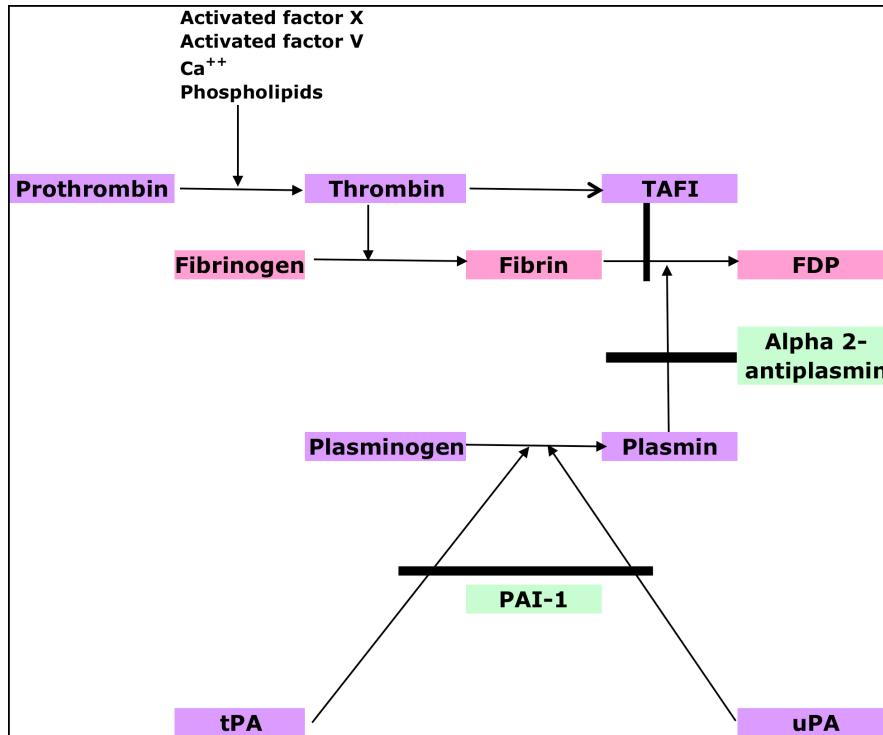
Figure 1. Clot formation



Clot formation through the extrinsic and intrinsic pathways via activation of Factor X [81].

The fibrinolytic system is necessary for processes involved in building and degrading tissues (Fig 2). Fibrin degradation is mandatory for recanalisation and/or dissolution of a thrombus, and disorders of the fibrinolytic system could result in thrombotic activation. Therefore, the system is important with regulation and control, which is mediated by interactions between its components, controlling synthesis, and the release and clearance of activators and inhibitors [82].

Figure 2. Outline of the haemostatic system



Fibrin degradation by the fibrinolytic system. Plasminogen is activated to plasmin by tPA or uPA. These enzymes are regulated by PAI-1. Fibrin is degraded to FDP by plasmin, which is regulated by α -antiplasmin. Prothrombin is converted to thrombin by the enzyme complex including factor Xa, factor Va, phospholipids, and Ca²⁺ ions. Thrombin not only converts fibrinogen into fibrin, but also activates TAFI, which inhibits fibrinolysis by modifying the fibrin substrate [83, 84].

Plasminogen and plasmin

The enzyme that degrades fibrin is plasmin, the active form of plasminogen, which is produced by the liver and circulates in the plasma at concentrations of 200 mg/L. Human plasminogen is a 92 kDa glycoprotein that is converted to plasmin by cleavage [85]. Plasminogen has a high affinity for the fibrin clot; when the clot is formed, a large amount of plasminogen is trapped in it [86]. Plasmin can be generated by two physiological plasminogen activators that are present in human plasma. In the first pathway, plasminogen is converted to plasmin by urokinase (uPA). The second pathway is important for dissolving thrombi in the vessels and involves tissue type plasminogen activator (tPA) [87,

88]. The affinity of tPA for plasminogen is higher when plasminogen is connected to fibrin, which means that fibrinolysis partly takes place in the fibrinolytic clot instead of systemically [89, 90].

Tissue type plasminogen activator (tPA)

Produced in the vascular endothelium, tPA is a 70 kDa serine protease found at a concentration of approximately 5-10 ng/mL in plasma [91]. The secretion of tPA is both constitutive and stimulated by several factors, including activated platelets, thrombin, epinephrine, stress, coffee, exercise, venous occlusion, and ischemia [92-95]. Local regulation determines the release rate of tPA; consequently, systematic levels of tPA do not reflect local levels of tPA. Furthermore, it is the local capacity of tPA secretion by the endothelium that determines the amount of available active tPA in the vascular bed of an organ [94]. The half-life of circulating tPA is 3-5 minutes, and it is cleared by the liver [96]. The main activator of plasminogen to plasmin in the plasma is tPA, and it does so in two phases. First, tPA activates plasminogen on the intact fibrin surface. After the degradation of fibrin, new binding sites for plasminogen and tPA are exposed [88]. The main inhibitor of tPA is PAI-1, but it is also inhibited slowly by the C1-inhibitor, α 2-antiplasmin, and α 2-macroglobulin [97, 98].

Urokinase type plasminogen activator (uPA)

The enzyme uPA was first isolated from urine [99] and binds to a specific cellular uPA receptor, which leads to enhanced activation of cell-bound plasminogen. This enzyme is the main activator of plasminogen in the induction of pericellular proteolysis during tissue remodelling and repair (a function of macrophages), ovulation, embryo implantation, and tumour invasion, including cleaning the tubuli from excreted protein [82, 87].

Plasminogen activator inhibitor (PAI)

PAI-1 is a glycoprotein belonging to the serpins and is the main inhibitor of the fibrinolytic system [100]. PAI-1 is produced in different cell types, including vascular endothelial cells, smooth muscle cells, spleen cells, fibroblasts, platelets, macrophages, hepatocytes, and adipocytes [100, 101]. In platelets, PAI-1 is stored in α -granulae and released when platelets are activated after vessel trauma. Plasma PAI-1 levels are 5-50 ng/mL, and normal levels of PAI-1 activity are 3-30 U/mL [91]. Active PAI-1 is unstable with a half-life in plasma of 8 to 10 minutes, and it is cleared by the liver [100, 102]. The production of PAI-1 is stimulated by various cytokines, thrombin, and hormones, including insulin and leptin, and by activation of the renin-angiotensin-aldosterone

system [101, 103, 104]. During the early stages of inflammation, the liver produces PAI-1 antigen as an acute phase protein, but the endothelium contributes during sepsis [82]. PAI-1 is secreted and circulates in plasma as a complex with vitronectin, which protects it from inactivation and stabilises its activity [105, 106]. PAI-1 also forms a stable 1:1 complex with tPA, the tPA/PAI-1 complex, which restricts fibrinolysis by limiting the activation of plasminogen to plasmin [107]. A several-fold excess of PAI-1 over tPA exists in the circulation, so most tPA circulates as a tPA/PAI-1 complex [82].

PAI-2 is produced by the placenta and, during pregnancy, the plasma concentration increases from <1 µg/L to 200 µg/L [87, 108].

α₂-antiplasmin

The glycoprotein α₂-antiplasmin has a molecular weight of 70 kDa and is found at a concentration of approximately 70 µg/mL in the plasma [109, 110]. α₂-Antiplasmin inhibits the fibrinolytic system by forming an inactive 1:1 complex with plasmin [111]. The antiplasmin-cleaving enzyme (APCE) splits α₂-antiplasmin, and the new form binds approximately 13 times more rapidly to fibrin during clot formation than the native form, more efficiently protecting the clot from fibrinolysis [112].

Thrombin-activatable fibrinolysis inhibitor (TAFI)

TAFI is a newly discovered inhibitor of the fibrinolytic system and represents a link between coagulation and fibrinolysis [113, 114]. TAFI is a glycoprotein with a molecular weight of 60 kDa [115]. After cleavage, TAFI is converted to activated TAFI (TAFIa) [116]. Thrombin not only converts fibrinogen to fibrin, but also slowly activates TAFI, which inhibits the fibrinolytic system by modifying the fibrin substrate, a process accelerated 1000-fold by thrombomodulin [117]. The result is strongly reduced binding to plasminogen and a concomitant reduction in the activation of plasminogen on the fibrin surface [118]. Furthermore, TAFI is both activated and inactivated by plasmin [117]. Under physiological conditions, TAFIa is relatively unstable with a half-life of roughly 8-15 minutes at 37°C [119].

von Willebrand Factor (vWF)

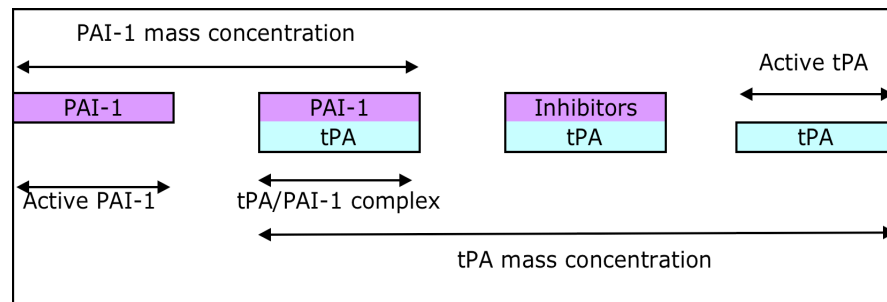
The vWF is an adhesive glycoprotein that plays a key role in primary haemostasis produced and secreted by endothelial cells and by the α-granulae in megakaryocytes and platelets. The plasma levels of vWF are expressed as a percentage of normal, and the normal range is 50 to 200%. Circulating levels of vWF in the plasma relate to the production by endothelial cells as the platelets only release their α-granule content when activated. vWF is cleared from plasma by the liver with a half-life of 12 to

18 hours. Deranged vWF levels lead to bleeding (defected vWF, von Willebrand disease) or thrombosis (elevated levels) [120, 121]. Factor VIII circulates in the plasma in an inactive form complexed with vWF and is activated by thrombin or active factor X. In von Willebrand disease, factor VIII also has decreased activity [122]. The secretion of vWF is stimulated by various factors, including thrombin, fibrin, and histamine. The synthesis of vWF increases with vascular injury or stress and, thus, plays a role as an acute phase protein [123-126]. Release of vWF mediates both platelet adhesion to the subendothelial tissue and the aggregation of platelets [127], and vWF binds to both collagen and the platelet receptor GPIb and GPIIb/IIIa [128]. Subjects with the type O blood group have 30% lower levels of vWF than subjects with the type AB blood group [129]. The concentrations of vWF increase with increasing age, diabetes, hypertension, inflammatory vascular disease, and peripheral artery disease [128].

Measurement of components in the fibrinolytic system

A measurement of the PAI-1 mass concentration (also known as PAI-1 antigen) includes both PAI-1 activity and inactive PAI-1 in complex with tPA (tPA/PAI-1 complex) (Fig 3). The total tPA mass concentration (tPA antigen) includes a mixture of the tPA/PAI-1 complex (estimated to be the largest portion of tPA mass), tPA bound to other inhibitors, and active tPA, which represents approximately 20% of tPA mass [130, 131]. In contrast, the measurement of tPA activity includes only active tPA. Consequently, high concentrations of tPA/PAI-1 complex, tPA mass, and PAI-1 mass reflect deteriorated fibrinolytic activity, whereas high tPA activity indicates high fibrinolytic activity.

Figure 3. Measurement of components in the fibrinolytic system



Schematic presentation of the relationships between tPA antigen and activity, PAI-1 antigen and activity, and the tPA/PAI complex [132].

Leptin

The protein

A gene located in chromosome 7q31.3 encodes the protein leptin and is translated into a protein 167 amino acids in size. After the translocation of leptin into microsomes, a signal peptide is removed to create a protein 146 amino acids in length [133, 134] with a molecular weight of 16 kDa [62, 134]. The main producer of leptin is the adipose tissue and adipocytes, but several other tissues also express the protein, including placenta, ovaries, skeletal muscle, bone marrow, mammary epithelium, and the fundic epithelium. Leptin circulates in a free form or bound to binding proteins and is cleared by the kidneys. The half-life of free leptin in plasma is approximately 3-4 minutes, but is much longer for the bound form at 25 minutes. The relative amount of free leptin is higher in obese humans compared to lean humans [135-145]. In humans, insulin, glucocorticoids, TNF- α , oestrogen, IL-1, and alcohol are associated with increased concentrations of leptin, whereas androgens, catecholamines, growth hormone, somatostatins, and smoking are related to lower levels [142, 146].

Leptin is involved in several metabolic and hormonal processes, including the regulation of pancreatic islet cells, growth hormone levels, immunology, homeostasis, hematopoiesis, angiogenesis, wound healing, osteogenesis, and gastrointestinal function [147, 148]. Furthermore, leptin is important for controlling food intake and the expenditure of energy by acting on the hypothalamus [149]. Leptin circulates partially bound to plasma proteins and enters the central nervous system (CNS) by diffusion through capillary junctures in the median eminence and by saturable receptor transport in the choroid plexus. In the hypothalamus, leptin binds to receptors that stimulate anorexigenic peptides, such as proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), and inhibit orexigenic peptides, such as neuropeptide Y (NPY) and the agouti gene-related protein (AgRP) [150].

The leptin production rate seems to be related to the degree of adipocyte mass. Individuals with obesity have higher levels of leptin, probably due to greater production per unit of body fat and increased production due to the increased body mass. These individuals also have greater subcutaneous fat compared to visceral fat [141, 142, 151]. Ethnic differences in circulating levels of leptin may also exist, with higher levels in Asian Indians [152].

Receptors

Leptin action is mediated by plasma membrane receptors, and at least six long and short receptor isoforms are known. The short isoforms are

expressed in several tissues, including adipose tissue, adrenal gland, choroid plexus, gonads, heart, kidney, liver, lung, lymphocytes, macrophages, pancreas, platelets, skeletal muscle, small intestine, spleen, testis, and vascular endothelium. The long form isoform is found in the hypothalamus. However, many tissues contain a heterologous mix of leptin receptors [135, 142, 148, 153, 154]. All isoforms of the receptor share an identical extracellular domain but have cytoplasmic domains of different lengths. Five of the isoforms contain transmembrane domains. One isoform lacks both transmembrane and cytoplasmic domains and circulates as a soluble receptor [155]. Leptin binds to the long form of the receptor in the hypothalamus and activates Janus kinase (JAK2)-signal transducer and activator of transcription (STAT3) and the phosphatidylinositol-3 kinase (P13K) pathway to increase the metabolic rate and sympathetic tone and suppress feeding, decreasing body weight [156]. In addition, leptin inhibits the activity of AMP-activated protein kinase (AMPK) in the hypothalamus in order to suppress food intake [157]. The short isoform can transduce signals through insulin receptor substrates and JAK-dependent signalling to mitogen-activated protein kinase (MAPK) pathways. The short form plays a role not only in transport, but also clearance and as a source of soluble receptor [158].

