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Weight loss and fat distribution in obese women

Effects on leptin, cardiovascular risk factors and cardiac parasympathetic activity

Doctoral dissertation

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ABSTRACT

Obesity is one of the most prevalent public health problems in Finland, since about 20 % of adults of working age are obese (body mass index, BMI $\geq$ 30 kg/m^2). The location of fat depots is more important than the excess fat per se. Abdominal fat, especially visceral fat, is an independent risk factor for cardiovascular diseases (CVD). This increased risk is suggested to be mediated via insulin resistance and hyperinsulinaemia frequently accompanied by glucose intolerance, dyslipidaemia, hypertension, abnormalities in blood coagulation and fibrinolysis and elevated leptin levels. Decreased cardiac parasympathetic activity (PSA) may also predispose obese subjects to CVD.

This study was undertaken to investigate leptin, cardiovascular risk factors characteristic of insulin resistance and cardiac PSA in relation to weight loss and fat distribution in healthy women participating in three European weight reduction trials using orlistat for six to twelve months.

The women lost weight between 8.4 and 9.5 kg during the first six months of the weight loss regimen. From six to twelve months 41 % of the women continued to lose weight, 24 % had stable weight and 35 % regained weight. Weight loss was accompanied by significant decreases in plasma leptin, serum insulin, and total and LDL cholesterol concentrations, activities of plasminogen activator inhibitor-1 (PAI-1) and factor VII (FVII) in plasma and systolic (SBP) and diastolic blood pressure (DBP) and by an increase in cardiac PSA. Serum glucose, HDL cholesterol, triglycerides and plasma fibrinogen remained unchanged. The decrease in serum insulin was related to decreases in plasma leptin, activities of PAI-1 and FVII, but not with cardiac PSA. Serum insulin and the activities of PAI-1 and FVII rose with weight regain, but the concentration and activities remained below the six month values when the weight loss was sustained or continued. LDL cholesterol, and SBP and DBP rose in all women after six months, including those who continued to lose weight.

None of the anthropometric measurements of abdominal fat (waist circumference, waist-to-hip ratio, waist-to-height ratio, sagittal and transveral diameter) was significantly superior to others to measure the association between total abdominal fat and cardiovascular risk factors. 'Upper lumbar fat' (the area between the first and fourth lumbar vertebrae) associated with most of the cardiovascular risk factors.

Plasma leptin decreased proportionally with decreasing body fat. In addition to total body fat, plasma leptin reflected adipose tissue distribution, especially peripheral and abdominal subcutaneous fat. The rise of cardiac PSA was not associated with decrease of abdominal fat.

Conclusions: Modest weight loss improves most cardiovascular risk factors in obese women. The maintenance of weight loss is associated with long-term benefits of serum insulin and activities of PAI-1 and FVII, while LDL cholesterol and blood pressure tend to rebound independently of weight change. The improvement of hyperinsulinaemia relates to the changes in plasma leptin, PAI-1 and FVII, but not cardiac PSA. Total body fat and changes in its amount are more important regulators of plasma leptin than fat distribution. The loss of abdominal fat is not associated with the change in cardiac PSA. Any of the anthropometric measurements of visceral fat and 'upper lumbar fat' assessed by DXA measurements can be used to measure abdominal obesity in obese women.

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ABBREVIATIONS

ASF	Abdominal subcutaneous fat
AVF	Abdominal visceral fat
BIA	Bioelectrical impedance
BMI	Body mass index
CT	Computed tomography
CVD	Cardiovascular diseases
DBP	Diastolic blood pressure
DXA	Dual-energy X-ray absorptiometry
FFM	Fat free mass
FVIIc	Factor VII coagulant activity
HDL	High-density lipoprotein
HF	High frequency
HRV	Heart rate variability
LDL	Low-density lipoprotein
MRI	Magnetic resonance imaging
PAI-1	Plasminogen activator inhibitor-1
PSA	Parasympathetic activity
RSA	Respiratory sinus arrhythmia
SBP	Systolic blood pressure
STR	Sagittal-to-transverse ratio
TC	Total cholesterol
TG	Triglycerides
UWW	Underwater weighing
WHR	Waist-to-hip ratio
WHTR	Waist-to-height ratio

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by Roman numerals **I-IV**:

I   Rissanen P, Hämäläinen P, Vanninen E, Tenhunen-Eskelinen M, Uusitupa M. Relationship of metabolic variables to abdominal adiposity measured by different anthropometric measurements and dual-energy X-ray absorptiometry in obese middle-aged women. Int J Obes 1997;21:367-371.

II   Rissanen P, Mäkimattila S, Vehmas T, Taavitsainen M, Rissanen A. Effect of weight loss and regional fat distribution on plasma leptin concentration in obese women. Int J Obes 1999;23:645-649.

III   Rissanen P, Vahtera E, Krusius T, Uusitupa M, Rissanen A. Weight change and blood coagulability and fibrinolysis in healthy obese women. Int J Obes 2001; 25:212-218.

IV   Rissanen P, Franssila-Kallunki A, Rissanen A. Cardiac parasympathetic activity is increased by weight loss in healthy obese women. Obes Res 2001;9:637-643.

CONTENTS

Appendix: ORIGINAL PUBLICATIONS I-IV

1. INTRODUCTION

Obesity has become a worldwide epidemic (World Health Organization 1998). In Finland it is one of the most important public health problems. About 20 % of adults of working age are obese (body mass index, BMI$\geq$30 kg/m^2) (Lahti-Koski et al. 2000a). The proportion of severely obese subjects (BMI$\geq$35 kg/m^2) has increased from 2 % to 5 % in men and from 5 % to 7 % in women between 1982 and 1997 (Lahti-Koski et al. 2000a). In addition, abdominal obesity has increased in men and women, independently of changes in BMI (Lahti-Koski et al. 2000b).

Already in the mid-forties it was noticed that the complications commonly observed in obese subjects were more closely related to the location of fat depots rather than to the amount of excess fat per se (Vague 1947). Now it is well known that abdominal fat and in particular the fat stored in the abdominal cavity (visceral fat) is metabolically more active than the subcutaneous fat in the gluteal and femoral region. However, the effects of different abdominal fat depots on metabolic disorders are still unclear.

Abdominal obesity is an independent risk factor for type 2 diabetes and cardiovascular diseases (CVD) (Rexrode et al. 1998). This increased risk is suggested to be mediated via insulin resistance and hyperinsulinaemia, frequently accompanied by glucose intolerance, dyslipidaemia, hypertension, abnormalities in blood coagulation and fibrinolysis and increased leptin levels.

Several potential new risk factors for CVD have been identified in recent studies. Left ventricular hypertrophy, oxidative stress and markers of inflammation such as C-reactive protein and serum amyloid A are among these risk factors (Harjai 1999). It is also possible that decreased cardiac parasympathetic activity can be added as a CVD risk factors in future. Decreased activity of the cardiac parasympathetic nervous system has been suggested to increase the risk of coronary heart disease and to predispose to arrhythmias and sudden death.(Liao et al. 1997). Interestingly, obese subjects have been shown to have lower cardiac parasympathetic activity than normal weight subjects (Piccirillo et al. 1996, Karason et al. 1999, Emdin et al. 2001).

Even modest weight loss can reduce hyperinsulinaemia and improve related cardiovascular risk factors (Tuomilehto et al. 2001). The reported effects of weight loss on blood coagulation and cardiac parasympathetic activity have been inconsistent (Folsom et al. 1993, Svendsen et al. 1996, Karason et al. 1999, Emdin et al. 2001). As most obese subjects regain their weight after weight loss, it is important to know how cardiovascular risk factors respond to weight regain. Moreover, there is a lack of knowledge of the effect of weight maintenance after weight loss on cardiovascular risk factors.

The association between abdominal obesity and weight loss with cardiovascular risk factors has been mainly studied in men (Desprès et al. 2001). However, the results may be different in women since they have less visceral fat and more abdominal subcutaneous fat.

The main purpose of this study was to investigate the effect of weight loss and weight change after weight loss on circulating leptin levels, cardiovascular risk factors and cardiac parasympathetic activity in obese women. Furthermore, the association between fat distribution and leptin, cardiovascular risk factors and cardiac parasympathetic activity was studied.

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2. REVIEW OF THE LITERATURE

2.1. Definition and classification of obesity

Obesity is defined as a condition of excessive fat accumulation in adipose tissue to the extent that health may be impaired (Garrow 1988). The body mass index (BMI, weight, kg/ height, m^2) is a commonly used crude measure of obesity (Garrow and Webster 1985). BMI does not distinguish between weight associated with muscle and weight associated with fat, and it does not reflect body fat distribution. Thus, owing to differences in body composition, BMI may not correspond to the same degree of fatness across populations (Swinburn et al. 1996). In the individual level, BMI can increase e.g. due to oedema, enhanced muscularity or decreased height in kyphosis and ageing.

In the 1970's and 1980's, normal weight was suggested to be a BMI between 20 and 25 kg/m^2, overweight a BMI between 25 and 30 kg/m^2 and obesity a BMI above 30 kg/m^2 (Bray 1978, Garrow 1981). In the 1990's several organisations have developed guidelines for the prevention and treatment of obesity. The World Health Organization (WHO) (1998), the National Institutes of Health (NIH) and the National Heart, Lung, and Blood Institute (NHLBI) (1998) have published classifications of overweight and obesity that applies to both men and women and to all adult age groups (**Table 1**). The classification is based on total mortality rates. However, one must keep in mind that the disease risk associated with obesity is affected by a wide range of factors including diet, level of physical activity, ethnicity and fat distribution.