Different levels in men and women

In humans, differences are found between men and women in both mRNA and circulating leptin levels and the correlation between leptin and fat mass. Women have higher expression and higher circulating levels than men and the correlation with fat mass is stronger. [159-162]. Women have more than three times higher levels of circulating leptin compared to men despite similar general and central obesity. Differences in the numbers and size of adipocytes, and the amount of subcutaneous versus visceral adipose tissue, may contribute to this effect [163, 164]. Furthermore, the stimulating role of oestrogens and/or the suppressive effect of androgens may contribute to this [159-162]. The brains of male and female rats are differentially sensitive to the catabolic actions of small doses of leptin and insulin, and oestrogens can alter hypothalamic sensitivity to leptin, possibly by stimulating the expression of the long leptin receptor [158, 165-167]. The increased leptin levels in females suggest that differences may exist in leptin transport across the blood-brain barrier or intracellular signalling cascades.

Leptin and endothelial dysfunction

In humans, the role of leptin in the regulation of endothelial function is controversial. High concentrations of leptin may elicit endothelium-dependent NO-mediated vasorelaxation in vitro [168-170], and acute

leptin administration in pharmacological doses increases plasma concentrations of NO metabolites and cGMP, its second messenger [171-174]. Leptin up-regulates inducible NO synthase, large amounts of which may impair endothelial function. Furthermore, in concentrations relevant to obesity, leptin impairs NO-dependent vasorelaxation induced by acetylcholine both in vitro and in vivo [175, 176]. In ob/ob mice, which cannot produce leptin due to a mutation in the leptin gene, endothelial function is impaired and leptin therapy improves NO-dependent relaxation of the isolated aorta [177]. In humans, however, plasma leptin levels inversely correlate with adenosine-induced (NO-dependent) coronary vasorelaxation in healthy obese males [178].

Leptin and lipids

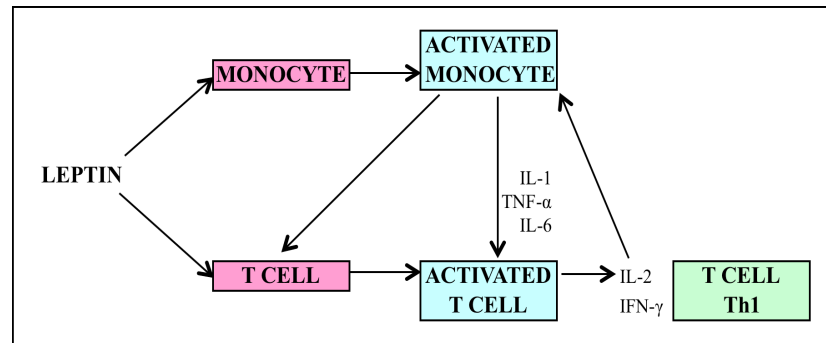
In cultured human and murine macrophages, leptin stimulates the secretion of lipoprotein lipase (LPL) [179]. LPL is considered to be pro-atherogenic as it promotes the accumulation of lipoproteins in the subendothelial space. In addition, leptin (especially at high glucose concentrations) increases the accumulation of cholesterol esters in foam cells [180]. In humans, an inverse relationship between leptin and HDL-cholesterol, and apolipoprotein A1, has been reported. Interestingly, ob/ob mice have high levels of HDL-cholesterol [181-183].

Leptin and inflammation

In humans, leptin dose-dependently enhances the proliferation and activation of human circulating T lymphocytes in the presence of co-stimulation with phytohemagglutinin (PHA) and concanavalin (Con A) [184]. This observation demonstrates the presence of the leptin receptor in human T lymphocytes and a role of leptin as a modulator of lymphocyte stimulation. In addition, leptin appears to be an enhancer of T lymphocyte stimulation with a shift towards a Th1 cytokine production profile [184, 185].

Leptin has been suggested to be a link between nutritional status and the immune system. In malnourished infants, low leptin levels are associated with suppression of the lymphoproliferative response and weight gain is followed by increasing levels of circulating leptin with a significant increase in Th1 activity [186-188]. Leptin also modulates monocyte-macrophage function and regulates the pro-inflammatory response. In vitro, leptin dose-dependently stimulates the proliferation of human peripheral blood mononuclear cells [184, 185, 189-192]. According to the stimulating effect on monocyte activation and proliferation, leptin is also able to induce the expression of monocyte cytokines IL-6 and TNF- α [192, 193] (Fig 4).

Figure 4. Simplified model of immune system modulation by leptin



Leptin directly activates monocytes and co-stimulated T cells. The system includes a Th1 response, which amplifies the pro-inflammatory response [186].

C-reactive protein (CRP) is an independent risk factor for cardiovascular events [194, 195]. CRP has a pro-atherogenic capacity by hampering endothelial NO production, activating vascular smooth muscle cells (VSMC), and stimulating monocyte adhesion to the endothelial surface [196]. High levels of circulating leptin correlate with increased inflammation [197-199], and leptin correlates with CRP in both lean and obese subjects [199, 200]. In subjects with BMI < 25 kg/m², CRP correlates with leptin but not BMI [199]. Furthermore, the administration of physiological levels of leptin increases CRP in women with a normal weight during acute caloric deprivation [201]. CRP production in the liver is stimulated by IL-6, but the association with leptin could be mediated by endotoxins, such as lipopolysaccharides [202].

Leptin and paraoxinase 1 (PON1)

PON1 is synthesized and secreted by hepatocytes and circulates bound to HDL. PON1 is important for protection from atherosclerosis, partly due to a prevention of plasma lipoprotein oxidation [203]. Many patients with known risk factors (e.g., hypercholesterolemia, obesity, DM, and smoking) have reduced PON1 activity [204]. In a prospective study, low PON1 activity predicted coronary events [205]. In rats, leptin decreases the effect of risk factors on PON1 activity, and PON1 activity is low in obese women and inversely correlates with plasma leptin levels [206, 207].

Leptin and vascular smooth muscle cells (VSMC)

Locally produced leptin is suggested to mediate VSMC hypertrophy via paracrine and autocrine pathways [208], and the expression of matrix

metalloproteinase-2 (MMP-2), which is important for migration of VSMC from the media to the intima and in plaque rupture [209, 210]. Leptin stimulates the migration and proliferation of VSMC from rat aorta in vitro [211]. In addition, leptin has been shown to be involved in the proliferation of VSMC from human aorta [209].

Leptin, fibrinolysis and vWF

Several studies, including paper I in this thesis, have shown positive associations between leptin and dysfibrinolysis (high PAI-1 levels) in both healthy subjects and patients with hypertension and ischemic heart disease (IHD), but is not always independent of confounders [212-216]. Mechanistically, it was recently shown that leptin up-regulates the expression of PAI-1 in human coronary artery endothelial cells in vitro [104].

We previously reported an independent association between high circulating levels of leptin and a low tPA activity in men and postmenopausal women [213, 215]. In both men and women without CVD, leptin positively correlates with vWF [217, 218].

Leptin and platelets

Leptin is associated with platelet aggregation [219]. In obesity, high concentrations of leptin (50-100 ng/mL) promote platelet aggregation, and the intracellular mechanisms by which leptin enhances platelet aggregation have been partially elucidated. In contrast, lower concentrations of leptin (< 10 ng/mL) do not potentiate agonist-induced platelet aggregation [220, 221].

Leptin-deficient ob/ob mice are protected from the arterial thrombosis or neointimal hyperplasia induced by arterial injury [222], which indicates that hyperleptinemia may contribute to atherogenesis in obese persons. The ob/ob (lacking leptin) and db/db (lacking the functional leptin receptor) mice have impaired thrombus formation compared to normal mice in a model of carotid injury [223]. The administration of leptin to ob/ob and wild-type mice enhances injury-induced arterial thrombosis, indicating an important role of the leptin receptor. Accordingly, animal experiments have shown that the long isoform of the leptin receptor expressed on platelets is required for normal thrombosis [224]. In obese women without known cardiovascular risk factors, leptin correlates with platelet activity measured as the urinary excretion of 11-dehydrothromboxane B2 [225].

Leptin and the sympathetic nervous system

Leptin administered intravenously, intracerebroventricularly, or into hypothalamic nuclei in rodents is associated with increased sympathetic

outflow to the kidneys, adrenal gland, adipose tissue, and skeletal muscle vasculature [226-229]. Related to this observation, the concept of partial leptin resistance was introduced as leptin may have full effects on the sympathetic system despite leptin resistance in respect to weight control [229].

In both adipocyte cell cultures and experimental animals, catecholamines (norepinephrine, epinephrine, and isoprenaline) have been found to have an inhibitory effect on leptin synthesis and release [230, 231]. Thus, a feedback loop between the sympathetic system and the adipose tissue is indicated. Leptin acts within the hypothalamus, causing the activation of central sympathetic outflow. Additionally, adrenal medullary release of epinephrine and renal sympathetic outflow is stimulated, and the sympathetic nervous system works to inhibit the release of leptin from white adipose tissue [232-234].

Leptin and renal Na⁺/K⁺-ATPase

Abnormal renal sodium metabolism is thought to play a main role in obesity associated hypertension [235, 236]. Hyperleptinemia decreases natriuresis by up-regulating the Na⁺/K⁺-ATPase in the renal cortex and medulla and stimulates tubular sodium reabsorption in rats. In addition, abnormal renal sodium retention and vasoconstriction is associated with NO deficiency [237].

Leptin and the renin-angiotensin-aldosterone system (RAS)

Obese subjects have elevated levels of angiotensinogen and renin, and ACE has a higher activity [238]. In both normotensive and hypertensive lean and obese subjects, leptin correlates with plasma renin activity and serum angiotensinogen [239, 240]. In addition, leptin synthesis is increased by angiotensin II in vivo in rats, and leptin mRNA expression and release increases in cultured adipocytes [219, 241, 242]. Related to these observations, treatment with an angiotensin II type I blocker significantly decreases subcutaneous and mesenteric adipose tissue with improved insulin resistance. Furthermore, the blocker reduces the circulating levels of leptin and leptin mRNA in adipose tissue [243].

Leptin and hypertension

Several mechanisms exist by which leptin could induce hypertension, as reviewed by Beltowski [244]. Studies have shown significant associations between plasma leptin and BP. These associations persist in both normotensive and hypertensive subjects, independent of body weight [245-247]. Furthermore, humans with inherited leptin deficiency are normotensive despite massive obesity [248]. However, the precise mechanism underlying the role of leptin has not been fully elucidated.

Leptin and the adiposity-pancreatic axis

The leptin-deficient ob/ob mouse is obese and characterised by insulin resistance and glucose intolerance [153], aberrations that normalise after the administration of leptin. However, human obesity is characterised by high leptin levels due to leptin resistance, and the administration of leptin does not rectify the metabolic disturbances [249] except for rare cases of congenital leptin deficiency [250].

The concept of an adiposity-pancreatic axis has been presented in which leptin is an important part of the bi-directional feedback and pathway between adipocytes and pancreatic β -cells [251, 252].

The insulin producing β -cell expresses functional leptin receptors [253], and leptin interacts with insulin released from isolated pancreatic β -cells [254-257] and suppresses proinsulin mRNA expression in human islets [253]. Leptin is also associated with the apoptosis of β -cells and the inflammatory response in β -cell islets [258, 259]. In the adipocyte, insulin stimulates leptin production by increasing secretion and gene expression [260, 261]. Finally, leptin is a prospective and independent predictor of T2DM, notably only in men in the Mauritius study [262, 263].

Adiponectin

The protein

Adiponectin is produced mainly in adipocytes [264]. However, Pineiro et al. suggested that human cardiomyocytes may also synthesize and secrete adiponectin [265]. Lean individuals have high circulating levels of adiponectin and, conversely, lower levels are seen those with obesity, particularly in viscerally obese individuals. Thus, adiponectin is negatively correlated with BMI [266, 267].

A locus associated with T2DM, the MS, and ischemic coronary disease has been identified on chromosome 3q27 [268-270]. This genetic sequence also codes for adiponectin. Full-length adiponectin contains 247 amino acids, resulting in a molecular weight of approximately 28 kDa [264, 271]. Adiponectin accounts for 0.01% of total plasma protein [266].

In plasma, adiponectin combines and forms various multimer complexes. The protein circulates in three different complexes: a low molecular weight trimer, a middle molecular weight hexamer, and a high molecular weight 12- to 18-mer [272-274]. The high molecular weight form of adiponectin is thought to be more clinically relevant in respect to diabetes and CAD [275-277].

The secretion of adiponectin from adipocytes decreases following stimulation with insulin, TNF- α , endothelin-1, and glucocorticoids, and transcription increases with IGF-1 treatment [278]. Adiponectin mediates

its effect via the activation of AMPK, peroxisome proliferator activated receptor (PPAR)- α , and p38 mitogen-activated protein kinase (MAPK) signalling pathways. The circulating levels of adiponectin in the plasma are 3-30 $\mu\text{g/L}$ and reach the cerebral spinal fluid (CSF) via the blood circulation. The measured levels in the CSF are roughly 0.1% of plasma levels [279, 280].

Different levels in men and women

Women have higher levels of adiponectin than men, probably due to androgens [281, 283]. Females also have higher levels of the high molecular weight form [277].

Receptors

Two receptors for adiponectin have been cloned, AdipoR1 and AdipoR2, which are expressed predominantly in skeletal muscle and liver, respectively. However, both AdipoR1 and AdipoR2 are found in the brain: in the hypothalamus and on endothelial cells [280, 284]. The effect on the hypothalamus is thought to be important in weight regulation. However, AdipoR1 and AdipoR2 are also expressed on other endothelial cells [285], cardiomyocytes [265], and pancreatic β -cells [286]. AdipoR1 has been suggested to be a receptor for globular adiponectin, and AdipoR2 is a receptor for full-length adiponectin [287].

In an animal model, the expression levels of AdipoR1 and AdipoR2 in the liver and skeletal muscle increase after fasting and are restored after refeeding. During a hypoinsulinaemic and hyperglycaemic state, AdipoR1 and AdipoR2 mRNA increase in skeletal muscle, and the levels are restored by insulin treatment [288].

Adiponectin and insulin sensitivity

In the Pima Indians of Arizona, a population with a high prevalence of obesity, T2DM, and insulin resistance, low levels of adiponectin correlate with reduced insulin sensitivity, and high levels reduce the risk of developing T2DM (incidence ratio 0.65, 95% confidence interval (CI) 0.43-0.92) [289]. This correlation was also shown in a Japanese population followed for 5 years; those in the lowest tertile of adiponectin developed diabetes nine times more often than those in the highest tertile [290].

In a study based on the hyperinsulinaemic-euglycaemic clamp, the glucose infusion rate, which is a measure of insulin sensitivity, correlated strongly with circulating levels of adiponectin [291]. Adiponectin-related insulin sensitization is mediated partly by the activation of AMPK in skeletal muscle and liver tissues, which increases the oxidation of fatty acids and reduces hepatic glucose production [292, 293]. Adiponectin

also increases the combustion of fatty acids and energy consumption via PPAR α activation, leading to decreased triglycerides in the liver, which contributes to the increased insulin sensitivity [294], [295]. Furthermore, thiazolidinediones (glitazones), oral agents for treating T2DM, increase the secretion of adiponectin by activating PPAR- γ in adipocytes [296, 297].

Adiponectin, fibrinolysis, and vWF

Adiponectin is inversely and independently (from BMI) associated with PAI-1 antigen in overweight hypertensive subjects [214] and negatively correlates with PAI-1 activity but not vWF in patients with angiographically confirmed stable CHD [298]. In a prospective study by Wannamethee et al., adiponectin negatively correlated with tPA antigen and positively correlated with vWF after adjusting for waist circumference [299].

Adiponectin and inflammation

Adiponectin indirectly decreases CRP and IL-6 levels by inhibiting TNF- α [300]. CRP mRNA is expressed in adipose tissue, and there is an inverse correlation between CRP and adiponectin mRNA in the subcutaneous adipose tissue of patients with angiographically demonstrated coronary atherosclerosis. An inverse relationship has also been reported between plasma high sensitive C-reactive protein hs-CRP and adiponectin [282, 301]. In addition, the expression and secretion of adiponectin is reduced by TNF- α [302]. This reciprocal association supports the concept that adiponectin is protective against developing atherosclerosis and vascular inflammation.

Adiponectin and vascular disease

In patients with T2DM, low levels of circulating adiponectin correlate with high levels of triglycerides and low levels of HDL-cholesterol; in non-diabetic females, adiponectin is inversely related to apolipoproteins B and E and positively related to apolipoprotein A-1 [303, 304]. Levels are lower in subjects with CVD [303, 305], hypertension [306], and MS [307], and these low levels correlate with insulin resistance [308-310]. In both men and women with T2DM, adiponectin levels are lower compared to age- and BMI-matched controls, and those with macrovascular disease have lower levels compared to those without the disease [303].

Low adiponectin levels are negatively associated with the intima-media thickness of the carotid artery and the presence of atherosclerotic plaque as assessed by ultrasound [311]. High levels of adiponectin are positively associated with less arterial pathology in the elderly, both men and women. In addition, circulating adiponectin is inversely associated with

heart systolic function in elderly men, and the association between adiponectin and heart function has been speculated to involve natriuretic peptides [312, 313].

Anti-atherogenic effects of adiponectin

Adiponectin may protect the vascular wall in several ways. The protein inhibits the proliferation of VSMC and arterial remodelling. Adiponectin also reduces the expression of VCAM-1 mRNA, suppressing monocyte adhesion to the endothelium, and it suppresses the transformation of macrophages to foam cells by suppressing the expression of class A scavenger receptor [314-318]. In addition, adiponectin increases anti-inflammatory cytokine IL-10 in human macrophages [319]. It has also been reported that adiponectin facilitates the removal of apoptotic cells by macrophages and modulates inflammation processes [320]. Furthermore, in human aortic endothelial cells, the surface expression of vascular adhesion molecules, which are known to modulate endothelial inflammatory responses, is decreased in vitro in an adiponectin dose-dependent manner [300], and high concentrations stimulate NO production in cultured aortic endothelial cells [321, 322]. In addition, low levels of adiponectin are associated with impaired endothelium-dependent vasorelaxation [306].

Circulating endothelial progenitor cells (EPCs) are believed to contribute to the modulation of the vascular response. Adiponectin levels are associated with the number of EPCs in patients with CAD [323]. Incubating human peripheral blood mononuclear cells with adiponectin increases the number of EPCs and the effectiveness of mobilization [324]. In an animal model of corneal angiogenesis, adiponectin stimulates blood vessel growth and stimulates endothelial cell migration and differentiation into capillary-like structures [325]. In vitro, adiponectin prevents endothelial apoptosis [276]. Finally, adiponectin decreases ischemia-reperfusion injury in acute MI via both COX-2 and AMPK-mediated effects [326].

Physical activity and adipokines

Physical activity is thought to prevent CVD and CVD-related death [327]. We hypothesise that this prevention can be partly explained by an effect on adipokines. However, divergent results are found in the literature regarding the effects of physical activity on circulating adipokine levels. Prolonged endurance exercise, such as marathon running [328] and elite gymnastics for both men and women of pubertal age [329], has been shown to decrease circulating leptin levels. Furthermore, endurance exercise training decreased leptin levels in men, independent of insulin levels and anthropometry [330]. In contrast,

several studies have not shown any effect on leptin levels after adjusting for changes in body fat mass [331, 332]. Kraemer et al. could not find an independent increase in circulating adiponectin levels in trained runners [333], and submaximal aerobic workouts did not improve adiponectin levels 48 hours post-exercise in healthy overweight men [334]. In a randomized controlled trial, middle-aged men with T2DM were randomly assigned to 8 weeks of training or to a control group. No significant changes were seen in circulating levels of leptin or adiponectin, despite a 44% decrease in abdominal fat and 58% improved insulin sensitivity [335]. However, 50 overweight men aged 65 to 78 years were voluntarily admitted to four groups (control, low-intensity, moderate-intensity, and high-intensity training) for six months; adiponectin significantly increased in the high-intensity group, whereas leptin decreased in all treatment groups [336]. In a cross-sectional study, energy expenditure in physical activity, measured with a tri-axial accelerator, explained 43% of the variation in adiponectin levels after adjusting for central fat mass in young non-obese women, whereas no association was seen with the variation in leptin levels [337]. Additionally, physical training enhances the expression of adiponectin receptors [338], and leptin mRNA is down-regulated after acute exercise [339].

The impact of adipokines and fibrinolytic factors on CVD risk

Leptin

The first study showing an association between high leptin levels and CVD was from Northern Sweden. In a nested case-control prospective study, 62 men were identified with a first MI. The participants were recruited from the WHO MONICA and VIP surveys. After adjusting for BMI, hypertension, diabetes, smoking habits, blood lipids, and PAI-1, high levels of leptin predicted first MI (odds ratio (OR)=9.0, 95% CI 1.7-46.5) [340].

The West of Scotland Coronary Prevention Study (WOSCOP) was a large randomised controlled trial studying the effect of the lipid lowering drug pravastatin in a male population with high cholesterol levels but no previous CVD. Several post-hoc studies have been performed, and a prospective nested case-control study looking at the impact of baseline levels of leptin and incident CHD (fatal and non-fatal MI or revascularization procedure) reported a 20% increase (95% CI 1.0-1.4, $p=0.03$) in CHD per 1 standard deviation increase in leptin levels after adjusting for several risk factors (i.e. age, BMI, lipids, and systolic BP). This study included 377 cases and 783 controls, which was representative of the whole WOSCOP cohort (580 cases and 1160 controls). Cases and

controls were matched for age and smoking, and the follow-up time was 5 years [341].

In the PRIME study, a nested case-control prospective study, high levels of leptin increased the risk of a future CHD event in the unadjusted model. After adjusting for traditional risk factors (i.e. blood lipids, diabetes, hypertension, and smoking status), these associations did not remain. The study included 617 cases and 1215 controls (all males) matched for study centre, age, and time of entry into the study. Controls were also free from CHD at the date of the ischemic event in the case. Follow-up time was 10 years. A first CHD event was defined as angina pectoris, unstable angina pectoris, MI, or coronary death [342].

In a sub-study to the LIPID trial, 184 men with a history of stable CHD with a recurrent cardiovascular event (cardiovascular death, non-fatal MI, or ischemic stroke) and matched controls without a recurrent event were compared in relation to leptin levels; leptin, but not adiponectin, independently predicted (adjusted for BMI, randomized treatment, other drugs, the LIPID score [HDL- and total cholesterol, age, sex, smoking status, nature of prior acute coronary syndrome, revascularization and history of stroke, diabetes, and hypertension] in separate models) a recurrent event. Follow-up time was 4 years. Furthermore, patients randomly allocated to pravastin had approximately 6% lower leptin levels compared to those randomly allocated to placebo [343].

Couillard et al. could not find any association between high levels of leptin and incident IHD events (defined as typical effort angina, coronary insufficiency, non-fatal MI, or coronary death) in the Quebec Cardiovascular Study, which included 2103 men free of IHD at baseline. During a 5-year follow-up, 114 developed an IHD event. Only 86 cases and 95 controls remained after exclusions. No significant differences were found between cases and controls in regards to the levels of leptin at baseline (risk related to 1-SD increase in leptin in the multivariate model: OR=1.0, 95% CI 0.8-1.4) [344].

In the British Women's Heart Health Study, which included 4286 British women aged 60 to 79 years in 23 British towns, 165 (137 non-fatal and 28 fatal) cases and 335 age-matched controls were selected to study the impact of adipokines. Both cases and controls were free from previous CHD at baseline. Outcome was defined as death due to CHD, MI, first diagnosis of angina, coronary artery bypass, or angioplasty and the mean follow-up time was 4 years. Leptin was not found to be associated with future CHD in any model, including the univariate analysis [345]. In contrast, Piemonte et al. presented a small prospective study (follow-up 7 years) that included 207 women (patients with T2DM, impaired glucose tolerance and normal glucose tolerance) aged 49 to 73 years with a BMI

of 25 to 37.7 kg/m². Leptin independently protected the women from CVD (hazard ratio 0.88, $p < 0.02$) [346].

The British Regional Heart Study included 5661 men aged 40 to 59 years. In a sub-study, baseline leptin did not predict cardiovascular events during 16 years of follow-up. Altogether, 550 cases with non-fatal MI or coronary death were compared with 1184 controls free from CHD. Analysed as tertiles, the top tertile did not associate with an increased risk in the univariate or multivariate logistic regression analysis. Excluding men with pre-existing diabetes or CHD did not change the results [347].

In the same paper, Sattar et al. presented a meta-analysis based on eight prospective studies that included a total 1335 cases and 3407 controls with a weighted mean age of 55 years and a follow-up time of approximately 10 years. The top third and bottom third leptin levels were compared. The combined risk ratio of all studies was 2.3 (95% CI 1.4-3.7) when only adjusted for age and sex. After further adjustments for established risk factors, the risk ratio was attenuated to 1.4 (95% CI 0.95-2.2).

A nested case-control study from Northern Sweden that included 276 cases (157 men and 119 women) with a first stroke (234 with ischaemic stroke and 42 with haemorrhagic stroke) analysed the impact of adipokines. After stratification for sex, a high leptin level independently (adjusted for BMI, cholesterol, diabetes, smoking, and hypertension) predicted stroke in men (OR=2.5, 95% CI 1.1-5.6) but not in women. The median time between survey and stroke was 4.9 years [348].

Adiponectin

Low adiponectin was an independent predictor of MI in the Health Professionals Follow-up Study, which included 18,225 male participants aged 40 to 75 years and free of cardiovascular disease at baseline. Follow-up time was 6 years. Outcome was non-fatal MI or fatal CHD. In a nested case-control study, 266 cases were identified and 532 controls matched for age, date of blood draw, and smoking status were selected in a 2:1 ratio. Subjects in the highest quintile had a decreased risk of MI compared to those in the lowest quintile (RR 0.4, 95% CI 0.2-0.6). After adjusting for family history of MI, BMI, alcohol, physical activity, and history of diabetes and hypertension, the RR was 0.4 (95% CI 0.2-0.7). After further adjustments for blood lipids, the RR was 0.6 (95% CI 0.3-0.99) [349].

In the Strong Heart Study (SHS), a prospective case-control study based on 4549 participants of American Indian heritage with an increased risk of obesity, T2DM, and CHD, Lindsay et al. did not find that low levels of adiponectin predict MI. Adjustments or stratification for diabetes mellitus did not change the results. Altogether, 251 cases (both

men and women) were identified with CHD (69 fatal and 182 non-fatal), and controls were matched for sex, diabetes status, geographic area, and serum creatinine [350].

Kizer et al. presented a prospective nested case-control study of older adults aged 65 to 100 years in which high adiponectin levels were associated with an increased risk of CHD. A total of 604 cases (282 men and 322 women) and 782 controls (366 men and 416 women) were identified from a cohort of 3857 participants free of prevalent CVD at baseline and matched for age, sex, race-ethnicity, subclinical disease status, and study centre. Outcome was non-fatal MI and fatal CHD, and the risk (OR) related to the highest quintile was 2.2 (95% CI 1.4-3.5) after adjusting for diabetes, blood lipids, CRP, and fibrinogen [351].

In both the PRIME and LIPID studies described above [342, 343], adiponectin did not predict a first CHD event or recurrent CVD. In the study from Northern Sweden described above with stroke as outcome, adiponectin did not predict a first stroke in men or women [348].

The adiponectin sub-study of the British Regional Heart Study included 589 cases and 1231 controls. The outcome was defined as fatal CHD or non-fatal MI. For the top third adiponectin levels, the OR for CHD was 0.9 (95% CI 0.7-1.2) compared to the bottom third levels in the fully adjusted model (age, town, BMI, total cholesterol, HDL cholesterol, log triglycerides, smoking, alcohol, physical activity, social class, and systolic BP). This result did not change after excluding subjects with pre-existing diabetes mellitus. In addition, baseline levels between cases and controls did not differ (10.2 versus 10.8 $\mu\text{g/mL}$, $p=0.5$). In the same paper, a meta-analysis of seven earlier studies (including his own study) was presented. A total of 1313 cases were identified with a mean age at entry of 59 years with a mean follow-up time of 9.7 years. The OR for CHD was 0.8 (96% CI 0.7-1.0) and, thus, of borderline significance [352].

Fibrinolytic factors

In 2004, meta-analysis of seven prospective studies of tPA and MI risk using an ELISA method to study activity/mass in plasma, included a total of 2119 cases and 8832 controls. After adjusting for age and sex, the top third tPA had an OR of 2.2 (95% CI 1.8-2.7) compared to the bottom third. After further adjustments (smoking, BP, blood lipids, and BMI), tPA remained associated with future MI (OR=1.5, 95% CI 1.2-1.8) [353].

PAI-1 was similarly analysed in a meta-analysis of five studies with CHD as the outcome. The studies included 833 cases and 3122 controls, but different assays were used. Both PAI-1 activity and PAI-1 mass (antigen) were included, though one study did not specify the assay method used. The analysis found that PAI-1 is not associated with CHD (OR=1.0, 95% CI 0.5-1.8) [353].

The impact of vWF has also been analysed in a meta-analysis of 2459 cases with fatal and non-fatal CHD and 3969 controls, finding an OR of 1.2 (95% CI 1.1-1.3) [123]. In the PRIME study, high levels of vWF was an independent risk factor for both fatal and non-fatal MI; comparing the highest quartile with the lowest gave an OR of 3.0 (95% CI 1.6-5.8) [354]. Finally, in the Swedish SHEEP study, both the tPA-PAI-1 complex and vWF predicted recurrent MI in a population with previous MI [355].

Epidemiology and risk factors

Epidemiology is the science of illness among people (epi=among, demos=people, and logos=science), defined in a dictionary as: “the study of distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems.” The Greek physician Hippocrates of Cos (~460-377 B.C.) is considered to be the earliest known authority who tried to explain disease on a rational basis, and is therefore called the first epidemiologist. Later, in the 1850s, Dr. John Snow studied cholera and its spread in London using an epidemiological approach.