Table 1 Classification of overweight and obesity by BMI, waist circumference and associated disease risk according to WHO (1998) and NIH, NHLBI (1998).

Classification	BMI (kg/m²)	Risk of comorbidities[a]	Disease risk[a] relative to normal weight and waist circumference	
			Men, ≤ 102 cm Women, ≤ 88 cm	Men, > 102 cm Women, > 88 cm
Underweight	<18.5	Low		
Normal range	18.5-24.9	Average		
Pre-obese	25.0-29.9	Increased	Increased	High
Obese class I	30.0-34.9	Moderate	High	Very high
Obese class II	35.0-39.9	Severe	Very high	Very high
Obese class III	≥40	Very severe	Extremely high	Extremely high

[a] Disease risk for type 2 diabetes, hypertension and CVD.

Abdominal fat mass can vary widely within a narrow range of total body fat and BMI (Smith et al. 2001). The excess of abdominal fat mass is an independent predictor of type 2 diabetes, hypertension and CVD (Blair et al. 1984, Chan et al. 1994, Rexrode et al. 1998). Therefore, the amount of abdominal fat mass has been taken into account in risk assessment (NIH,

NHLBI 1998). The sex-specific cut-off points identify the increased risk for the development of obesity-associated diseases in most adults with a BMI between 25 and 34.9 kg/m^2. If BMI is equal to or greater than 35 kg/m^2 the waist circumference seems to lose its incremental predictive power.

Globally applicable cut-off points for waist circumference cannot be developed since populations and ethnic groups differ in the level of risk associated with a particular waist circumference (Dowling and Pi-Sunyer 1993, Okosun et al. 2000). The cut-off points in **Table 1** are based on studies of Lean et al. in the United Kingdom (Han et al. 1995, Lean et al. 1995, Lean et al. 1998). Other cut-offs for Caucasians have been proposed by Dutch and Canadian investigators (Han et al. 1995, Lemieux et al. 1996a). None of these cut-off points take age into account, even though it is known that with increasing age there is a selective deposition of visceral adipose tissue (Lemieux et al. 1996b). A middle aged man with a waist measurement of 95 cm, has on average more visceral fat than a young adult man with a similar waist circumference (Lemieux et al. 1996a). Age and ethnicity have been included in the American recommendations of waist circumference for established levels of overweight (BMI 25-29.9 kg/m^2) and obesity (BMI $\geq$ 30 kg/m^2) (Okosun et al. 2000).

2.2. Assessment of total body fat

2.2.1. Reference methods

BMI is not an accurate measure of total body fat and changes in BMI do not reflect changes in fat mass. One of the difficulties is that there is no true 'gold standard' in evaluating in vivo total body fat. Cadaver analysis is often cited as the ultimate reference method, but in practice it is impossible and is not devoid of its own errors like loss of solids and water during the handling of material (Jebb 1998). Underwater weighing (UWW) is another reference method. The method is based on an assumption that the density of fat is 0.9 kg/l and that of fat free mass (FFM) is 1.1 kg/l (Siri 1956). The limitation of the method is that it assumes the density of FFM to be constant (Jebb 1998). Deviations in body density can occur because of changes in hydration or the proportion of bone mineral. In recent years multi-compartment models have been suggested to be accurate reference methods (Cohn et al. 1984, Heymsfield et al. 1991, Wang et al. 1998). In addition to fat, they take into account water, protein, bone and soft tissue minerals and even glycogen in skeletal muscle (Heymsfield et al. 1991). Some of the methods estimating total body fat and fat distribution are presented in **Table 2**.

Table 2 Assessment of some methods in the estimating of total body fat and abdominal fat distribution. Modified from Kvist et al. 1988, Wang et al. 1998, Clasey et al. 1999, Nordhamn et al. 2000, Ellis 2001.

Method	Costs	Ease of use	Validity	Reproducibility	Measurement of abdominal fat distribution
Anthropometric methods					
Weight	Low	Easy	Moderate	Good	No
BMI (kg/m^2)	Low	Easy	Moderate	Good	No
Waist circumfererence	Low	Easy	Moderate	Moderate	No
Waist-to-hip-ratio (WHR)	Low	Easy	Moderate	Moderate	No
Abdominal sagittal diameter	Low	Easy	Moderate	Good	No
Abdominal transverse diameter	Low	Easy	Moderate	Not known	No
Prediction methods					
Skinfold thickness	Low	Easy	Low	Poor	No
Infrared interactance	Low	Easy	Low	Poor	No
Bioelectrical impedance (BIA)	Low	Easy	Low	Poor	No
2-compartment methods					
Isotope dilution (TBW)	High	Difficult	Moderate	Good	No
Total body potassium (TBK)	High	Difficult	Moderate	Good	No
Underwater weighing (UWW)	High	Difficult	Moderate	Good	No
Air-displacement plathysmography (ADP)	High	Difficult	Moderate	Good	No
Multi-compartment model	High	Difficult	High	Good	No
Novel methods					
In vivo neutron activation analysis (IVNAA)	High	Difficult	High	Good	No
Dual-energy X-ray absorptiometry (DXA)	High	Difficult	Moderate	Good	Yes
Ultrasound	Moderate	Difficult	Moderate	Moderate	Yes
Computed tomography (CT)	High	Difficult	High	Good	Yes
Magnetic resonance imaging (MRI)	High	Difficult	High	Good	Yes

2.2.2. Dual-energy X-ray absorptiometry (DXA)

DXA is widely used for the measurement of bone mineral content, but it also divides the soft tissue into fat and FFM (lean+bone) providing a 3-compartment model. The result of a measurement is based on the attenuation of X-rays at two low dose energies in fat, bone mineral and fat free soft-tissues (Pietrobelli et al. 1996). Concerns have risen about the validity of DXA in measuring fat percentage. DXA programming software assumes an uniform and fixed constant for the hydration of fat-free mass, 0.73 ml/g, which is not accurate for many hospitalised patients, elderly individuals and obese subjects (Roubenoff et al. 1993). Studies with hemodialysis patients have reported that fat mass and bone density measured by DXA were unaffected by fluid changes (Fromica et al. 1993, Stenver et al. 1995), indicating that changes in hydration of soft tissue does not affect the validity of a measurement of fat percentage by DXA.

The validity of DXA technique has been assessed against multi-compartment methods (Wang et al. 1998, Clasey et al. 1999). The mean differences between the methods have been reported in several ways, e.g. in fat-kg or in fat-%, which has made difficult the comparison of the results. In general the mean difference between DXA and multi-compartment models (4- and 6-compartment models) has been modest but individual errors have been large (Wang et al. 1998, Clasey et al. 1999). It has been suggested that errors might be associated with varying subject thickness and the estimation of soft tissue composition in regions surrounding bone (Kohrt 1995). DXA can measure subjects with a limited range of thickness (e.g. 15-22 cm) and soft tissue pixels that do not contain bone mineral (Snead et al. 1993). DXA measured fat mass is not solely adipose tissue, but a sum of all the fatty elements in the soft tissue (Svendsen et al. 1993a). Thus, fat mass by DXA and adipose tissue by multi-compartment methods are different measures of fatness that may not be directly comparable.

2.2.3. Bioelectrical impedance analysis (BIA)

The BIA measurement is performed by attaching a pair of electrodes at the wrist and at the ankle so that a weak alternating current can be passed through the body. BIA is based on the premise that the voltage drop between two electrodes is proportional to the body's fluid volume in that region of the body. Water and electrolytes in FFM are good conductors of current, while fat mass is a poor conductor. The voltage drop is measured and the resistance (R) calculated. To estimate the volume of total body water (TBW), BIA uses three assumptions: the whole body acts like a cylindrical conductor, the length of the conductor is proportional to the subject's height and the reactance component of the voltage can be disregarded (Ellis 2001). The impedance index (height^2/R) is assumed to be proportional to the volume of TBW. FFM is determined on the basis of TBW from the prediction equations, and fat mass can be defined as the difference between body weight and FFM.

The validity of BIA has been studied against multi-compartment methods and underwater weighing (UWW). BIA using the equations of Segal (1985) underestimated body fat percentage compared to the 6-compartment model (Wang et al. 1998), and similarly BIA using the equations of RJL (RJL Systems Ins., Detroit, MI) to UWW (Fogelholm and van Marken Lichtenbelt 1997). The equations of Lukaski et al. (1985, 1986) overestimated body fat percentage. This may reflect the complex geometry of the body, which cannot be adequately described using the model of a simple cylinder. On the other hand, the prediction equations should be population specific (Elia 1992). BIA measurements also require normally hydrated patients. Obese subjects have been reported to have oedema, which increases body water volume and is interpreted by BIA as an expansion of lean tissue. BIA may also be insensitive in detecting variations in body composition of the trunk region (Gray et al. 1989).