Six main questions are proposed to be “The Epidemical Questions”:
What health related events are occurring? **Who** is affected? **Where** are events taking place? **When** are events taking place? **Why** is it occurring? **How** can it be influenced? [356, 357]

Different designs can be used in epidemiological studies. In the papers this dissertation is based on, we have used several designs: cross-sectional, observational experimental, randomised controlled trial, and prospective nested case control study. To evaluate the causality in a study, results from a prospective cohort or nested case-control study are more reliable than results from an ecological or cross-sectional study. This approach is an analytical method of epidemiological study in which a subset of a defined population can be identified that is, has been, or may in the future be exposed or not exposed in different degrees to a factor or factors hypothesised to influence the probability of the occurrence of a given disease or other outcome. Using the “nested” design means a case control study in which cases and controls are drawn from the population of a cohort study. As some data are already available about both cases and controls, the effect of some potential confounders are reduced or possibly eliminated [357, 358]. However, cross-sectional studies are important for finding associations and generating a hypothesis. This study design examines the relationship between diseases or other health-related characteristics and other variables of interest as they exist in a defined population at one particular time. Disease prevalence, rather than incidence, is normally recorded in a cross-sectional study, but the temporal sequence of cause and effect cannot be determined. In an

experimental study, one or more factors are added to a selected population under controlled conditions, and the effects of this regime are measured by comparing the outcomes in the groups. In a randomized controlled trial (a form of an experimental study), individuals are allocated by randomization with the intention that the groups should be so similar that they only differ with respect to the tested regime. An observational study is an epidemiological study in which no interventions are performed, and the groups are followed over time to register changes in different characteristics.

The definition of a risk factor varies. The WHO (1993) has described a risk factor as follows: “The term risk factor is commonly used to describe factors that are possibly associated with the risk of development of a disease,” but that a risk factor per se is not sufficient to cause the disease [359]. In Last’s 2001 *Dictionary of epidemiology* [357], a risk factor is defined as “an aspect of personal behaviour or lifestyle, an environmental exposure, or an inborn or inherited characteristic, that, on the basis of epidemiological evidence, is known to be associated with health-related condition(s) considered important to prevent.” To define criteria for the probability of clinical causality (rule out chance, bias, or confounding), Hill presented a systematic approach in 1965: 1) temporality, cause must precede effect or disease, which is most often not possible in cross-sectional or case-control studies, but this criterion is fulfilled in prospective studies; 2) strength of the relation, a strong association between cause and effect are more likely to be causal; 3) plausibility, if there are possible mechanisms of action or experimental studies that support the hypothesis; 4) consistency, the causality is supported by consistency if several studies of varying design and in different populations provide the same result; 5) specificity, a measure of the probability of correctly identifying a non-diseased person with a screening test; 6) dose-response relationship, when changes in the level of a possible cause are related to changes in effect; 7) reversibility, if removal or reduction of a factor leads to reduced disease; and 8) study design, different study designs have different bearing on the inference of causality [360].

Aims of this dissertation

The general aims of this dissertation are:

- To evaluate the associations between circulating levels of leptin and fibrinolytic variables (i.e. PAI-1 activity, tPA mass and activity, and the tPA/PAI complex) and other variables associated with the metabolic syndrome (i.e. insulin sensitivity, testosterone, blood lipids, anthropometry), particularly whether there is a sex difference in these associations.
- To investigate the effect of heavy exercise on circulating levels of adipokines (i.e. leptin and adiponectin) and fibrinolytic/thrombotic variables (i.e. PAI-1 activity, tPA activity and mass, and vWF).
- To investigate the effect of an ACE inhibitor on circulating levels of leptin and fibrinolytic/thrombotic variables (i.e. PAI-1 mass, tPA mass, tPA/PAI complex, and vWF), and to explore determinants of changes in the circulating levels of these factors.
- To test the hypotheses that leptin and adiponectin independently predict a first ever myocardial infarction, and to evaluate whether these associations differ between men and women.

Material and methods

Study populations

MONICA, VIP, and MSP

The WHO MONICA (multinational MONitoring of trends and determinants in CARDiovascular disease) study was described in detail by Söderberg [361]. The study started in the beginning of the 1980s. In short, the overall objective was “to measure trends in cardiovascular mortality and coronary heart disease and cerebrovascular morbidity and to assess the extent to which these trends were related to changes in known risk factors, daily living habits, health care, or major socioeconomic features measured in the same time in defined communities in different countries” [362, 363]. The strongest arguments for starting the study were changes in mortality statistics and the need for validation and international comparisons.

The WHO MONICA study was started in 26 countries in 39 different populations. The majority of participating populations were from Europe, but North America, Russia, and China were also represented. Two populations from Sweden participated, one in the southwest (Göteborg) and one in the north (Norrbotten and Västerbotten).

Cardiovascular disease monitoring consisted of continuous recording of acute MI (AMI) in 25 to 64-year-olds for a 10-year period. Stroke events were also registered in most of the populations. During the same time period, at least two population surveys focusing on risk factors were performed. The BP, total cholesterol, HDL-cholesterol, and BMI were measured. Smoking habits, level of education, and history of previous CVD were determined by a questionnaire. The global part of the project was finished after 10 years, and the findings were reported in *The Lancet* in 2000 [12, 364].

Population-based AMI and stroke registers in the two northern-most counties of Sweden (Norrbotten and Västerbotten) were started in 1985, and all cases of AMI (age 25-64 years) and stroke (age 25-74 years) have been recorded since that time.

Cross-sectional and population-based studies were performed within the MONICA project in 1986, 1990, 1994, 1999, 2004, and 2009, examining a total 10,585 persons (14,000 were invited). In each survey, randomly selected samples of 2000-2500 inhabitants of Västerbotten and Norrbotten counties (age 25-74 years) were invited. In addition, 4177 subjects from the 1986, 1990, and 1994 studies were resurveyed in 1999.

Since 1985, the county of Västerbotten has been engaged in an ongoing community intervention program concerning CVD and diabetes

prevention – The Västerbotten Intervention Program (VIP). In VIP, subjects are asked to participate in a health examination with a similar design as the MONICA population surveys at their primary health care centre the year they turn 30, 40, 50, and 60 years old (since 1996: 40, 50, and 60 years). The participation rate was 57% in the early nineties but has increased to 65%. Comparisons of social characteristics between participants and non-participants have shown little evidence of selection bias [9, 365]. Between 1985 and 2000, roughly 66,300 individuals took part in the VIP health surveys, and the numbers are increasing exponentially due to re-surveys.

Regular mammography screening is offered to all women older than 50 years of age in Västerbotten county as part of the mammary screening project (MSP), and 28,400 unique women participated between 1995 and 2000.

All participants within these three surveys were asked to donate blood to the Northern Sweden Biobank. Subjects from the surveys in 1990 and 1994 were recruited for the follow-up studies described in Paper I, and cases and controls in Paper IV were recruited from MONICA, VIP, and MSP after cross-matching with the MONICA AMI registry.

The Igloo study

The father of the Igloo study described in Paper II was Doctor Gideon Hellsten, general practitioner at the Health Care Centre in Norsjö, Västerbotten, Sweden. Hellsten had noticed that the woodmen in the community lived a long and healthy life, probably due to hard physical work. Earlier, Hellsten had conducted “igloo tours” through the Swedish Alps (Sarek, a National Park in Northern Sweden) as a tour leader for the Swedish Tourist Association (STF), and a pilot study took place in 1987. Based on this experience, a hypothesis of the beneficial effects of hard physical activity on lipid levels was tested in the Igloo study. Including Hellsten, 20 well-trained men aged 18 to 55 years were invited and voluntarily applied for participation in a 14-day cross-country skiing tour (“igloo-tour”) in the Swedish Alps in February 1991. The participating men had to be enthusiastic and in good physical fitness in order to minimize the risk of dropouts. The participants were randomly allocated to one of two different diets. Diet A included 40% energy derived from fat and diet B included 30% energy derived from fat. No limitations were set according to energy intake, but due to careful packaging of the food, the individual food consumption could be estimated afterward and the intake of energy and fat calculated.

The physical “exposure” could be expressed as follows. Each participant carried a backpack weighing 25 to 30 kilos, skied between 12 and 20 km over the course of 10 hours each day, and slept in a snow-cave

built by himself. The outdoor temperature was between -10 to -25°C. Measurements, anthropometry, BP, and blood samples were taken at baseline, after one and two weeks of skiing, and six weeks after coming home. All participants completed the study.

The Enalapril study

Between 1995 and 1996, a clinical double-blind single centre randomised trial took place at Skellefteå Hospital, Sweden, with the aim of evaluating the effects of the ACE inhibitor enalapril on fibrinolytic variables in patients with an uncomplicated MI. To be eligible for the study, the following criteria had to be fulfilled: age ≥ 18 years when admitted for chest pain lasting more than 20 minutes and within 24 hours of onset, and ECG changes (ST elevation in two adjacent leads or new Q waves) or the elevation of biochemical markers above normal. Chest x-rays and echocardiography were performed if clinically indicated, and patients were excluded from the study if congestive heart failure emerged on clinical or radiological findings or as an ejection fraction below 40% in the acute phase or any time before randomisation. Furthermore, patients with any contraindication to an ACE_i or with any severe disorder that affected short-term prognosis were excluded. At least 2 months from the qualifying AMI (median 12 months, interquartile range 8–22 months), men and women were asked to participate in this study. The risk factors for AMI, medical treatment, use of thrombolytics, and invasive procedures were recorded, and eligible subjects were randomised to enalapril or matching placebo in a blinded fashion. From an initial dose of 2.5 mg daily, the dose was up-titrated to 20 mg given as a single dose. Subjects were then followed for one year and cardiovascular complications (i.e. death, AMI, unstable angina, new stable angina, and heart failure) were recorded. Altogether, 83 participants were recruited, 46 men and 37 women.

Study designs and characteristics

Paper I

This study was a cross-sectional study designed to represent a wide range of insulin sensitivity and body weight. The aim was to explore sex-related differences in associations between variables related to the MS, mainly dysfibrinolysis and leptin. Subjects were recruited from the WHO MONICA population living in the health care districts of Umeå and Luleå hospitals in Northern Sweden. From the MONICA survey in 1990 and 1994, 40 men and 41 women representing the highest and lowest quartiles of fasting insulin levels were identified (fasting insulin was measured at baseline in the MONICA surveys). These subjects were

invited to participate in this study, the men in 1997 and women in 1999. Three men were excluded due to the use of oral glucocorticoids (n=1) and low levels of testosterone, suggesting hypogonadism (n=2). Additionally, five more men did not complete the study due to private reasons. One woman was excluded due to diabetes mellitus. These participants were extensively characterised, including the hyperinsulinaemic-euglycaemic clamp, bioimpedance, and blood sampling. Results related to the HPA axis and incretins were published earlier [366].

Paper II

This was an experimental observational study with a component of randomisation (diet). Data regarding the effects of heavy exercise on haemostasis and fibrinolysis were published earlier [367]. In this study, blood samples were retrieved from the Northern Sweden Biobank and tested for adipokines in order to explore the effect of exercise.

Paper III

This study was designed as a randomised, double-blind, single centre clinical trial and performed at the Department of Medicine and Geriatrics, Skellefteå Hospital, Sweden. Results based on the a priori aim were already published [368]. Paper III is based on a new analysis of leptin in blood samples stored in Skellefteå. The aim of this re-evaluation was to explore whether enalapril affects leptin levels, and if changes in circulating leptin levels are associated with changes in fibrinolytic status.

Paper IV

This was a prospective nested case-control study within the frame of the MONICA, VIP, and MSP surveys. After cross-matching the MONICA AMI registry and MONICA, VIP, and MSP surveys, subjects aged 25-74 years with AMI or cardiac death between 1985 and 1999 and previous participation in the MONICA, VIP, or MSP surveys were selected. Altogether, 564 subjects (390 men and 174 women) were identified (446 from VIP, 48 from MONICA, and 70 from MSP). Two controls per case were randomly selected and matched for sex, age (± 2 years), date of health survey (± 4 months), type of survey (MONICA, VIP, MSP), and geographical region. Case identification followed the MONICA guidelines [363, 369]. Possible MI events were identified by screening hospital discharge records, general practitioners' reports, and death certificates with ICD 8 and 9 codes 410-413 corresponding to ICD 10 codes I20-I24. For death certificates, the codes 414 and 798-799 (ICD 8 and 9), and I25 and R96-99 (ICD 10) were also screened. Data collection included information on medical history, symptoms, examinations, and presenting electrocardiogram (ECG). The number of subjects with MI included in the

Northern Sweden MONICA registry who were not willing to participate in further studies after information was obtained averaged two per year (0.2%). An AMI met one of the following criteria: 1) typical ECG progression according to the Minnesota criteria, 2) ECG progression (estimated as probable) with cardiac enzymes elevated to more than twice the upper limit of normal, or 3) typical symptoms of MI with elevated cardiac enzymes as above. From the late 1990s, troponins were introduced for the diagnosis of MI. Based on these parameters, survivors were diagnosed as definite MI or non-MI. Subjects who died within 28 days of the onset of MI were recorded as fatal cases, including pre-hospital deaths, patients who died in hospital, and patients who were discharged alive but died outside the hospital within 28 days. An event was considered to be the first for the patient if the patient's history was free from a previous clinically recognized MI. For fatal events, both definite and possible infarctions were included. For this purpose, information was also obtained from death certificates and necropsy reports when available, and the frequency of necropsy was 16.4%.

“Silent” MIs were not included as the date of occurrence could not be determined, and thus not possible to match. All cases and controls with a previous history of MI or stroke any time before the survey, or cancer less than 5 years before or 1 year after MI, were excluded. In order to validate the data from the registries, all surviving cases and controls were asked to complete a questionnaire about previous CVD and cancer.

Blood samples from all subjects were identified in the Northern Sweden Biobank and tested for adipokines. Other biomarkers were tested by other groups and made available for our analysis. Our aim was to evaluate the importance of leptin and adiponectin as predictors of a first-ever MI.

Anthropometry and blood pressure

BMI was calculated as the total body weight in kilograms divided by the height in meters squared; BMI <25 identified normal weight, BMI 25-30 indicated overweight, and BMI >30 was considered obese. Weight was measured without shoes in light indoor clothing and recorded to the nearest 0.2 kg. The waist-hip ratio (WHR) was calculated as the ratio of the circumference of the narrowest part of the waist divided by the broadest part of the hip. Height was measured to the nearest centimetre without shoes. Body composition was determined by bioelectrical impedance analysis (101F; Akern-RJL System bioelectrical impedance instrument, EL.Dot, Fredriksvaerk, Denmark) (Paper I).

The BP was recorded with a mercury sphygmomanometer after 15 minutes of rest with the subject in the supine position (Papers I, II, and III). In Paper IV, baseline BP was measured with the random zero

method in MONICA [363], and with a mercury sphygmomanometer in VIP and MSP. However, BP was measured in the recumbent position in VIP and adjustments were made for the sitting posture BP measurements. This adjustment was based on a comparison between the sitting and recumbent position in 1850 subjects from the VIP health survey [370]. Hypertension was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg, and/or anti-hypertensive therapy.