2.2.4. Anthropometric measurements

Circumferences, abdominal diameters, their ratios and skinfold thicknesses have been used to estimate both overall and abdominal obesity. None of these measurements can distinguish between visceral and subcutaneous abdominal fat, but they correlate with cardiovascular risk factors and are proxy measures for public health initiatives (**Table 2**). WHR is calculated as the ratio of waist circumference to hip circumference. It correlates moderately with CT- or

MRI-measured visceral fat and is a widely used measure of abdominal fat and even an estimate of visceral fat (Ashwell et al. 1985, Zamboni et al. 1992, van der Kooy et al. 1993, Pouliot et al. 1994). Waist circumference correlates with abdominal subcutaneous fat and visceral fat (van der Kooy et al. 1993). A large waist circumference is associated with cardiovascular risk factors in both genders (Lean et al. 1998).

The measurement of abdominal diameters is based on the premise that the accumulation of visceral fat would maintain the depth of the abdomen in a sagittal direction (sagittal diameter), while subcutaneous fat would reduce the abdominal depth due to force of gravity (transverse diameter) (Sjöström et al. 1991). Sagittal diameter has been shown to correlate with both subcutaneous abdominal fat and visceral fat, while transverse diameter correlates only with subcutaneous abdominal fat (van der Kooy et al. 1993, Pouliot et al. 1994). Sagittal diameter has the highest reproducibility compared to waist circumference, WHR and skinfold thickness measurements (Nordhamn et al. 2000). Sagittal diameter also maintains its reproducibility with overweight and obesity (Nordhamn et al. 2000). Waist circumference and sagittal diameter are better correlated with the change of visceral adipose tissue than WHR (Lemieux et al. 1996b). Simultaneous changes in waist and hip circumferences result in a stable WHR during weight loss (Desprès et al. 2001).

2.3. Assessment of fat distribution

2.3.1. Reference methods

Abdominal fat consists of subcutaneous abdominal fat and intra-abdominal fat. Intra-abdominal fat is composed of visceral fat, which consists of omental, mesenteric and retroperitoneal fat (van der Kooy and Seidell 1993). Subcutaneous abdominal fat is devided into two fat layers by the fascia superficialis; deep and superficial subcutaneous abdominal fat (Smith et al. 2001).

Computed tomography (CT) and magnetic resonance imaging (MRI) can provide a direct measure of fat mass at specific sites of the body and thus also in the abdominal and intra-abdominal region (**Table 2**). A particular advantage of these techniques is that they can discriminate between omental, mesenteric, retroperitoneal and abdominal subcutaneous fat depots. This distinction is important since intraperitoneal and abdominal subcutaneous fat depots are associated with metabolic disorders (Björntorp 1990, Desprès 1992, Abate et al. 1995, Goodpaster et al. 1997, Smith et al. 2001). The validity and reproducibility of CT and MRI measurements have been shown to be good compared to cadaver analysis (Rössner et al. 1990, Fowler et al. 1992). Therefore, CT and MRI are used as the reference methods for the measurement of abdominal fat distribution.

2.3.2. DXA

In addition to the measurements of total body fat mass, DXA can analyse specific regions of the body, e.g. arms, legs, trunk and separately total abdominal fat mass. The validity of DXA-measured total abdominal fat (subcutaneous and intra-abdominal fat) has been shown to be good compared to CT-measured total abdominal fat (Jensen et al. 1995). However, some

investigators have speculated that DXA measures soft tissue inaccurately, especially in the abdominal region. In the experimental studies exogenous fat (lard) was more correctly assessed by DXA when it was placed on the thighs than on the abdomen (Snead et al. 1993, Milliken et al. 1996). The authors suggest that in individuals with increased upper body adipose tissue (obese, older individuals) DXA may underestimate abdominal fat and thus total body fat (Snead et al. 1993, Milliken et al. 1996). The estimation of intra-abominal fat by DXA is questionable. By subtracting anthropometrically predicted subcutaneous adipose tissue from DXA-measured abdominal fat it is possible to get an estimate of intra-abdominal fat (Svendsen et al. 1993b, Jensen et al. 1995, Treuth et al. 1995). The accuracy of this method is only modest compared to CT (Svendsen et al. 1993a, Jensen et al. 1995). The other way to estimate intra-abdominal fat is to measure the region from the L2 to L4 vertebra (Campbell et al. 1996). The region of interest has been drawn lateral to inner costal margins to exclude abdominal subcutaneous fat and the rest of fat is thought to be intra-abdominal fat (Campbell et al. 1996). However, the validity of this method is unknown and thus cannot be recommended in clinical studies (Jebb 1998).

2.3.3. Ultrasonography

Ultrasonography has been used as a non-invasive technique for measuring abdominal subcutaneous and intra-abdominal fat (Armellini et al. 1990). The distance between the linea alba and the dorsal border of the abdominal aorta describes the intra-abdominal fat and the thickness of subcutis in the same sagittal line the subcutaneous abdominal fat (Armellini et al. 1990). The method has been criticised because of its reported low reproducibility and moderate validity in the measurement of intra-abominal fat (Armellini et al. 1990, Bellisari et al. 1993). The sources of poor reproducibility are e.g. too short measurement time and difficulties in identification of tissue interfaces, especially the aorta and the linea alba. Their position may be affected by respiratory and intestinal motion and the expansion and contraction of the aorta (Bellisari et al. 1993, Tornaghi et al. 1994). Intestinal gas may also cause poor visibility of the aorta and the linea alba. The validity of the method can be improved by standardising the technique. After standardisation, validity has been found to be better than that of waist-to-hip-ratio (WHR) or sagittal diameter compared to CT measurements (Tornaghi et al. 1994).

2.4. Obesity-related metabolic changes

The strong association between obesity, especially, abdominal obesity and cardiovascular diseases and type 2 diabetes is mediated via various metabolic alterations. Reaven (1988) proposed that a common feature underlying a cluster of metabolic alterations is insulin resistance, a failure of insulin to act normally on target tissues. Insulin resistance, in turn, is frequently accompanied with e.g. impaired glucose tolerance, dyslipidaemia, hypertension, abnormalities in blood coagulation and fibrinolysis and elevated leptin levels (De Courten et al. 1997, Bosello and Zamboni 2000). Clustering of these risk factors is called e.g. metabolic syndrome or insulin resistance syndrome, but the term metabolic syndrome is now favoured (Alberti and Zimmet 1998).

The so called Portal Theory describes the idea how insulin resistance and many of its deleterious features arise through high rates of free fatty acid (FFA) liberation from visceral adipose tissue (Arner 1997). The release of FFAs into portal vein have crucial effects on hepatic metabolism (Östman et al. 1979). Increased portal FFA availability reduces insulin binding to the hepatocytes, causing insulin resistance in the liver (Peiris et al. 1986, 1987). Thus, hepatic insulin clearance is reduced and pancreatic insulin secretion is increased due to obesity-induced insulin resistance, leading to more marked elevation of peripheral insulin concentrations (Peiris et al. 1986, 1987). Elevated hepatic concentrations of FFAs also lead to increased hepatic glucose production, alterations in plasma lipid profile and increased systemic concentrations of FFAs (Kissebah et al 1976, Bevilacqua et al. 1987). An increased supply of FFAs in the periphery results in increased FFA uptake and re-esterification in muscle leading to skeletal muscle insulin resistance and decreased glucose oxidation and glycogen synthesis in skeletal muscle and thus impaired glucose uptake in skeletal muscle (Groop 2000).

Obesity-related changes in leptin, insulin and lipid metabolism, hypertension and blood coagulation and fibrinolysis will be discussed in detail below.

2.5. Role of leptin in obesity

2.5.1. Definition of leptin

Leptin, a 167-amino acid protein, is a product of the *ob* gene (Zhang et al. 1994, Halaas et al. 1995). In humans leptin messenger ribonucleic acid (mRNA) has been found e.g. in white mature adipocytes (Masuzaki et al. 1995), placenta (Green et al. 1995, Masuzaki et al. 1997), heart (Green et al. 1995), ovaries and mammary gland (Smith-Kirwin et al. 1998). In mouse, leptin mRNA is also expressed in brown adipose tissue (Oliver et al. 2000). The main source of circulating leptin in humans is white adipose tissue, but also the placenta during pregnancy (Masuzaki et al. 1995, Masuzaki et al. 1997).

The effect of leptin is mediated by the leptin receptor encoded by *db* gene (Lee et al. 1996). Leptin circulates in a bound and free form in the blood stream (Lee et al. 1996). In lean subjects 50 % of leptin circulates in the free form, while in obese subjects about 80 % circulates in the free form (Sinha et al. 1996). Elevated levels of the free form of leptin has been suggested to be a sign of leptin resistance (Sinha et al. 1996, Lahlou et al. 2000).

2.5.2. Leptin and energy metabolism

Acute changes in energy balance alter leptin levels in humans (Boden et al. 1996, Kolaczynski et al. 1996). Leptin concentrations decrease in response to a state of negative energy balance produced by fasting or short-term low energy intakes when no change in the amount of adipose tissue triacylglycerol concentration is observed (Jenkins et al. 1997, Pratley et al. 1997, Dubuc et al. 1998, Chin-Chance et al. 2000). Conversely, during short-term overfeeding, plasma leptin levels increase (Kolaczynski et al. 1996, Chin-Chance et al. 2000). In animal models changes in leptin secretion affect food intake (Rosenbaum et al. 1997a, Woods et al. 1998). Leptin is thought to both increase and decrease hypothalamic neuropeptides that regulate food intake (Stephens et al. 1995, Schwartz et al. 1996, Schwartz

et al. 1997, Sahu et al. 1998, Kristensen et al. 1998, Schwartz et al. 2000). In humans, circulating leptin is suggested to be more a long-term regulator of energy balance than a short-term satiety signal (Keim et al. 1998). Leptin also signals to other systems (reproduction, T-cell -mediated immunity and secretion of hormones, including insulin, cortisol, growth hormone and thyroxine) whether adipose tissue stores are sufficient or insufficient for their active function (Boden et al. 1997, Gracia-Mayor et al. 1997, Heiman et al. 1997, Mantzoros et al. 1997, Tannenbaum et al. 1998).

2.5.3. Leptin in human obesity

Human obesity is nearly always associated with elevated leptin levels (Flier 1998). Serum leptin concentrations correlate strongly with percentage body fat or BMI (Maffei et al. 1995, Considine et al. 1996), but still vary widely at a given level of fat mass. Thus, leptin concentrations of some obese subjects with a BMI more than 40 kg/m^2 may be equivalent to that of subjects with a BMI less than 20 kg/m^2 (Maffei et al. 1995), suggesting that other factors than body mass or fat mass are also involved. Adipose tissue distribution may be such a factor (Maffei et al. 1995). Abdominal subcutaneous adipocytes are about 50 % larger than omental ones, and they express more leptin mRNA compared to visceral adipocytes (Masuzaki et al. 1995, Lönnqvist et al. 1997a, Montague et al. 1997, van Harmelen et al. 1998, Lefebvre et al. 1998).

However, studies relating abdominal fat distribution to plasma leptin have yielded controversial results. Some studies show a strong association between both visceral and subcutaneous fat and circulating leptin concentration or total abdominal fat and plasma leptin levels (Couillard et al. 1997, Liuzzi et al. 1999). Other studies have demonstrated significant correlation of plasma leptin with abdominal subcutaneous fat or with visceral fat (Caprio et al. 1996, Giower et al. 2000, Tai et al. 2000). Furthermore, peripheral fat (gluteal femoral fat) has also been suggested to be an important determinant of plasma leptin concentration (Bennet et al. 1997, Lönnqvist et al. 1997b, Niskanen et al. 1997). According to some studies circulating leptin concentrations may not be related to regional body fat distribution at all (Haffner et al. 1996, Rönnemaa et al. 1997).

2.5.4. Leptin and weight loss

Weight loss is associated with decrease in serum leptin and leptin mRNA content of adipocytes (Maffei et al. 1995, Considine et al. 1996, Niskanen et al. 1997, Torgerson et al. 1999). The decrease of leptin with a modest energy intake deficit (1.0-2.9 MJ deficit/day) has been proportional to the decline of actual amount of adipose tissue (Nicklas et al. 1997). Leptin concentrations per actual body fat mass have decreased significantly with the diet containing energy of 3.4-4.0 MJ/day (Rosenbaum et al. 1997b, Wadden et al. 1998). A six-week low-energy diet of 4.0 MJ/day reduced serum leptin concentration by 55 %, which was 10 times more than the body weight reduction (Wadden et al. 1998). When the energy intake was increased to 5.0-7.6 MJ/day, changes in leptin became more related to changes in weight and fat than to the energy intake (Wadden et al. 1998). Strict energy restriction may be one reason for that leptin concentration decreases disproportionately with adipose tissue during weight loss.

2.6. Cardiovascular risk factors in obesity

2.6.1. Hyperinsulinaemia and insulin resistance

Both hyperinsulinaemia and insulin resistance have been suggested to accelerate atherosclerosis (Folsom et al. 1994, Salomaa et al. 1995). Obesity, hyperinsulinaemia and insulin resistance are strongly associated (Desprès and Marette 1999). BMI and weight change after the age of 20 years were the strongest predictors of fasting insulin levels in middle-aged women (Wing et al. 1992). Furthermore, weight gain during a three-year follow-up was the strongest predictor of further increases in insulin levels (Wing et al. 1992). In the prospective Nurses' Health Study, the risk of type 2 diabetes increased with greater BMI (Colditz et al. 1995). Additionally, the relative risk for type 2 diabetes was doubled in women with weight gain of 5.0 to 7.9 kg in 14 years compared to women who had stable weight. In contrast, weight loss more than 5.0 kg reduced their risk for type 2 diabetes by 50 % or more.
In addition to BMI, WHR has been associated with hyperinsulinaemia in prospective studies in men (Folsom et al. 1996, Lakka 2001). Men who had BMI $\geq$ 26.7 kg/m^2 had a 8.1 -fold risk of developing hyperinsulinaemia compared to men without overall and abdominal obesity (Lakka 2001). If men had BMI $\geq$ 26.7 kg/m^2 and WHR$\geq$0.95, the risk of hyperinsulinaemia was 10.5-fold. Thus, overall and abdominal obesity as well as weight gain during adulthood are associated with increased risk of developing hyperinsulinaemia.

Intentional weight loss decreases serum insulin concentrations in short- and long-term (Wing et al. 1995a, Ditschuneit et al. 1999, Tuomilehto et al. 2001). Weight regain after weight loss may be associated with increased insulin levels, and weight maintenence with sustained improvements or a partial regain in insulin levels (Wing et al. 1995a, Wing et al. 1995b, Fogelholm et al. 2000).

2.6.2. Hypertension

Hypertension is one of the main risk factors for atherosclerotic vascular diseases (WHO 1999). In most studies, overweight and obesity is associated with a manyfold increase in the prevalence of hypertension or in the risk of developing hypertension even after adjusting for age and lifestyle factors like smoking, alcohol consumption and physical activity (MacMahon et al. 1987, Jousilahti et al. 1995, Lean et al. 1999). The association has been stronger in men than in women. Especially, abdominal obesity has been related to blood pressure (Blair et al. 1984, Jousilahti et al. 1995, Lean et al. 1999, Mikhail et al. 1999). In contrast to several studies, the findings of the Framingham study and the Normative Ageing study showed that the correlation between obesity and hypertension is weak and BMI and WHR are unrelated to blood pressure after adjustment for insulin (Kannel et al. 1967, Ward et al. 1996). However, additional support to the existence of the association of obesity with hypertension provides the decrease of blood pressure with weight reduction (Wing et al. 1995a, Ditschuneit et al. 1999, Tuomilehto et al. 2001). Changes in blood pressure after weight loss also correlate with changes in body weight (Dornfeld et al. 1985, Wing et al. 1995a, Ditschuneit et al. 1999, Flechtner-Mors et al. 2000). Weight regain results in elevation of blood pressure, and weight maintenance sustains blood pressure at the level it was after weight loss (Wing et al. 1995a). However, some recent studies suggest that reduced blood pressure is not sustained even

though reduced weight is maintained (Wing et al. 1995b, Fogelholm et al. 2000, Sjöström et al. 2000, Stevensen et al. 2001).

2.6.3. Dyslipidaemia

Dyslipidaemia, i.e. elevated serum total and LDL cholesterol and triglycerides and a low HDL cholesterol, is associated with overweight and obesity even after controlling for age and lifestyle factors in both genders (Lean et al. 1999). With increasing total body fat and WHR, total and LDL cholesterol levels have been found to be elevated in cross-sectional population studies (Seidell et al. 1990, Bertrais et al. 1999). This relation is less clear in women than in men. Upper-body obese women had similar LDL cholesterol concentrations to lower-body obese women (Dennis et al. 1993). HDL cholesterol levels tend to decrease and triglyceride levels to increase with increasing WHR in both genders, after adjustment for age and lifestyle factors (Bertrais et al. 1999).

Weight reduction by dieting decreases total- and LDL cholesterol and triglycerides (Dattilo and Kris-Etherton 1992). HDL cholesterol declines during active weight loss and increases when weight is stabilised (Dattilo and Kris-Etherton 1992). Men who were matched to women with regard to age, height, body weight and WHR showed greater improvements in serum triglycerides and HDL cholesterol than women (Wirth et al. 1998). This difference was suggested to be caused by greater visceral fat reduction in men than in women. Also in women the improvement of lipid profile has been associated with the reduction of abdominal fat (Dennis et al. 1993, Han et al. 1997). Waist reduction (adjusted for age, smoking, alcohol consumption, diet treatment) correlated significantly with decrease in total and LDL cholesterol, but not with changes in HDL cholesterol or triglyceride levels (Han et al. 1997). Contrary to this result, HDL cholesterol levels rose and triglycerides fell irrespective of abdominal or total body fat only in upper-body obese women (Dennis et al. 1993).

Weight regain after weight loss elevates LDL cholesterol and decreases HDL cholesterol (Wing et al. 1995b). If the entire weight loss result is sustained it seems that total- and LDL cholesterol slightly increases, but increased HDL cholesterol levels and decreased triglyceride levels are maintained in long-term (Wing et al. 1995a). If weight maintenance is only partial, subjects who maintained losses > 10% of initial weight had significantly greater reductions in total and LDL cholesterol than subjects who maintained losses of only 5 to 10 % of initial weight (Wadden et al. 1999).