Smoking status

Smoking habits were self-reported. In MONICA and VIP, daily smokers were defined as smokers, ex-smokers, occasional smokers, and non-smokers. The two latter together were defined as non-smokers. In the enalapril study, participant smoking status was defined as smoker or non-smoker.

Diabetes

Diabetes was excluded by self-reporting and fasting glucose measurements in Papers I and II. Diabetes was defined by self-reporting in Paper III. In Paper IV, the majority of subjects had a 2-hour 75 g oral glucose tolerance test (OGTT), and fasting and post-load glucose levels were measured. The presence or absence of diabetes was based on self-reported data and/or fasting plasma glucose levels ≥ 7.0 mmol/L and/or post-load plasma glucose levels ≥ 11.0 mmol/L (≥ 12.2 mmol/L in the VIP based on capillary plasma). Impaired fasting glucose (IFG) was defined as a fasting glucose level ≥ 6.1 and < 7.0 mmol/L, and impaired glucose tolerance (IGT) as a post-load glucose level ≥ 7.8 and < 11.1 , or ≥ 8.9 and < 12.2 in VIP, together with a non-diabetic fasting glucose level [371].

Electrocardiogram (ECG)

The classification of MI into subtypes in Paper IV was done according to the Minnesota code 9-2 [372], and non-ST-elevated myocardial infarction (STEMI) and STEMI were defined. If the ST segment could not be evaluated (e.g., in the presence of a bundle branch block or in pacemaker-electrocardiogram) or if no electrocardiogram was investigated because of sudden cardiac death, the cases were categorized as uncodable MI cases.

Blood sampling

Samples were drawn in the morning after at least 4 hours of fasting. A minimum of stasis was used. Plasma samples were stored in a deep-freeze blood bank at -70 to -80°C until analysis.

Samples for leptin and adiponectin were collected in plasma vacuum tubes, and fibrinolytic samples were collected in Stabilyte tubes.

Laboratory procedures

Fibrinolytic and thrombotic factors

The activity of tPA was determined by a parabolic rate assay based on stimulation of the tPA-mediated conversion of plasminogen to plasmin in the presence of fibrin. The activity is reported in U/mL by reference to the activity of the First International tPA Standard, coded 83/517 (National Institute for Biological Standards and Control, Potters Bar, London), using Chromolize tPA (Biopool AB, Umeå, Sweden). The standard curve was linear between 0 and 0.2 IU/mL. Within and between assay coefficients of variation (CVs) were 7.0% and 5.3%, respectively, at low levels (mean 0.43-0.48 IU/mL) and 3.9% and 5.2% at mid-range levels (mean 1.25-1.32 IU/mL) according to the manufacturer.

The plasma mass concentration of tPA (antigen) was determined with an enzyme-linked immunosorbent assay. The reagent kits for assaying tPA antigen were purchased from Biopool AB (Umeå, Sweden). According to the manufacturer, the detection range was 1.5-30 ng/mL. The intra- and inter-assay CVs were 8% and 10%, respectively. In the Igloo study (Paper II), samples for tPA mass and activity were also taken after venous occlusion (VO) of the arm with a manometer cuff at 100 mmHg for 10 minutes.

PAI-1 activity was determined by an activity assay based on the addition of excess tPA to the sample (40 IU/mL) and quantifying the remaining tPA with the activity assay as described above. The activity is given in arbitrary U/mL, where 1 U of PAI-1 is defined as the amount of PAI-1 that inhibits 1 U of the International tPA Standard (above). Chromolize PAI-1 reagent was purchased from Biopool AB (Umeå, Sweden). The detection range estimated by the manufacturer was 2.5-50 IU/mL. Within-run and run-to-run CVs were 3.7% and 16.9%, respectively, at low levels (2 IU/mL) and 2.6% and 3.6%, respectively, at high levels (36 IU/mL). The PAI-1 mass concentration (antigen) was determined by enzyme-linked immunosorbent assays [98] using a reagent kit from Biopool AB (Umeå, Sweden). The CV was 9% according to the manufacturer.

To quantitatively estimate the tPA-PAI complex, an enzyme immunoassay using a polyclonal antibody against tPA and monoclonal antibody against PAI-1 was used. According to the manufacturer, the limit of detection was 0.25 ng/mL. Within and between assay CVs were 6.8% and 5.7%, respectively, at low levels (2.5 ng/mL) and 3.2% and 2.1% at high levels (16.5 ng/mL).

Circulating levels of vWF were also determined by enzyme-linked immunosorbent assays. Reagents were purchased used from DAKO

(Denmark). The values were expressed as the percent of normal subjects. The CV at a level of 138% was 11.7% (n=41) (Papers II and III) [373].

Glucose, insulin, and insulin sensitivity

Insulin was measured by a microparticle enzyme immunoassay (MEIA) on an "AXSYM" instrument (Abbott Laboratories, IL, USA) in Paper I. The working range for this method is 1.0–600 mU/L and the interassay CV was 6.7% at the level of 7.9 mU/L. Cross reactivity with c-peptide/ glucagon was non-detectable and 0.005% with proinsulin. In Paper II, the Pharmacia Insulin 100 kit (Uppsala, Sweden) was used. Glucose was measured by either using the hexokinase method (Boehringer Mannheim) (Paper II) or capillary using a glucose dehydrogenase method in a HemoCue B-glucose analyzer (HemoCue AB, Sweden) (Paper I). Insulin sensitivity was estimated in Papers I and II. In Paper I, insulin sensitivity was estimated by the hyperinsulinaemic-euglycaemic clamp, which was done in the morning after an overnight fast. Synthetic human insulin (Actrapid 40 IE/mL, NovoNordisk, Malmö, Sweden) was given as a weight-adjusted infusion during the first 10 minutes, followed by a continuous infusion for 110 minutes in order to maintain steady-state hyperinsulinaemia (mean plasma insulin concentration during steady-state 97.7 ± 26.6 mU/L). To maintain a blood glucose level of 4.5 mmol/L during the clamp, 20% glucose solution was infused, and the infusion rate was adjusted according to repeated blood glucose measurements (every fifth minute). Serum insulin was measured just before the clamp and after 60, 90, and 120 minutes. The amount of glucose metabolised by the individual (M-value) was calculated for the steady-state period as glucose infusion (mL) \times glucose concentration (mg/mL) \times body weight (kg)⁻¹ \times time (minutes)⁻¹. The adjusted M-value (M/I) was calculated as the M-value divided by the mean insulin concentration during the steady state period (the last 60 minutes of the clamp). In Paper II, insulin sensitivity was calculated according to the homeostatic model assessment model (HOMA): insulin sensitivity = fasting insulin (mU/L) / (22.5 * e^{-ln fasting glucose (mmol/L)}).

Leptin and adiponectin

Leptin and adiponectin were analysed by double-antibody radioimmunoassays (RIA) (Linco Res., St. Louis, MO, USA). The detection level of leptin in this assay is 0.5 ng/L. Total CV for leptin was 4.7% at both low (2–4 ng/mL) and high (10–15 ng/mL) levels, and for adiponectin the total CV was 15.2% at low levels (2–4 µg/mL) and 8.8% at high levels (26–54 µg/mL).

Testosterone and sex hormone-binding globulin (SHBG)

Serum concentrations of testosterone and SHBG were determined by commercial RIAs from Diagnostic Products Corporation (Coat-a-Count® for testosterone; Los Angeles, CA, USA) and Eurodiagnostics AB (SHBG; Malmö, Sweden). In the testosterone assay, the samples were extracted with diethyl ether and, following evaporation of the ether, the extracts were dissolved in zero calibrator and analysed according to the manufacturer's protocol. This extra step was included in order to avoid possible interference from SHBG in the native serum samples. Detection limits and within and between assay CVs were testosterone 0.1 nmol/L, 6%, and 10%, respectively, for testosterone, and 0.05 nmol/L, 4%, and 8%, respectively, for SHBG.

Lipids

In Paper I, blood lipids (total cholesterol, triglyceride, and HDL) were measured by dry chemistry using a multianalyser (Vitros 950 IRC; Johnson and Johnson, NY, USA). In Papers III and IV, the total cholesterol was measured by using either a benchtop analyser (Reflotron®; Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) or an enzymatic method (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) at a central laboratory. Low density lipoprotein (LDL)-cholesterol was calculated according to Friedewalds formula. Apolipoproteins A-1 and B were determined by immunoturbidimetry (Dako, Glostrup, Denmark) (Paper IV). In Paper II, total cholesterol and triglycerides were determined using enzymatic method kits (Boehringer Mannheim GmbH Diagnostica, Mannheim, FRG). HDL-cholesterol was measured after precipitating the other lipoproteins with sodium phosphowolframate/magnesium chloride.

C-reactive protein (CRP)

High-sensitivity (hs) CRP was determined using IMMULITE (Diagnostic Products Corporation, USA) (Paper IV).

Statistical analysis

Skewness was determined by formal tests and inspection of Q-Q plots, and skewed variables were ln transformed to obtain a normal distribution. In all studies, baseline characteristics are expressed as (geometric) means with 95% CI. Differences between baseline groups were explored by Student t-test (Papers I-IV), analysis of variance (ANOVA) (Paper III), and Chi square tests (Paper IV). Associations between variables were explored with bivariate (Person's) and partial correlation analysis in Papers I, II, and IV. In Papers I and III, univariate and multivariate linear regressions were used with a stepwise method for

testing associations. Two-tailed tests were used and a p-value <0.05 was considered significant (Papers I-IV). In Papers II and III, repeated measures ANOVA was used to analyse changes over time. Because the study groups were small in both Papers II and III, a post-hoc power analysis was done. Leptin data were missing at 16 occasions in Paper III, and the analysis was done both with missing data and after replacement with the mean of two adjacent measurements. Missing values in continuous variables in Paper IV were replaced with the median value in controls, separately for men and women. In Paper IV, continuous variables were categorized into quartiles, and the distribution of cases and controls (test for trend) was tested with a chi-square test. The distribution among controls (men and women separately) was used for calculating the quartile cut-offs. Missing values were treated as a separate category and omitted from the tables. Conditional logistic regression was used to estimate the OR and 95% CI. To estimate the interaction/antagonism, synergy indices with 95% CI were calculated (>1.00 indicates synergistic interaction). A Kaplan-Meier analysis and Cox regression analysis were used to evaluate the impact of leptin and adiponectin on time to MI. In Papers I-IV, all calculations were performed with the statistical program SPSS (version 18, Chicago, IL, USA). In Paper IV, STATA (version 10, College Station, TX, USA) was used for the conditional logistic regression analysis. All calculations were performed with a Macintosh computer.

Ethical considerations

The Ethics Committee at Umeå University approved all studies included in this thesis, and all participants provided oral and written consent when applicable.

Results

Paper I

The association between circulating levels of leptin and fibrinolytic variables (i.e. PAI-1 activity, tPA mass and activity, and tPA-PAI complex) were investigated in both men and women with high and low levels of insulin sensitivity. Men had higher levels of total and free testosterone. Women had higher levels of SHBG, HDL-cholesterol and leptin. Fibrinolytic variables, anthropometry (except WHR) and estimated insulin sensitivity did not differ.

In bivariate correlations, high levels of leptin were associated with high PAI-1 activity, high levels of tPA mass, and PAI-tPA complex in men. In women, high levels of leptin were associated with high PAI-1 activity, high levels of tPA mass, high levels of PAI-tPA complex, and low tPA activity. After adjusting for age, fat mass, and insulin sensitivity, these associations only remained for the men, additionally high levels of leptin also associated to low tPA activity. In Fig 5-8, leptin is expressed as the leptin/fat mass ratio in order to adjust for obesity and is interpreted as “secretion rate” (Reproduced from Paper I)

Figures 5 to 8. Associations between fibrinolytic variables and secreted leptin (leptin/fat mass ratio) in men (blue) and women (red)

Figure 5: Men: $r=0.36$, $p<0.05$; women: $r=0.13$, $p=0.4$

Figure 6: Men: $r=-0.32$, $p<0.07$; women: $r=-0.33$, $p<0.05$

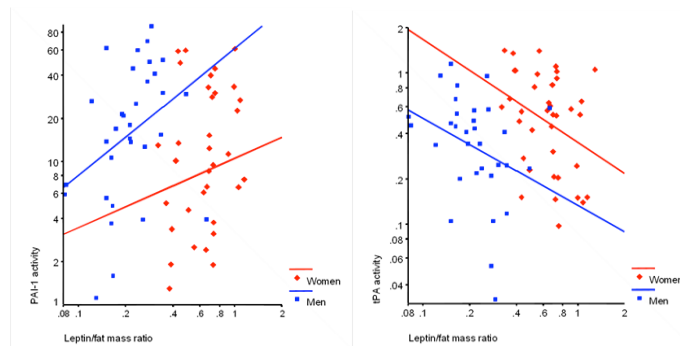
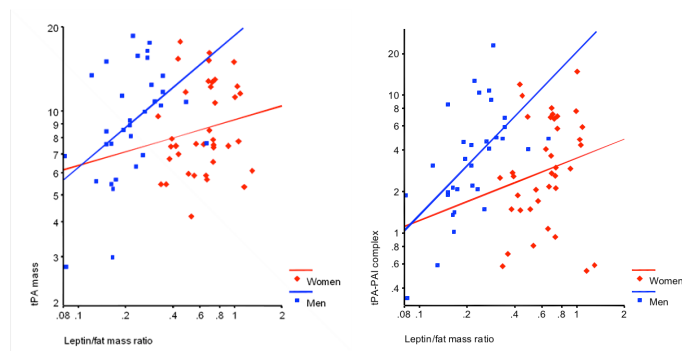


Figure 7: Men: $r=0.46$, $p<0.01$; women: $r=0.16$, $p=0.3$

Figure 8: Men: $r=0.59$, $p<0.001$; women: $r=0.18$, $p=0.3$



In multivariate linear regression analysis, fat mass, leptin, free testosterone, insulin sensitivity, triglycerides, and smoking were used in the model to determine predictors for the fibrinolytic factors. In men, high levels of leptin predicted high PAI-1 activity, tPA mass, and PAI-tPA complex together with low tPA activity. Additionally, high levels of free testosterone predicted high PAI-1 activity. Insulin sensitivity and smoking predicted high tPA activity. Insulin sensitivity and triglycerides predicted high levels of tPA mass (Table 1).

Table 1. Multivariable analysis in men

	Beta	SE (B)	St Beta	p-value	R²
PAI-1 activity					
Leptin	1.025	0.253	0.617	<0.001	36.2
Free testosterone	0.844	0.369	0.348	0.030	
tPA activity					
Leptin	-1.210	0.287	-0.979	<0.001	37.3
Smoking	1.138	0.423	0.418	0.012	
Insulin sensitivity	-0.781	0.297	-0.589	0.014	
tPA mass					
Leptin	0.542	0.157	0.767	0.002	41.7
Insulin sensitivity	0.379	0.162	0.500	0.027	
Triglycerides	0.228	0.109	0.330	0.047	
PAI-tPA comp					
Leptin	0.808	0.211	0.586	0.001	31.9

Model included fat mass, leptin, free testosterone, insulin sensitivity, triglycerides, and smoking. Beta=regression coefficient, SE (B)=standard error of beta, St Beta=standardised regression coefficient, R²=adjusted R².

In women, obesity and low insulin sensitivity predicted PAI-1 activity. Low leptin and high insulin sensitivity predicted high tPA activity. Obesity and high levels of triglycerides predicted tPA mass. Insulin sensitivity, obesity, and triglycerides predicted the level of PAI-tPA complex (Table 2).

Table 2. Multivariable analysis in women

	Beta	SE (B)	St Beta	p-value	R²
PAI-1 activity					
Fat mass	2.233	0.565	0.530	<0.001	52.6
Insulin sens	-0.808	0.323	-0.336	0.018	
tPA activity					
Leptin	-0.746	0.224	-0.478	0.002	44.8
Insulin sensitivity	0.438	0.191	0.329	0.029	
tPA mass					
Fat mass	0.545	0.147	0.476	0.001	45.6
Triglycerides	0.350	0.104	0.435	0.002	
PAI-tPA comp					
Insulin sens	-0.489	0.211	-0.314	0.027	62.0
Fat mass	1.268	0.326	0.462	<0.001	
Triglycerides	0.540	0.237	0.279	0.030	

Model included fat mass, leptin, free testosterone, insulin sensitivity, triglycerides, and smoking. Beta=regression coefficient, SE (B)=standard error of beta, St Beta=standardised regression coefficient, R²=adjusted R².

Waist circumference was used instead of fat mass in all models, but the results did not differ significantly.

Paper II

Levels of leptin, adiponectin, and fibrinolytic variables (i.e. PAI-1 activity, tPA activity, and mass) were studied in men during and after strenuous physical exercise. No differences were found in any variable when comparing the two diet groups at baseline. The levels of total and

LDL-cholesterol differed between diet groups after completion of the tour, with higher levels observed in the group with a high fat diet.

Table 3. Subject characteristics

	Baseline	2 weeks	6 weeks	p
Age yrs	35.8 (30.8-40.7)			
Weight kg	76.9 (70.9-82.9)	75.8 (69.9-81.6)	74.2 (68.8-79.7)	<0.001
BMI kg/m ²	23.7 (22.1-25.3)	23.4 (21.9-24.9)	22.8 (21.5-24.2)	<0.001
Waist cm	84.1 (79.0-89.1)	79.4 (75.2-83.6)	83.2 (79.5-86.9)	<0.001
SBP mmHg	122 (117-126)	120 (116-124)	121 (115-126)	0.7
DBP mmHg	73 (69-76)	70 (68-72)	71 (67-74)	0.4
PAI 1 act U/mL	7.5 (3.1-11.8)	2.7 (1.4-4.0)	5.3 (3.4-7.1)	0.006
tPA act U/mL	0.28 (0.13-0.42)	0.17 (0.08-0.26)	0.30 (0.24-0.36)	0.039
tPA mass µg/L	5.0 (4.0-6.0)	3.3 (2.9-3.8)	4.4 (3.6-5.2)	<0.001
Insulin sens	0.92 (0.71-1.19)	0.49 (0.42-0.58)	0.78 (0.60-1.02)	<0.001
Leptin ng/mL*	2.2 (1.5-3.1)	1.3 (1.1-1.5)	1.6 (1.1-2.2)	0.001
Adipon µg/mL	9.7 (8.4-11.0)	7.6 (14.8-20.4)	9.0 (7.4-10.5)	<0.001
vWF (%)*	112 (94-133)	121 (103-142)		0.1

* Means (geometric) 95% CIs and p-value for time trend (repeated measurement, Greenhouse-Geisser). SBP=systolic blood pressure, DBP=diastolic blood pressure.

The levels of adipokines, fibrinolytic variables, and estimated insulin sensitivity changed during the expedition and returned to baseline levels after recovery (Table 3). The levels of leptin decreased 56% and adiponectin increased 81%. The activity of PAI-1 decreased 64% and tPA activity 38%. Levels of tPA mass decreased 33%. Estimated insulin sensitivity improved 51% (Fig 9 to 14) (Fig 9-11 reproduced from Paper II)

Figures 9 to 14. Levels of fibrinolytic variables, leptin, adiponectin, and insulin sensitivity at the start and after 1, 2, and 6 weeks of recovery

Figure 9. Adiponectin ($\mu\text{g/mL}$)

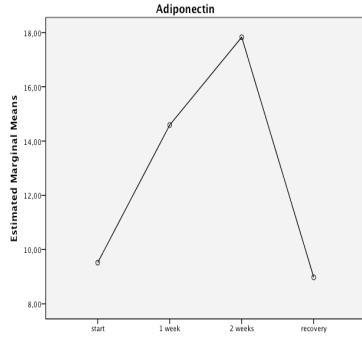


Figure 10. Leptin (ng/mL)

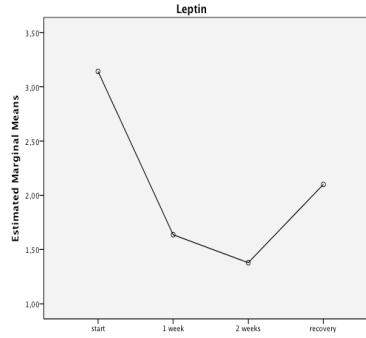


Figure 11. PAI-1 activity (U/mL)



Figure 12. tPA activity (U/mL)

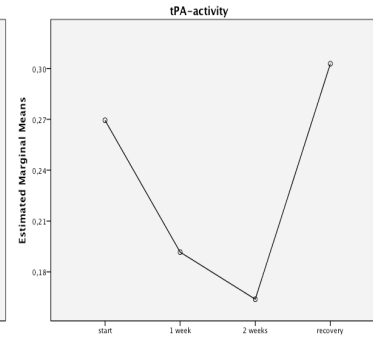
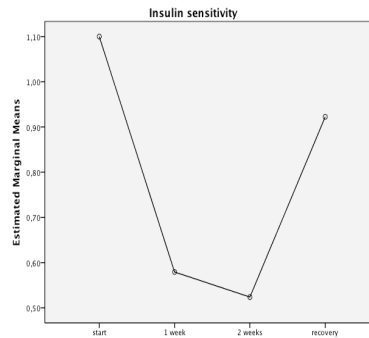
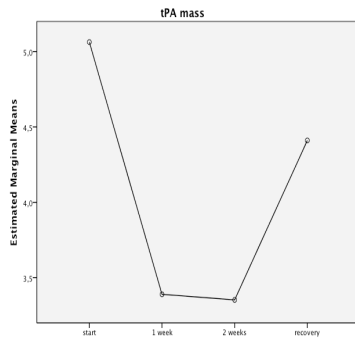


Figure 13. tPA mass levels ($\mu\text{g/mL}$) Figure 14. Insulin sensitivity (HOMA)



The correlation coefficients between the adipokines (leptin and adiponectin) and anthropometry (weight, waist, and BMI) differed at baseline, during the expedition, and recovery, most notably for adiponectin (Table 4). No relationship was found between the estimated insulin sensitivity and adiponectin before, during the expedition, and at recovery.

Univariate and multivariable regression models were calculated for changes during the expedition and recovery. In the univariate analysis, age, increased insulin sensitivity, decreased leptin levels, and increased adiponectin levels were associated with decreased PAI-1 activity during the expedition. Increased adiponectin levels and age were also associated with decreased tPA mass. After adjusting for age, changes in waist circumference, and insulin sensitivity, changes in leptin and adiponectin remained associated with decreased PAI-1 activity. After recovery, decreasing adiponectin levels associated independently with increasing PAI-1 activity. Changes in leptin and adiponectin were not associated with changes in vWF during the expedition.

Changes in tPA activity and mass after VO were not associated with changes in the levels of leptin and adiponectin. Adjusting for lipids or diet group, or using BMI instead of waist circumference in the models, did not change the results. vWF was not measured during recovery.

Table 4. Changes in associations (bivariate and partial) between adipokines and anthropometry at the start of exercise, during heavy exercise, and after recovery

	Leptin	Age, group, and IR	Adiponectin	Age, group, and IR
Start				
Weight	0.757***	0.735**	-0.338	-0.343
Waist	0.657**	0.605*	-0.382	-0.364
BMI	0.875***	0.864***	-0.471*	-0.433
End exp				
Weight	0.316	0.448	0.544*	0.446
Waist	0.480*	0.620**	0.597**	0.413
BMI	0.502*	0.647**	0.671**	0.463
Recovery				
Weight	0.562*	0.508	-0.217	-0.374
Waist	0.662**	0.540*	-0.224	-0.318
BMI	0.710**	0.599*	-0.402	-0.577*

* p < 0.05, ** p < 0.01, *** p < 0.001. IR=insulin resistance.

Paper III

The effect of treatment with enalapril on circulating leptin levels was explored in a population of men and women with a previous AMI. The association between changes in circulating leptin levels and changes in fibrinolytic status was investigated. Women were older than men at baseline (70.1 vs. 63.2 years). More women had hypertension (men 32.6% and women 56.8%). Women were included later in the study than men (men: median 10.0 months after MI, women: median 19.0 months after MI, $p < 0.001$). BMI, smoking, diabetes, thrombolytic treatment, history of coronary bypass, and medical treatment did not differ between men and women. No differences were measured at baseline between treatment allocation groups.

Focusing on changes in leptin levels, the univariate test of the within subject effect showed a significant overall time effect of increasing leptin levels during one year. None of the time*factor interactions were significant. The linear time trend for leptin was significant, and the trend increased in both men and women but was not significant due to the small study groups. As expected, differences in circulating levels were related to sex and BMI, but not to enalapril treatment.

Increasing leptin levels were associated with increased levels of tPA mass, PAI-1 mass, and tPA-PAI complex in both groups of men (Table 5). After stratification for enalapril treatment, changes in leptin levels were associated with changes in levels of tPA mass and tPA-PAI complex in men on placebo. Enalapril treatment was also associated with changes in PAI-1 mass after adjusting for changes in leptin. In women, changes in circulating leptin levels were not associated with changes in the levels of fibrinolytic variables. Changes in the concentration of vWF were not associated with changes in leptin levels in men. In women, changes in leptin levels associated to changes in vWF concentration.

Table 5. Multivariable linear regression with changes in fibrinolytic variables as the outcome

	tPA mass			PAI-1 mass			tPA-PAI comp		
	B	SE	P	B	SE	P	B	SE	P
Men									
Leptin	0.41	0.12	0.002	0.62	0.23	0.01	0.62	0.19	0.003
Enalapril	-0.13	0.08	0.12	-0.52	0.16	0.003	-0.05	0.12	0.66
Placebo									
Leptin	0.69	0.15	<0.001	0.66	0.35	0.08	0.69	0.27	0.02
Enalapril									
Leptin	0.22	0.27	0.42	0.68	0.51	0.21	0.90	0.45	0.08
Women									
Leptin	-0.14	0.18	0.45	-0.13	0.38	0.73	-0.14	0.30	0.64
Enalapril	-0.04	0.12	0.76	-0.04	0.26	0.89	0.15	0.20	0.46
Placebo									
Leptin	0.15	0.23	0.54	-0.34	0.33	0.34	0.37	0.35	0.33
Enalapril									
Leptin	-0.28	0.38	0.48	0.48	0.74	0.53	-0.13	0.53	0.81

Leptin denotes a change in leptin.

Paper IV

In this study, we explored the risk for a first MI related to circulating levels of leptin and adiponectin. Altogether, 564 cases were identified (174 women and 390 men). The time period between survey and event was 4.2 years in men and 3.2 years in women. Fifty-one percent of the MIs were classified as STEMI (52% in men and 48% in women), 29% were classified as nonSTEMI (29% in men and 28% in women), and 21% were unclassified (20% in men and 24% in women). Twenty-three percent of all cases were fatal within 28 days (23% in men and 24% in women). Established risk factors, such as high BMI, smoking, hypertension, and diabetes, were more common among cases than controls. Cases had impaired glucose tolerance more often than controls and higher levels of fasting and post-load glucose. Cases also had higher levels of hsCRP, lower levels of ApoA-1, higher levels of cholesterol and ApoB, and a higher ApoB/ApoA-1 ratio. In addition, adiponectin levels were lower and leptin levels higher in cases. The leptin/adiponectin ratio was also higher in cases. Risk for MI related to confounders was

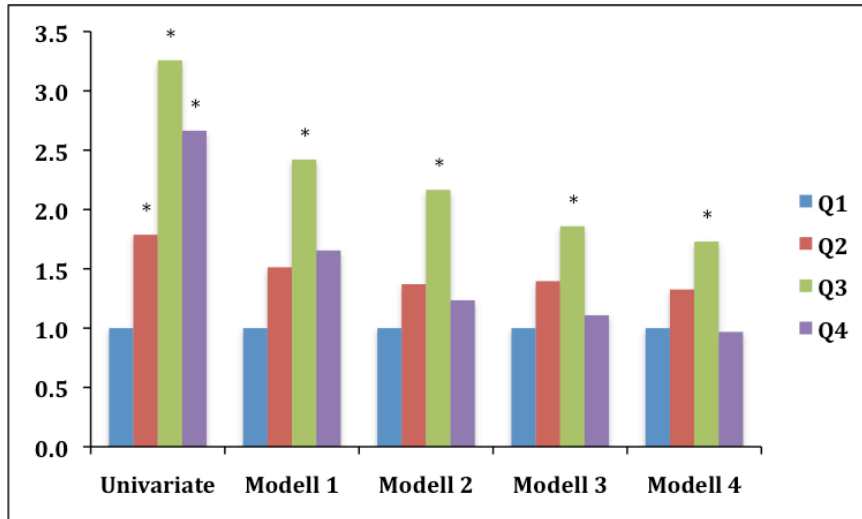
estimated. Traditional risk factors were associated to increased risk for future MI in both men and women.

Leptin independently predicted MI in men in different multivariable models including factors from the previous analysis. The first model included BMI, and the second included traditional risk factors such as BMI, hypertension, diabetes, smoking, and cholesterol. The third model was similar to the second except that total cholesterol was replaced with the apo B/apo A-1 ratio. Finally, a fourth model added hsCRP to the previous model.

In men, high leptin levels corresponding to the third quartile predicted MI in all models. In contrast, leptin did not remain associated with MI after adjustments in women (Fig 15 and 16). These models were tested without BMI, as BMI and leptin are strongly correlated, but the results did not change. High leptin levels were strongly associated with future STEMI in men but not in women. Leptin did not predict nonSTEMI in men or women, though a significant trend was observed in men. High levels of circulating leptin in men predicted MI in survivors but not those with fatal MI.