2.6.4. Abnormalities in fibrinolysis and coagulation

2.6.4.1. Blood coagulation and fibrinolysis

Blood coagulation is a complex cascade of chemical reactions in response to a rupture or cut of the blood vessel (Guyton and Hall 1996). The net result is the conversion of a soluble blood protein, fibrinogen, into an insoluble fibrin to form the clot or thrombosis (**Figure 1**). The forming of fibrin can occur by two different mechanisms. The slower intrinsic pathway is dependent on circulating coagulation factors, whereas the faster extrinsic pathway needs an extravascular factor, tissue factor (TF) which activates factor VII (FVII) (Guyton and Hall 1996). Contact with the traumatised blood vessel wall activates factor XII (FXII) and initiates

the intrinsic pathway. The final step in these intrinsic series of cascading reactions is the activation of factor VIII (FVIII). The active FVII and FVIII activate factor X (FX) which starts the final processes of coagulation before forming fibrin. The two activation pathways have been considered functionally separate but physiologically important. However, FVII deficiency is, unlike FXII deficiency, associated with increased bleeding tendency, indicating that the FVII-dependent TF pathway is the important route in vivo (Rapaport and Rao 1992, Rapaport and Rao 1995). The TF pathway may be especially important in arterial thrombosis. Atherosclerotic plaques have a high content of membrane-bound TF, which becomes exposed to the flowing blood when plaques rupture (Ardissino et al. 1997).
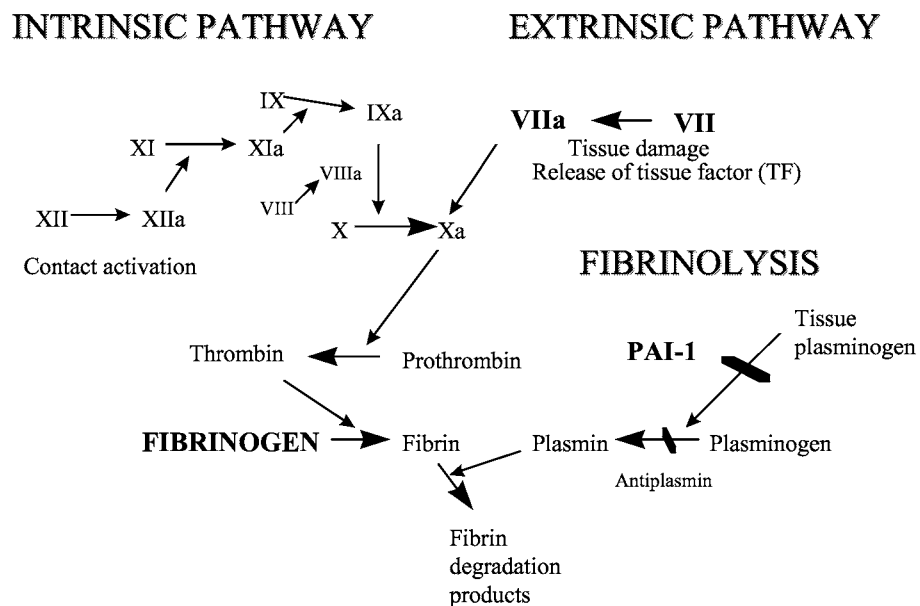
Figure 1 The coagulation and fibrinolytic pathways.

In dynamic equilibrium with the coagulation system is the fibrinolytic system (**Figure 1**) (Juhan-Vague et al. 2000). Plasmin is a proteolytic enzyme that digests fibrin fibers and causes the lysis of the clot (fibrinolysis). Plasmin is formed when plasminogen activators convert plasminogen to plasmin. Plasminogen activator inhibitor 1 (PAI-1) is the major inhibitor of fibrinolysis. PAI-1 is synthetised in an active form, and it is rapidly converted into an inactive (latent) form (van Meijer and Pannekoek 1995). This inactivation is avoided if PAI-1 binds to vitronectin which stabilises the inhibitor in its active conformation. The majority of PAI-1 in blood is active.

2.6.4.2. Fibrinogen and FVII

Fibrinogen is an acute-phase protein, synthesised by the liver. Older prospective and recent cross-sectional epidemiological studies have shown that high fibrinogen levels are associated with excess body weight (Ernst and Resch 1993, Cushman et al. 1996, Woodward et al. 1997).

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However, changes in fibrinogen were not related to changes in BMI over a 6-year follow-up (Folsom et al. 2000). Ageing, smoking and markers of insulin resistance (e.g. low HDL, elevated triglycerides and glucose intolerance) were associated with the increase in fibrinogen (Folsom et al. 2000). In clinical studies, the effect of weight on fibrinogen levels has been studied by reducing weight in obese subjects but no comparison between obese and normal weight subjcets has been made. The reported effects of weight loss on fibrinogen have been inconsistent. Weight loss has decreased fibrinogen concentrations (Primrose et al. 1992, Folsom et al. 1993, Marckmann et al. 1998a) or they have remained unchanged (Svendsen et al. 1996, Hankey et al. 1997). Only two studies have investigated the influence of weight change on fibrinogen after weight loss (Svendsen et al. 1996, Marckmann et al. 1998b). Fibrinogen concentrations were unaffected by weight change (Svendsen et al. 1996) and decreased concentrations were sustained with weight maintenance (Marckmann et al. 1998b).

Factor VII is also produced by the liver. The association of FVII with obesity has been found only in elderly women in cross-sectional studies (Cushman et al. 1996, Woodward et al. 1997). However, weight was a significant long-term determinant of FVIIc, as FVIIc increased with weight gain over a 6-year follow-up (Folsom et al. 2000). Like fibrinogen, adverse changes associated with insulin resistance are accompanied by increases in factor VII coagulant activity (FVIIc). FVIIc increases with elevated serum total and LDL cholesterol, triglycerides, insulin and smoking (Folsom et al. 1991, Cushman et al. 1996, Woodward et al. 1997, Folsom et al. 2000).

FVIIc falls during weight loss, but it is not known whether this beneficial change is maintained as body weight is sustained or regained after weight loss (Primrose et al. 1992, Hankey et al. 1997, Marckmann et al. 1998b).

2.6.4.3. Plasminogen activator-inhibitor-1 (PAI-1)

The PAI-1 gene is expressed in a variety of cells like vascular endothelial cells, vascular smooth muscle cells and platelets (Lupu et al. 1993). However, adipose tissue and the liver are considered to be the major sources of plasma PAI-1 in obese subjects (Björntorp 1990, Eriksson et al. 1998, Loskutoff et al. 1998, Frühbeck et al. 2001).

Visceral adipose tissue produces more PAI-1 than abdominal subcutaneous adipose tissue both in vitro (Alessi et al. 1997) and in vivo (Shimomura et al. 1996, Ferguson et al. 1998, Janand-Delenne et al. 1998, Giltay et al. 1998). However, accumulation of subcutaneous fat may also affect plasma PAI-1 levels, since human abdominal subcutaneous adipose tissue has been shown to express and secrete more PAI-1 in obese and morbidly obese subjects than in lean subjects (Eriksson et al. 1998, Alessi et al. 2000). Higher plasma PAI-1 activity in men than in women has been proposed to be due to greater visceral fat accumulation in men than in women (van Harmelen et al. 2000). Different estrogen status may also cause gender differences in PAI-1 levels. Premenopausal and postmenopausal women receiving oestrogen therapy have lower PAI-1 activity or antigen levels than men of the same age or postmenopausal women without therapy (Otavio et al. 1995, Meilahn et al. 1996, Vehkavaara et al. 2001).

In the short term plasma PAI-1 activity and antigen concentrations decrease with weight loss (Primrose et al. 1992, Folsom et al. 1993, Svendsen et al. 1996, Hankey et al. 1997, Lindahl et

al. 1998, Marckmann et al. 1998a, Kockx et al. 1999, Mavri et al. 1999). There is one study that also reported the dose-response relation of the amount of weight loss and PAI-1 levels (Folsom et al. 1993). Accordingly, at least 5 % weight reduction was needed before PAI-1 antigen concentrations declined in men. In women such a relationship was not noticed.

The long-term effects of weight maintenance on fibrinolysis have seldom been studied. In the study of Svendsen and co-workers (Svendsen et al. 1996), obese women lost an average of 9.6 kg (10 % of initial body weight) in 3 months and were encouraged to avoid weight regain thereafter. After an average body weight regain of 2 kg in 6 months, a significant increase in PAI-1 activity was observed. In the study of Marckmann and co-workers (Marckmann et al. 1998b), obese women lost 12.6 kg in 2 to 4 months. In the maintenance phase lasting for 6 months, patients gaining some weight (average 0.5 kg) and those who lost further weight (average 2.3 kg) maintained the improved fibrinolysis and coagulation. In a study of Mavri and co-workers (1999), obese women reduced their weight on average 14.0 kg in 3 months. After a five-month follow-up the decreased level of PAI-1 remained low in the women who maintained their reduced weight but increased in the women who regained (average 8.0 kg) weight. Thus, improved fibrinolysis by weight loss may be maintained after weight stabilisation and disappears with weight rebound.

2.7. Cardiac autonomic activity in obesity

2.7.1. Measurement of cardiac autonomic activity

Cardiac function is extremely sensitive to autonomic influences, and is therefore a good candidate to evaluate the status of autonomic nervous system. Cardiac parasympathetic activity reflects in general parasympathetic activity (PSA). Variations in heart rate, termed heart rate variability (HRV), has both sympathetic and parasympathetic elements that can be analysed. HRV assesses nerve activity, neurotransmitter release and end-organ responses. Heart rate or blood pressure cannot be used indicators of autonomic activity, since they are determined by unknown combination of sympathetic and parasympathetic inputs (Hirsch et al. 1991).