In both men and women, high levels of circulating adiponectin protected against MI (men=fourth quartile, women=second quartile) (Fig 17 and 18). In women, these associations remained after adjustments. In men, the association remained after adjusting for BMI but not after further adjustments. In the combined analysis, adiponectin was protective and remained protective after adjusting for traditional risk factors (model 2), but not in the models including apolipoproteins and hsCRP. After stratifying STEMI/nonSTEMI, similar patterns were seen in both men and women.

Figure 15. Leptin as a predictor of MI in men



Model 1: BMI; model 2: BMI, hypertension, diabetes, smoking, and cholesterol; model 3: BMI, hypertension, diabetes, smoking, and apo B/apo A-1; and model 4: BMI, hypertension, diabetes, smoking, apo B/apo A-1, and hsCRP. (Apply to figures 15 to 18.)

Figure 16. Leptin as a predictor of MI in women

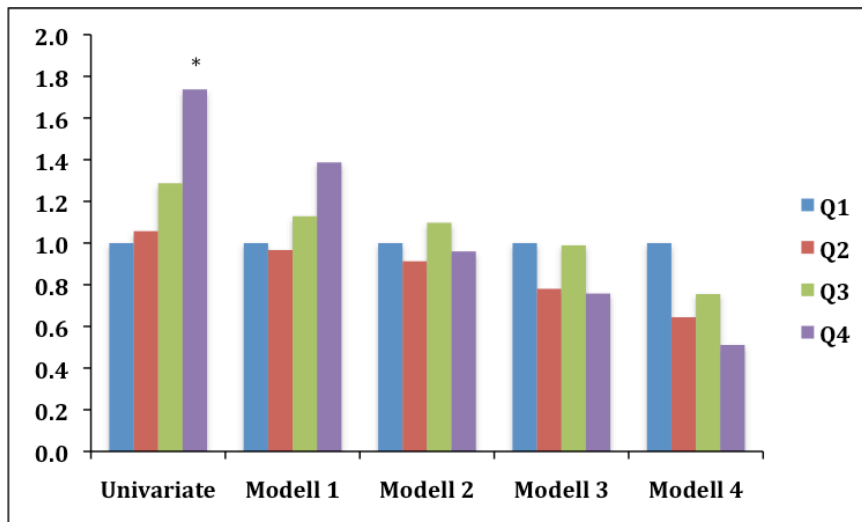


Figure 17. Adiponectin as a predictor of MI in men

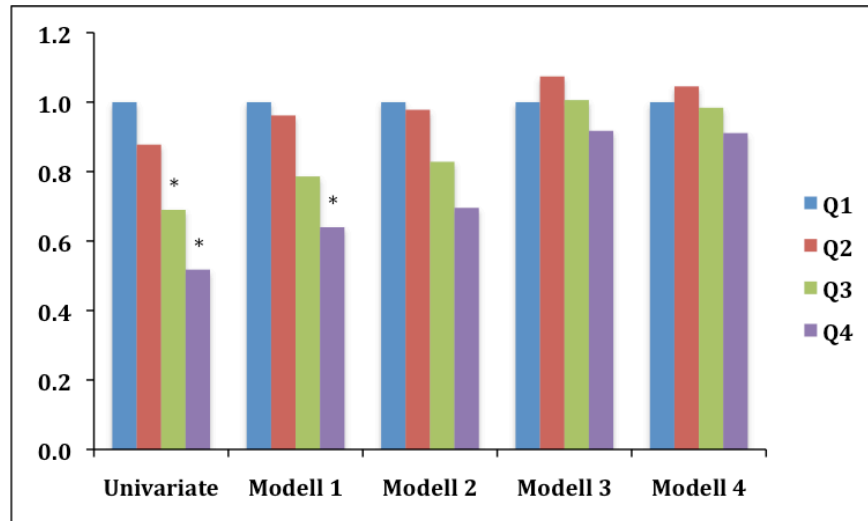
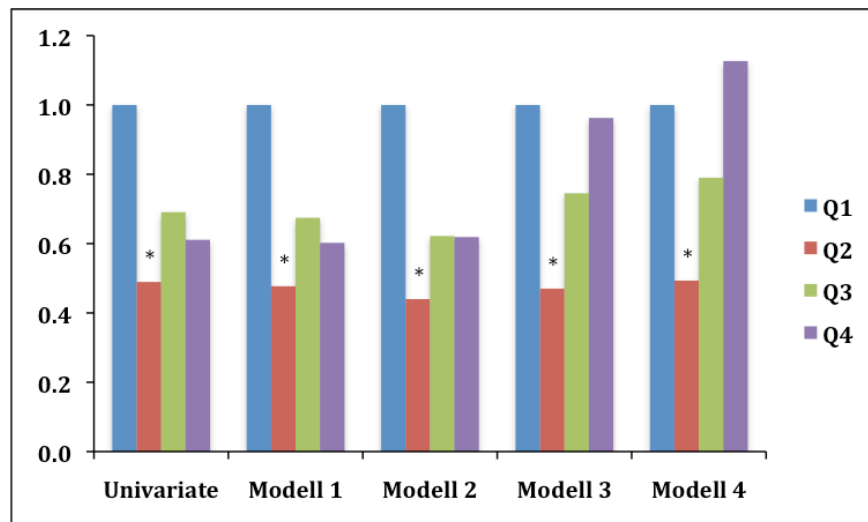


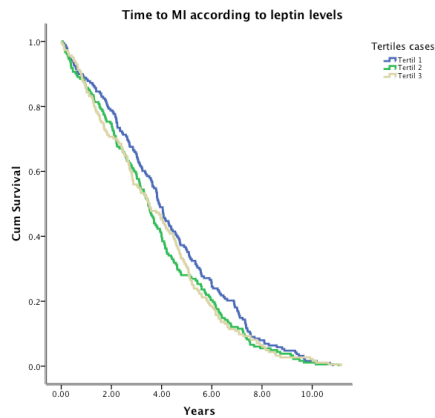
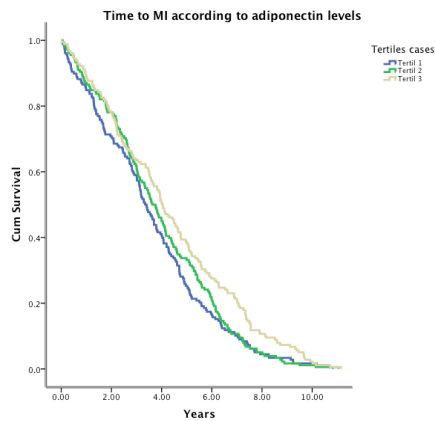
Figure 18. Adiponectin as a predictor of MI in women



Finally, we examined whether the levels of leptin and adiponectin affected the time between survey and event. The median time to MI was 4.0 years (3.6-4.5) for those with high levels of adiponectin at baseline compared to 3.3 years (2.9-3.7) for those with low levels (log rank 0.011). This pattern was also seen in men (high levels 4.5 years (4.0-5.0) and low

levels 3.6 years (3.0-4.3), log rank 0.013). The median time to MI was 3.5 years (2.8-4.2) for those with high levels of leptin at baseline compared to 3.9 years (3.5-4.2) for those with low levels (log rank 0.208). After stratifying sex, the median time to MI was 4.0 years (3.3-4.7) and 4.3 years (3.6-4.9) for men with high and low leptin levels, respectively (log rank 0.744). The difference between high and low levels in women was significant (2.6 years [2.0-3.3] vs. 3.4 years [2.8-4.0], log rank 0.030).

Figures 19 and 20. Time to MI as a function of baseline adiponectin and leptin



General discussion

Why new biomarkers?

In order to evaluate a patient, clinicians rely on biomarkers for assessing the risk of developing disease. Preserving health is important, as it results in socio-economic earnings of importance for society. Thus, one aim of risk factor research is to define validated prognostic biomarkers. However, the use of established risk factors is not enough for assessing cardiovascular risk [374-376], and it has been proposed that multiple biomarkers should be used to improve the prediction of cardiovascular risk or death due to CVD [376, 377].

The search for reliable biomarkers should, of course, include prospective studies, but also experimental studies to evaluate mechanisms and cross-sectional studies to test associations. Though the incidence of MI is decreasing, this change applies mainly to men in the county of Västerbotten, which implies that there is not enough knowledge about sex-specific mechanisms in various pathways.

A well-known relationship exists between leptin, adiponectin, and parts of MS including CVD. Though leptin and adiponectin are confounders for obesity, whether they play important mechanistic roles in atherosclerotic processes (a casual relationship) cannot be determined in these studies.

Leptin

In our studies, high levels of leptin were independently associated with dysfibrinolysis in men (Papers I and III). In women, we found an inverse and independent association between leptin and tPA activity (Paper I). In addition, leptin was an independent predictor of a first MI in men. In women, leptin was only associated with future first-ever MI in the unadjusted model (Paper IV).

Divergent hypotheses have been presented about how leptin affects pathophysiology in CVD. Phylogenetically, leptin is important for the regulation of food intake and body weight. The amount of fat mass is rather stable over the course of a lifetime despite metabolic and behavioural alterations, which indicates strict regulation. Many physiological processes depend on energy storage as fat, including growth, puberty, fertility, and thyroid function [378], which indicates that hormonal signals regulate the mass and function of adipose tissue by interacting with neuroendocrine systems.

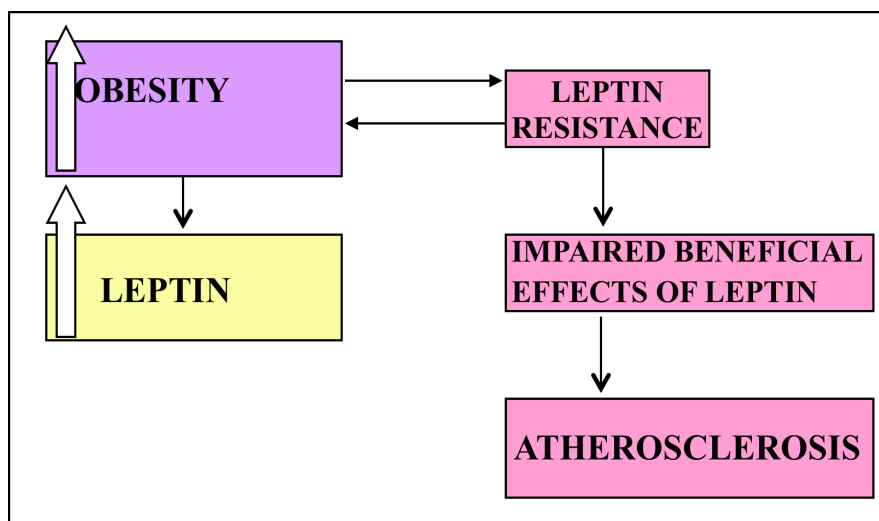
The etymology of the word “leptin” (from the Greek word “leptos”, meaning thin) suggests that the primary physiological function of leptin is to decrease the appetite or increase energy expenditure. Conversely, low

levels of leptin signal an increased requirement of food intake and decreased energy expenditure [379]. Leptin deficiency due to an inactivated leptin gene is the cause of morbid obesity in a minority of cases and was discovered in children with closely related parents (cousins) [61]. This condition can be treated with subcutaneous leptin administration [250], leading to normal growth, including normal puberty. In addition, a defect in the leptin receptor can cause a similar phenotype due to defective leptin signalling, and defects in the leptin receptor are found in 3% of patients with morbid obesity [380].

A dysfunctional endothelium is necessary for atherosclerotic plaque development; consequently, structural lesions develop that eventually lead to a cardiovascular event. Endothelial cells express leptin receptors, but how leptin affects the vascular wall is unclear.

In 2002, the concept of selective leptin resistance was introduced to explain how hyperleptinemia promotes atherosclerosis [381]. Leptin resistance can be more or less developed between central and peripheral locations, and between different organs and tissues (i.e. resistance against the central effects but sensitive to sympathetic stimulation, platelet stimulation, etc.). Therefore, at the peripheral level, leptin may promote atherosclerosis due to both direct effects and an absence of beneficial actions due to resistance (Figure 21).

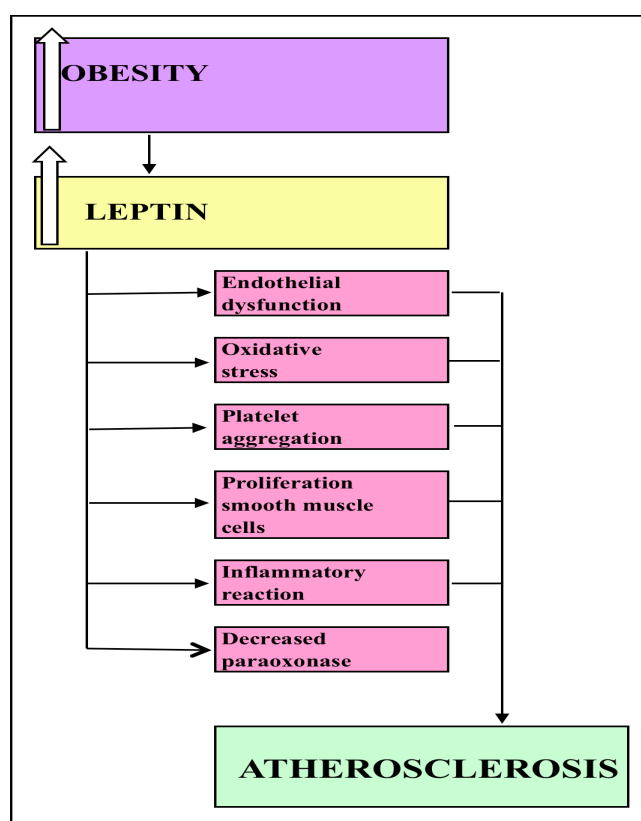
Figure 21. Theory of impaired beneficial leptin effects leading to atherosclerosis [382]



Thus, leptin could directly affect the vessel wall and be an important causal factor in the development of CVD.

The risk of future MI related to leptin showed a plateau effect (Paper IV), which could reflect selection in which subjects with very high levels of leptin have strong protective mechanisms if they survive. The effect may also be an expression of protection partly due to selective peripheral leptin resistance. Competing causes of MI, such as hypertension and dyslipidemia, are seen in obesity, and an increasing lifespan means longer exposure to high leptin levels. Whether leptin levels are high does not matter, and the expected survival age is approximately 30 to 40 years. Today, though the age at death could be between 70 and 100 years, hyperleptinemia may induce changes leading to atherosclerosis (Figure 22).

Figure 22. Theory of hyperleptinemia leading to atherosclerosis [382]



Our results in Paper IV are in line with earlier studies, including the WOSCOP study. In most studies with negative results, the definition of events differs from ours (i.e. including stable angina pectoris). However, the meta-analysis of eight prospective studies showed that leptin does not predict CVD after adjusting for sex, age, and traditional risk factors. A main difference between this analysis and our work is that we *stratified* for sex, whereas they *adjusted* for sex. We suggest that it is important to make stratified calculations due to biological differences between sexes, including circulating levels and hypothalamic sensitivity. Furthermore, we suggest that adjusting for BMI may produce different effects on these associations in men and women.

In Paper II, we demonstrated a strong association between decreasing leptin levels and decreasing PAI-1 activity during strenuous physical activity in men.

Leptin levels were independent of changes in waist circumference, and the correlation between leptin and measures of obesity changed during exercise, which indicates that measured leptin levels not only reflect the amount of body fat, but also other processes. In rat adipose tissue, acute exercise down-regulates the expression of leptin mRNA [339]. Exercise has also been suggested to alter the nutrient flux into cells (effect of the hexosamine pathway), which may affect the expression of the leptin gene [383]. Divergent results are found in the literature for humans. Some results report no acute or chronic effects on leptin levels, but others report positive effects, and variations in the strength and duration of exercise could be an explanation.

We hypothesised in Paper III that decreased leptin levels may mediate improved fibrinolysis together with enalapril treatment. Angiotensin II type 1 receptor blockers significantly decrease the mass of subcutaneous and mesenteric adipose tissue, improve insulin resistance, and reduce both leptin plasma levels and mRNA in adipose tissue [243]. Furthermore, treatment with ramipril decreases the levels of circulating leptin 11% [384]. However, in our study population, leptin levels increased over the course of a year. Unfortunately, BMI was only measured at the start of the study, and we speculate that the increasing levels of leptin were due to weight gain.

Adiponectin

Adiponectin was protective against a future first-ever MI. This protection was clearly seen in men, probably due to the number of participants and stronger statistical power compared to women. Several studies (in vitro, animal models, and humans) have indicated that adiponectin has favourable effects on angiogenesis and endothelial

function [266, 276, 315-317, 321, 325]. The production of adiponectin is down-regulated in obesity, possibly due to increased levels of TNF- α and IL-6, which reduce the expression of adiponectin mRNA [314, 385-387]. Reduced adiponectin function could also be due to the down-regulation of adiponectin receptors related to obesity [288, 388, 389].

Low levels of adiponectin are closely related to impaired insulin sensitivity, and women are more sensitive to insulin compared to men, which could explain the sexual dimorphism in circulating adiponectin levels [390]. Polymorphisms in the promoter region of the adiponectin gene could partly explain the association with insulin resistance, CVD, and T2DM [270, 391], as subjects with one genotype had a doubled risk of developing T2DM compared to those with the other genotype. Subjects with a missense mutation in the globular domain of adiponectin had lower levels of circulating adiponectin, independent of BMI, compared to those without the mutation [392, 393]. In patients with severe insulin resistance and dominant-negative PPAR- γ mutations, the circulating levels of adiponectin were suppressed five-fold [394].

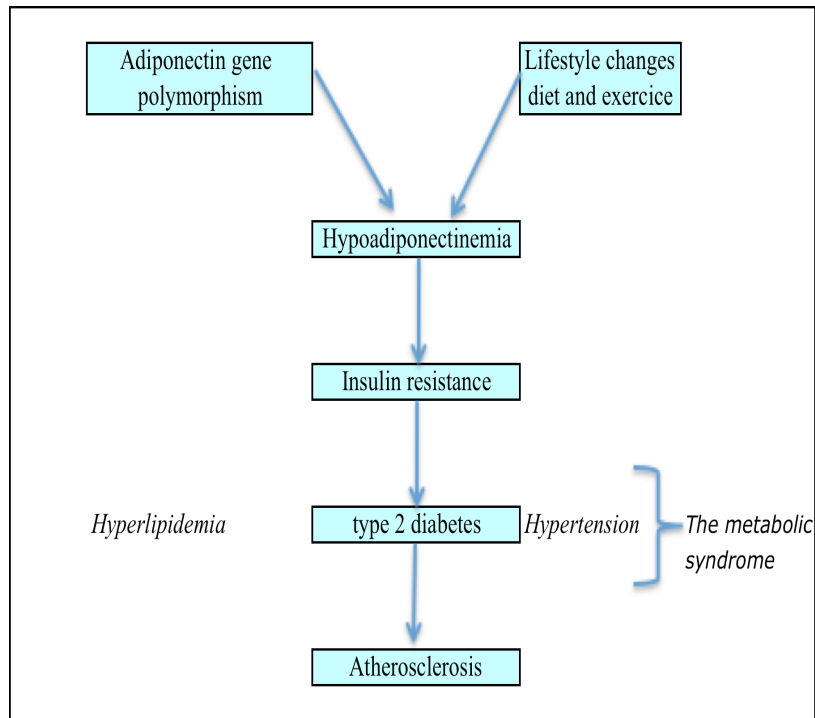
Earlier prospective studies on the impact of adiponectin showed both positive and negative results. This variation could be due to ethnicity, sex, and previous ischemic heart disease. A study of an elderly population showed that high levels of adiponectin increase the risk of CHD. A meta-analysis that included seven prospective studies yielded an OR of 0.84 with borderline significance. Notably, we and others measured total adiponectin and not the high molecular weight form of adiponectin, which is considered to be the biologically active form.

Interestingly, our results showed that adiponectin was independently protective for future MI in men until adjusting for apolipoproteins (apo B/apo A-1 ratio), which could indicate that the effect of adiponectin is mediated through apolipoproteins.

In Paper II, we demonstrated that strenuous exercise induced high levels of adiponectin in men, and that changes in adiponectin were strongly associated with improvements in PAI-1 activity. Interestingly, no associations were found between adiponectin and insulin sensitivity before, during, or after the skiing tour. Notably, our study group was exposed to extreme physical circumstances, which affected several physiological systems, including anthropometry, insulin sensitivity, and the endothelium.

Polymorphisms in the adiponectin gene, together with diet-induced obesity and low physical activity, could lead to hypoadiponectinemia, which promotes CVD (Figure 23).

Figure 23. Model of the role of adiponectin in developing CVD



Hypoadiponectemia and CVD [395].

Studies

MONICA, VIP, and MSP

We identified only 390 MIs in men and 174 MIs in women. The number of MIs according to the size of different study cohorts may appear low; however, only first-ever MIs were included, and all with previous MI and/or stroke, cancer, or incomplete blood samples were excluded. Additionally, only a few health care centres performing health examinations for the VIP study were active from the start, which means substantially fewer years of risk for many participants. In contrast, a linear growth of screened subjects within the VIP is seen today. In the beginning of the 1990s the study had a low participation rate, roughly 50%, which has recently increased [9]. This rate could introduce bias, though a previous analysis did not show this [365].

The MONICA surveys have several strengths, including an identical protocol since the first survey in 1986, a very high participation rate that has only recently been slightly decreasing, and strict population-based

samples. In Paper IV, we present data from a case-control study, a design prone to biases in both case and control selection. In our study, all cases were strictly validated according to the WHO MONICA manual, and all controls were matched with the cases within the surveys, reducing the risk of bias. Furthermore, all data for both cases and controls were collected before the MI, and in order to increase the number of women the MSP survey was used. These women were older than those participating in MONICA or VIP, and missing anthropometry and blood pressure data was common in that cohort. We checked the influence of this missing data with models without BMI, which provided similar results.

The Igloo study

Twenty well-trained men were invited to take part in the Igloo study, and they were exposed to extreme physical exercise for 2 weeks. This study had several limitations. Most importantly, the participants were already very well-trained at the start of the exercise in order to reduce the risk of drop-outs. Therefore, the external validity of the study should be questioned [396].

The enalapril study

The enalapril study included men and women with a previous MI and was performed in the mid-90s; thus, it reflects good clinical care from that period. First, most subjects had earlier thrombolytic treatment. Second, treatment with an ACE inhibitor was not yet routine post-MI in the absence of signs of heart failure. However, due to these considerations, this study is unique and it will not be possible again to test the effect of an ACE inhibitor on leptin and fibrinolytic variables post-MI against placebo.

Design and methodological considerations

Paper I

Subjects with the lowest and highest quartiles of fasting insulin levels at baseline in the MONICA surveys in 1990 and 1994 were chosen. The aim was to represent a wide range of insulin sensitivity, and there was a strong correlation between fasting insulin and estimated insulin sensitivity. Interestingly, most study variables from the re-examination in the late 1990s were reasonably normally distributed, which probably represents regression towards the mean. Furthermore, this was a cross-sectional study that involved observations from a subset of the population at a defined time with the aim to measure associations between dysfibrinolysis, leptin, and androgens in men and women. However,

selection biases could influence the results as only participants from Umeå and Luleå were included, and quite many men dropped out, mainly due to a long period between the initial clamp and the follow-up visits.

In a cross-sectional study design, establishing what represents the cause and what represents the effect is difficult. The mathematical meaning of cross-sectional is “the effective area” for collision (i.e. associations). The advantages of the study design are that it is easy to conduct, is good for descriptive analyses (i.e. easy to investigate many variables), and is good in generating hypotheses. Furthermore, the population could be very broadly characterised, as was the group in this study.

Paper II

Participants were randomised to two different diets in this experimental and observational study (clinical trial). In the post-hoc analysis, this diet allocation could theoretically influence the results. However, the effect was very limited to lipids, and all efforts were made to explore and adjust for this effect. The conditions were, in many ways, extreme, including cold weather, which could influence the results. However, leptin levels have been shown to not vary over the course of the year in our area [152].

Paper III

This study was partly supported by MSD, Inc. The risks for biases to yield favourable results for the sponsor through chosen study designs are well known. A publication bias also exists in which favourable results are more easily accepted and published. However, MSD, Inc was not involved in the acquisition or analysis of data presented in this study. Some of the patients were also treated with statins, acetylsalicylic acid, and beta-blockers, treatments we have not adjusted for. Effects of these treatments on the leptin levels and associations described in the paper could exist. Statin treatment has been associated with lower leptin levels [343, 397].

A main limitation of the study is that BMI was only measured at baseline; consequently, adjustments for BMI could only be done with this initial measure. The increase in leptin levels could indicate increased weight during the study year. However, the main study variables were the changes (delta-values), and we think that our findings represent a true interaction between leptin and fibrinolysis. Furthermore, the positive association between changes could also indicate decreasing levels, which is evident from the graphs presented in the paper.

Paper IV

There are several advantages to a nested case-control study. To evaluate potential risk factors for CVD, an observational study (cohort or case-control) is more appropriate for studying cause-effect relationships compared to randomised trials [398]. Compared to a traditional case-control study, both cases and controls represent random samples of the same study base, resulting in less risk of introducing selection bias. Because samples were collected before the outcome, the prospective design is less sensitive to the problems of reverse causality [399] and is not dependent on disease prevalence for the validity of relative risk estimates. A problem is the time axis and definition of case/control. A case becomes a case when a special event or outcome occurs during the determined cohort time axis, which means that a selected control could be a future case in the study population, with an implication of bias. However, the recommendation is to allow future cases as controls because exclusion could also create bias [400]. In this study, controls were not supposed to have had any MI or stroke, not even after the date that their matched case had an event, which means that the control population is healthier than the general population. Some variables used for adjustments are closely related, and the biological physiology is not always fully understood; interpretation of the adjustments for different variables can be difficult. Adjusting for BMI is questionable, as leptin and BMI are highly correlated. Analysing the leptin/BMI ratio could partly overcome this issue as an expression of a high secretion rate per unit of fat mass.

The univariate and multivariate analyses should be interpreted carefully, and the question of causality between adipokines and MI cannot be fully answered despite the prospective design. A potential limitation of the study is that baseline blood samples were analysed. We had no data on within-person variation in the studied variables, but repeated measurements could increase our knowledge of this issue [401]. However, low within-person variation was recently described in the MONICA survey for leptin over many years [152], and the fact that more than 27,000 subjects have participated twice in the VIP study provides good opportunities for evaluating this issue ([9].

The effect of a variable could also be under- or overestimated due to imprecise measurements [402]. Adipokines were tested only once, not in duplicate, in this study. However, adipokines, including leptin and adiponectin, are very robust and resistant to long-time storage and perform very well in tests (e.g., low CVs) [403, 404]. Furthermore, the same RIA tests from the same manufacturer were used for all studies of leptin and adiponectin in this thesis.

Strengths and limitations of this thesis

Statistics and power

We calculated the power of the association between leptin and PAI-1 activity, tPA activity, and tPA mass in Paper II. Due to unknown pre-test associations between adiponectin and fibrinolytic variables, the power calculations for these associations could not be performed. In Paper III, power was calculated for associations between leptin and PAI-1 mass in men. These calculations indicate that we can only detect an association above a certain level (β -coefficient), and there was no power to say anything about the lack of associations such as what was found in women. Consequently, Papers I-III were based on a limited number of subjects, which means that we were not able to detect weak associations. However, by using a repeated measures design in Papers II and III, we have probably strengthened the statistical power related to sample size. There are benefits of using mixed models, including the homogeneity of regression (modelling the variability in regression slopes). Because the subjects were constant, the variance due to subjects can be partitioned out of the error variance term, making the test more powerful [405]. Despite the benefits of repeated measures, there are disadvantages, and internal validity issues should be assessed. “Carryover” effects may lower the internal validity, for example, the effect of a drug treatment may affect the next treatment. This situation could be a possibility in Paper III [406].

Sex differences in associations between leptin and fibrinolysis

Despite different study populations and study designs, this dissertation has a clear message. In Papers I and III, differences in the circulating levels and associations were found between sexes. In men, high levels of leptin associated to reduced fibrinolysis, also after MI. In Paper II, these associations persisted during heavy physical strain. We suggest that this association may have an impact on the development and progression of ischemic heart disease. Most interestingly, these sex differences were also seen in the prospective nested case-control study (Paper IV).

Future implications

These findings demand better and deeper knowledge about sex differences and pathways in leptin and adiponectin physiology. Mendelian randomisation could be a tool to better understand causality between adipokines and CVD; this is a method for identifying spurious causes. Mendelian randomisation is based on Mendel’s second law, which states that the inheritance of one trait is not dependent on the inheritance of other traits. Common genetic polymorphisms that are known to

influence exposure are used to avoid misleading findings that can be due to confounding by socio-economic factors and behavioural and physiological factors related to both exposure and disease endpoints. Finally, future studies have to include a sufficient number of women in order to draw firm conclusions about adipokines and the risk for CHD.

Conclusions

1. High levels of leptin were independently associated with dysfibrinolysis (high PAI-1 activity, low tPA activity, and high levels of tPA mass and PAI-tPA complex), particularly in men.
2. Heavy exercise in men was followed by decreasing leptin and increasing adiponectin levels. Furthermore, heavy exercise improved the fibrinolytic capacity (PAI-1 activity and tPA mass) and estimated insulin sensitivity, and changes in leptin and adiponectin were associated with changes in fibrinolytic variables.
3. Post-MI treatment with enalapril did not affect leptin levels.
4. Changes in leptin levels were independently associated with changes in PAI-1 mass, tPA mass, and PAI-tPA complex in men during the course of one year after MI. Furthermore, elevated levels of leptin were associated with elevated levels of vWF in women.
5. High leptin levels predicted a first-ever MI with a sex difference, and possibly with a plateau effect. These associations were strongest for STEMI and non-fatal events. A high adiponectin level was protective, but without a sex difference, and time to MI was shorter for those with low adiponectin levels. The combination of high leptin and low adiponectin did not exhibit any synergy, and the leptin/adiponectin ratio did not add information.

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