HRV can be assessed with time- and frequency-domain analysis of R-R intervals. The time-domain measurement is the simplest test. Each QRS complex is detected in a continuous electrocardiographic (ECG) record and the normal-to-normal (NN) intervals (all intervals between adjacent QRS complexes resulting from sinus node depolarisations) are determined (Malik et al. 1996). Time-domain measures are divided into two groups: 1) those derived from direct measurements of the NN intervals, and 2) those derived from the differences between NN intervals. The standard deviation of the NN interval (SDNN) is an overall measure of HRV. Long-term HRV can be estimated by the standard deviation of a mean of RR values from all 5-minute segments (SDANN). The square root of the mean squared differences of successive NN intervals (RMSSD) is an estimate of short-term HRV describing mainly high frequency variations and thus parasympathetic control. Other time-domain measures also exist, but the European and North-American cardiologists recommend the use of SDNN, SDANN and RMSSD (Malik et al. 1996).

Frequency-domain analysis of R-R intervals allows measurement of total power (variance), different frequency bands and the concomitant assessment of cardiac sympathetic and

parasympathetic activity and their balance. The efferent vagal activity is a major contributor of the high frequency component (HF; 0.15-0.40 Hz) (Akselrod et al. 1981, Pomeranz et al. 1985, Malliani et al. 1991). Low frequency component (LF; 0.04-0.15 Hz) is considered as a marker of sympathetic modulation (Rimoldi et al. 1990, Malliani et al. 1991, Kamath and Fallen 1993, Montano et al. 1994) or as a marker of both sympathetic and parasympathetic modulation (Akselrod et al. 1981, Appel et al. 1989). The physiological correlates of very low frequency power (VLF; <0.04 Hz) are not known. In clinical studies, 5-minute recordings processed by frequency-domain methods and nominal 24-h recordings processed by time-domain methods are recommended (Malik et al. 1996). Standardised conditions are necessary because heart rate variability is influenced by factors such as respiratory rate, posture and time of the day. Respiratory sinus arrhythmia increases during the night and decreases in the morning and is augmented in the supine position (Pomeranz et al. 1985, Furlan et al. 1990).

Cardiac vagal tone measurement is also a frequency-domain analysis of R-R intervals. It differs from the above mentioned frequency-domain analysis by evaluating the respiratory sinus arrhythmia (RSA) in one of three frequency bands: 0.12 to 0.40 Hz (approximately 7.5-20 breaths per minute), 0.24 to 1.04 Hz (approximately 15-60 breaths per minute) and 0.30 to 1.30 Hz (approximately 20-80 breaths per minute) (Newlin et al. 1990). The accuracy of R-wave detection and R-R interval timing of the vagal tone monitor is +/- 1 ms (Newlin et al. 1990). RSA occurs naturally in correspondence with variations in respiratory phase and heart rate and provides an index of cardiac parasympathetic tone (Katona et al. 1975, Eckberg 1983, Porges 1986, Berntson et al. 1993). In the 1930s, physiologists discovered that vagal excitation of the sinus node is nearly absent during inspiration which increases the heart rate (Brooks et al. 1979). During expiration the situation is the opposite. This speeding and slowing of heart rate is a primary contribution to the variability in heart rate over time. Animal and human studies have shown that pharmacological blockade of vagal activity can abolish RSA and reduce heart rate variability (Akselrod et al. 1985, McCabe et al. 1985).

Cardiac autonomic activity can also be assessed by different functional tests which are based on the responses of blood pressure and heart rate to different stimuli (Ewing and Clark 1982, Valensi et al. 1993). A commonly used clinical test battery consists of five tests; three mainly parasympathetic tests (heart rate variation during deep breathing, heart rate response to Valsalva manoeuvre and immediate response to standing) and two sympathetic tests (systolic blood pressure response to standing and diastolic blood pressure response to isometric handgrip). Like HRV, functional tests assess nerve activity, neurotransmitter release and end-organ responses.

2.7.2. Cardiac autonomic activity in obesity

Obese subjects have a lower heart rate variability than normal weight subjects, suggesting that cardiac autonomic activity is altered in obesity (Zahorska-Markiewicz et al. 1993, Aronne et al. 1995, Petretta et al. 1995, Richter et al. 1996, Aronne et al. 1997). Which branch of cardiac autonomic control is changed depends e.g. on the subjects studied and the methods used. Decreased cardiac sympathetic and parasympathetic activity in obesity has been reported in frequency- and time-domain analysis of heart rate variability (Piccirillo et al. 1996, Karason et al. 1999, Emdin et al. 2001). Parasympathetic response has decreased and sympathetic response has remained unchanged in studies measuring cardiac autonomic response to several

functional tests (Rossi et al. 1989, Richter et al. 1996, Kansanen et al. 1998, Valensi et al. 1998), whereas neither sympathetic nor parasympathetic cardiac responsiveness was affected by moderate obesity in a study on identical twins (Piha et al. 1994). The passive head-up tilt test with frequency-domain analysis has shown a blunted sympathetic response and increased parasympathetic response in obese subjects (Piccirillo et al.1996). After sequential blockade of cardiac sympathetic and parasympathetic innervation, obese subjects had significantly lower parasympathetic control and higher sympathetic control (Peterson et al. 1988, Aronne et al. 1997). To summarise, when heart rate variability has been measured in obese subjects, cardiac sympathetic and parasympathetic activities have been decreased in most of the studies (Piccirillo et al. 1996, Karason et al. 1999, Emdin et al. 2001). In cardiac functional tests, mainly cardiac parasympathetic response has been found to be reduced and sympathetic response has been unchanged in obese compared to normal weight subjects (Peterson et al. 1988, Rossi et al. 1989, Richter et al. 1996, Kansanen et al. 1998, Valensi et al. 1998). Some studies also suggest that cardiac sympathetic response may be affected by obesity (Piccirillo et al. 1996, Aronne et al. 1997).

2.7.3. Cardiac autonomic activity and weight change

There are only two studies on the effect of weight gain on cardiac autonomic activity. Experimentally induced 10 % weight gain increased cardiac sympathetic control and decreased cardiac parasympathetic control both in obese and lean subjects (Hirsch et al. 1991, Aronne et al. 1995). However, findings concerning the effect of weight loss on cardiac autonomic activity have been inconsistent. Weight loss has increased only cardiac parasympathetic control or had no influence on it (Hirsch et al. 1991, Aronne et al. 1995, Karason et al. 1999, Emdin et al. 2001). Cardiac sympathetic activity has either decreased or increased after weight loss (Aronne et al. 1995, Karason et al. 1999, Emdin et al. 2001). In some studies neither sympathetic nor parasympathetic activity has been influenced by weight loss (Zahorska-Markiewicz et al. 1993, Kansanen et al. 1998). This variability in results may be due to methodological differences and short-term vs long-term recording of heart rate variability. Only few studies have taken gender (Zahorksa-Markiewicz et al. 1993, Petretta et al. 1995) and age into account (Karason et al. 1999, Emdin et al. 2001), although both affect heart rate variability (Stein et al. 1997, Ramaekers et al. 1998).

3. THE AIMS OF THE PRESENT STUDY

This study, carried out in healthy obese women, was undertaken to investigate risk factors characteristic of insulin resistance in relation to obesity, fat distribution and weight change (**I-IV**). The first study (**I**) was a cross-sectional study measuring fat distribution by different methods. The other three studies (**II-IV**) were weight reduction trials in which the subjects received dietary advice and orlistat or placebo. **Figure 2** illustrates the main parameters studied and their interrelationship.

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┌──────────────────┐        ┌──────────────────────┐      ┌──────────────┐
│     OBESITY       │ ←→    │      LEPTIN           │      │   WEIGHT     │
│                  │        │        ↕              │  ←→  │              │
└──────────────────┘        │  CARDIOVASCULAR       │      │   CHANGE     │
          ↕                 │  RISK FACTORS         │      │              │
┌──────────────────┐        │        ↕              │      │              │
│ FAT DISTRIBUTION  │ ←→    │  CARDIAC PARA-        │      │              │
│                  │        │  SYMPATHETIC          │      │              │
└──────────────────┘        │  ACTIVITY             │      └──────────────┘
                            └──────────────────────┘
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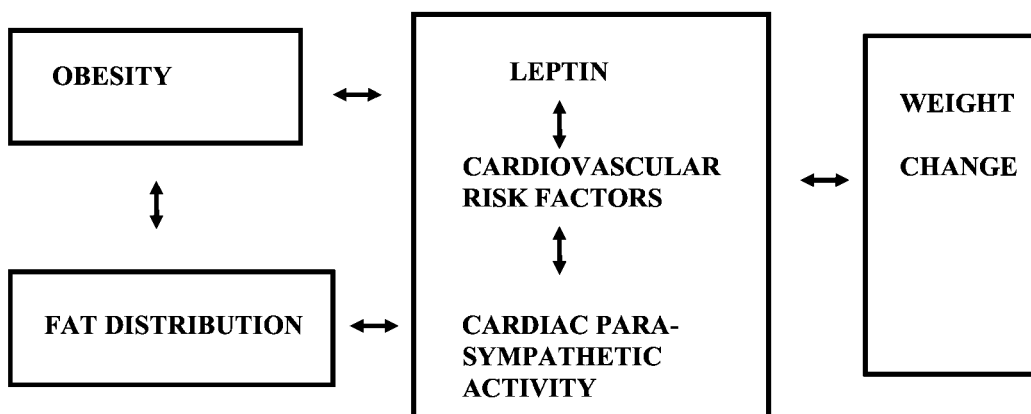
Figure 2 A schematic presentation of the main parameters studied and their interrelationships.

The specific aims of the present work were to examine:

1. Does the method used in the measurement of fat distribution affect the association of fat distribution with cardiovascular risk factors (serum insulin, glucose, HDL and LDL cholesterol, triglycerides) ? (**I**)

2. What is the effect of weight loss on circulating leptin concentrations, cardiovascular risk factors (serum insulin, glucose, HDL and LDL cholesterol, triglycerides, fibrinogen, FVIIc, PAI-1, systolic and diastolic blood pressure) and cardiac parasympathetic activity ? (**II-IV**).

3. What is the effect of weight change after weight loss on the above mentioned cardiovascular risk factors ? (**III**).

4. What is the association of the changes in cardiovascular risk factors with the changes in leptin, blood coagulation and fibrinolytic factors and cardiac parasympathetic activity with weight loss? (**II-IV**).

5. Does fat distribution affect circulating leptin levels and cardiac parasympathetic activity? (**II, IV**)

4. SUBJECTS AND METHODS

4.1. Subjects

The subjects of the present studies were Finnish female participants in three European multicentre double-blind placebo controlled weight reduction trials of orlistat. These studies have been described in detail by Sjöström et al. (1998), van Gaal et al. (1998) and Rössner et al. (2000). The Finnish study centres were the Department of Clinical Nutrition, University of Kuopio and Kuopio University Hospital and the Obesity Research Unit, Helsinki University Central Hospital. All subjects were non-diabetic and healthy as established by physical examination, medical and psychiatric history and routine laboratory tests (**Table 3**). None of the subjects had a history of eating disorders according to a clinical interview and the bulimia-questionnaire BITE (Henderson and Freeman 1987). Written informed consent was obtained from all subjects after a detailed explanation of the study protocol. The studies were approved by the Ethical Committees of the Helsinki University Central Hospital and the Kuopio University Hospital.

Table 3 The characteristics of the obese women in the beginning of studies I-IV.

Variable	Study I	Study II	Study III	Study IV
Number of subjects	43	38	51	52
Age (y)	45.6 (1.4)	44.3 (1.6)	44.0 (0.7)	44.0 (1.0)
BMI (kg/m^2)	34.0 (0.5)	34.0 (0.7)	36.2 (0.5)	34.3 (0.5)
Body fat (%)	40.8 (0.6)[a]	38.6 (0.6)[b]	39.8 (0.7)[b]	38.4 (0.6)[b]
Waist circumference (cm)	102.5 (1.5)	100.9 (1.6)	103.4 (1.4)	102.3 (1.3)
WHR	0.92 (0.01)	0.90 (0.01)	0.89 (0.01)	0.90 (0.01)
SBP (mmHg)	132.7 (2.5)	132.0 (2.3)	135.1 (2.0)	132.7 (1.9)
DBP (mmHg)	84.6 (1.5)	88.7 (1.6)	88.0 (1.0)	88.4 (1.3)
S-TC (mmol/l)	5.7 (0.1)	5.7 (0.2)	5.5 (0.1)	5.6 (0.1)
S-LDL (mmol/l)	3.7 (0.1)	3.7 (0.1)	3.7 (0.1)	3.7 (0.1)
S-HDL (mmol/l)	1.2 (0.0)	1.3 (0.1)	1.3 (0.0)	1.3 (0.0)
S-TG (mmol/l)	1.6 (0.1)	1.7 (0.1)	1.4 (0.1)	1.5 (0.1)
S-Glucose (mmol/l)	5.3 (0.1)	5.3 (0.1)	5.8 (0.1)	5.4 (0.1)
S-Insulin (mU/l)	15.0 (0.9)	15.0 (1.2)	15.0 (0.9)	14.3 (0.8)

Mean (standard error of mean). [a] measured by DXA, [b] measured by bioelectrical impedance.

4.2. Experimental design

4.2.1. General design

In all studies, a 4-week run in-period with a hypoenergetic (-2.4 MJ daily, corrected for daily activities) diet and placebo (single blind study) was followed by a randomisation of the subjects to groups receiving either orlistat or placebo (**Figure 3**). The doses of orlistat were 60 or 120 mg three times a day (Study I and III), 30 mg, 60 mg, 120 mg or 240 mg three times a day (Study II and IV). The diet was designed to cause a weight loss of 0.25 to 0.5 kg/week, with 30 % of energy as fat, 50 % as carbohydrate, 20 % as protein, and a maximum of 300 mg/day cholesterol. Alcohol consumption was limited to be no more than 150 g of alcohol per week. At 6 months the energy intake was reduced by an additional 1.3 MJ/day to compensate for the reduced energy requirements following weight loss (Study III and IV).

Run-in (weeks)   Intervention (months)
1   4   0   3   6   12

Diet

Placebo   Placebo or orlistat
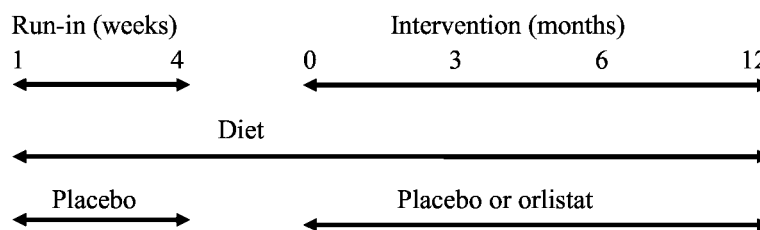
Figure 3 General design of the studies.

Study I

Sixty women from the European orlistat study (Rössner et al. 2000) were recruited to this cross-sectional study at the Department of Clinical Nutrition, University of Kuopio. Seventeen women were excluded for the following reasons: depression (n=1), uncontrolled hypertension (n=1), postsurgical adhesions (n=2), high liver enzymes (n=1), dyspepsia (n=1), hyperthyreosis (n=1), multiple gall stones (n=1), daily abdominal pain (n=1), deterioration of arthritis rheumatoides (n=1), weight loss of more than 4 kg in 3 months prior to screening (n=1), participation in a clinical trial within 30 days prior to the study entry (n=1), and unable to comply with the protocol requirements (n=5). Thus, data on 43 women were available in the statistical analyses.

Study II

Forty-seven women were recruited from the European orlistat study (van Gaal et al. 1998) and six of them were excluded before randomisation at the Obesity Research Unit, Helsinki University Hospital. The reasons for exclusions were hyperthyreosis (n=1), hypothyreosis (n=1), depression (n=1), cholelithiasis (n=1), weight loss more than 4 kg in 3 months prior to screening (n=1) and low serum vitamin E concentration (n=1). Thus, 41 women were randomised to this 6-month study, 38 completed the study and provided data used in the

statistical analyses. Premenopausal and postmenopausal women were analysed as one group, since plasma leptin levels adjusted for body fat percentage did not differ between the groups before weight loss (33.4±2.0 ng/ml vs (27.1±2.3 ng/ml, p=0.156).

Study III

Sixty women from the major European orlistat study (Sjöström et al. 1998) were recruited. Three were excluded before randomisation, due to depression (n=1), uncontrolled hypertension (n=1) and non-compliance with the study protocol (n=1). Two of the 57 women did not provide samples for analyses of FVII, fibrinogen and PAI-1. Therefore, fifty-five women participated in the weight reduction programme at the Department of Clinical Nutrition, University of Kuopio (n=16) and at the Obesity Research Unit, Helsinki University Hospital (n=39). The first study year was completed by 51 women (n=15 in Kuopio, n=36 in Helsinki). Thirty-seven of these were non-smokers and 14 were smokers.

Study IV

The subjects of this study were female participants in two European orlistat studies (Sjöström et al. 1998, van Gaal et al. 1998). The women were recruited from employees of Helsinki University Hospital. Of the 89 recruited women, seven were excluded due to previously unknown diseases including depression (n=2), thyroid disease (n=2) and cholelithiasis (n=2), or due to marked weight loss in three months prior to screening (n=1). None of the 82 women in these trials was receiving β-blockers or any drug known to affect autonomic nervous system activity. Fifty-two women had cardiac vagal tone measurements both before and after a 6-month weight loss and were included in this study. Forty of the women were non-smokers and 12 were smokers.

4.3. Methods

The measurements performed in studies I-IV are listed in **Table 4**.

Table 4 Measurements performed in studies I-IV.

Measurements	Study I	Study II	Study III	Study IV
Obesity				
Weight	x	x	x	x
Body mass index	x	x	x	x
Body composition				
Fat mass	x	x	x	x
Fat free mass	x	x	x	x
Fat distribution				
Waist circumference	x	x	x	x
Hip circumference	x	x	x	x
Waist-to-hip ratio	x	x	x	x
Waist-to-height ratio	x			
Sagittal diameter	x			
Transverse diameter	x			
Total abdominal fat	x			
Visceral fat		x		
Abdominal subcutaneous fat		x		
Metabolic studies				
Oral glucose tolerance test (75 g, 3 h)	x			
Serum glucose	x	x	x	x
Serum insulin	x	x	x	x
Plasma leptin		x		
Serum total cholesterol			x	
Serum low-density lipoprotein	x		x	
Serum high-density lipoprotein	x		x	
Serum triglycerides	x		x	
Haemostatic and haemodynamic studies				
Plasma plasminogen activator inhibitor 1			x	
Plasma factor VII			x	
Plasma fibrinogen			x	
Blood pressure and pulse			x	x
Cardiac parasympathetic activity				
Cardiac vagal tone				x
Menopausal status				
Serum follicle stimulating hormone		x		

4.3.1. Measurements of obesity

Standing height and weight were measured after voiding in light clothing without shoes (I-IV). Weight was measured to the nearest 0.1 kg using an electronic scale (Vogle&Halke Gmb&Co, Hamburg, Germany) and height was measured to the nearest 0.1 cm using a well-mounted stadiometer. Body mass index (BMI) was calculated [BMI= weight (kg)/ height (m^2)].

To study the impact of weight loss, weight maintenance and weight regain on the activities of FVII and PAI-1, the concentration of fibrinogen, serum lipoproteins, insulin and blood

pressure, the subjects were divided into three categories on the basis of their weight change from 6 to 12 months (III):

1. Regainers: subjects who regained more than 1 % of the body weight (n=18). The mean increase was 2.8 kg (range + 0.9 - + 6.8 kg).
2. Maintainers: women whose weight was within $\pm$ 1 % of their 6-month weight at 12 months (n=12). The mean change was + 0.1 kg (range - 0.7 - + 0.6 kg).
3. Weight losers: women whose weight was more than 1% smaller at 12 months than at 6 months (n=21). The mean decrease was 3.7 kg (range - 16.2 - - 1.2 kg).

4.3.2. Body composition

Body composition was determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System, RJL Systems, Detroit, MI) in studies II-IV and DXA in the study I. BIA measurements were performed as described by Lukaski et al. (1985). To calculate fat free mass (kg) for females the following equation provided by the manufacturer was used:
$5.091 + 0.6483$ x $height^2$/resistance + 0.1699 x weight. Fat mass was calculated as the difference between body weight and fat free mass.

4.3.3. Measurements of fat distribution

In studies I-IV, waist circumference was measured in standing position at the level midway between the lateral lower rib margin and iliac crest and hip circumference was measured at the levels of the trochanters, through the pubic symphysis (WHO 1988). The supine transverse diameter and the supine sagittal diameter were measured at the same level as waist circumference by a slide gauge on a hard couch in study I (Sjöström 1991). An average of two measurements was used for analysis in all above mentioned measurements.

DXA measurements were made by a densitometer (Lunar DPX, Lunar Radiation Corp, Madison, WI) using a medium speed total body scanning acquisition mode (Mazess et al. 1990, Haarbo et al. 1991) in study I. The software version 3.6z was employed for subsequent analyses. Each patient was positioned with the arms sufficiently separated from the trunk. The abdominal region of interest (ROI) was defined manually by adjusting the lines of rib box in four different ways (**Figure 4**). 'Abdominal fat' was measured from the dome of the diaphragm to the top of the femur. 'Upper lumbar fat' was measured between the first and the fourth lumbar vertebrae. 'Lower lumbar fat' was measured between the fourth and the fifth lumbar vertebrae. 'Hip fat' was gauged downwards from the trochanter major so that the height of the area was as high as the the height of 'lower lumbar fat' area.
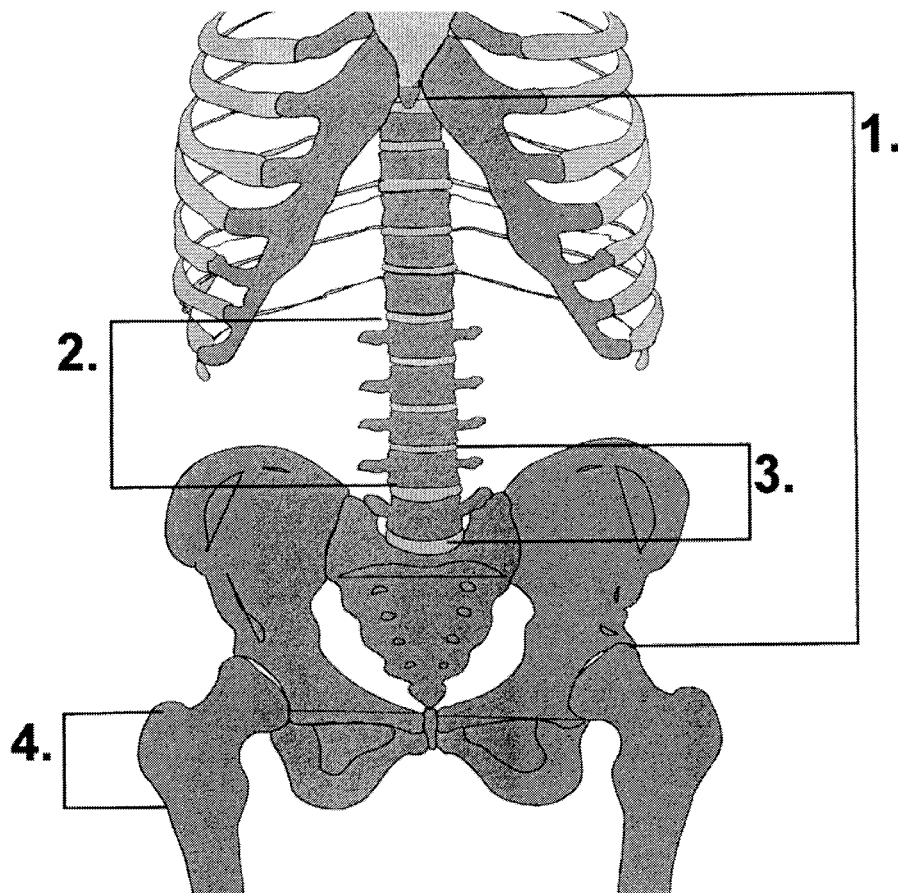
Figure 4 Schematic representation of the DXA measurement areas.
1. "Abdominal fat"; dome of diaphragm - top of femur.
2. "Upper lumbar fat"; 1st - 4th lumbar vertebrae.
3. "Lower lumbar fat"; 4th - 5th lumbar vertebrae.
4. "Hip fat"; downwards throchanter major, height as in lower lumbar fat.

The ultrasound measurements were performed using a Toshiba Sonolayer 100 A scanner with a 3.5 Mhz convex transducer for determining visceral fat and a 7.5 Mhz transducer for determining subcutaneous fat (II). Compression was carefully avoided. The error in identification of structures and measurements of the depth was diminished by having the copies checked afterwards by a trained operator. The measurements were performed at the level midpoint between the umbilicus and xiphoid process. This level reflects both total intraperitoneal and total abdominal subcutaneous fat well (Abate et al. 1997). The measurements were carried out by the method developed by Armellini and co-workers (Armellini et al. 1990). The distance between the linea alba and the dorsal border of the abdominal aorta was an estimate of visceral fat (**Figure 5**). The thickness of the subcutis in the same sagittal line was a measure of abdominal subcutaneous fat. Our previous data showed a significant correlation (r = 0.77) between the ultrasonography measured visceral fat and CT measured visceral fat (unpublished data).
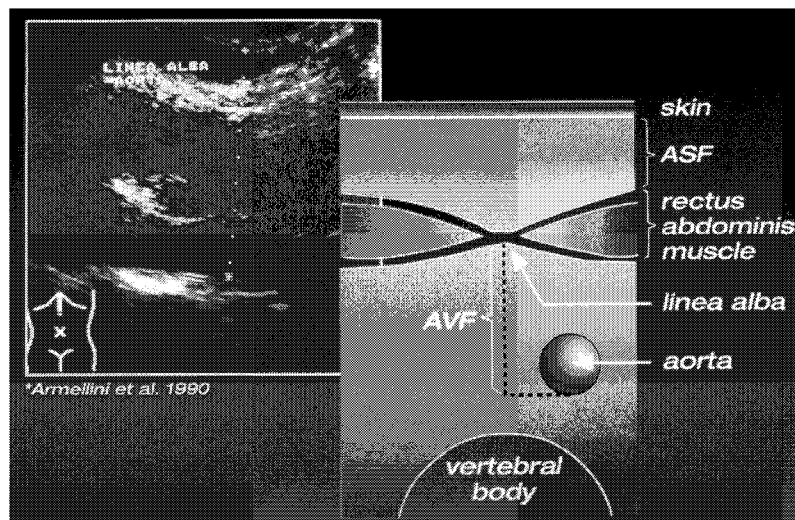
Figure 5 Schematic representation of the measurements of abdominal subcutaneous fat and visceral fat by ultrasonography. ASF, abdominal subcutaneous fat, AVF, abdominal visceral fat.

4.3.4. Collection of blood samples

In all studies (I-IV) venous blood samples were drawn between 7:30 and 10:00 a.m. after a 12-h fast and a 15-min rest in sitting position in studies I-IV. The subjects were advised not to smoke before drawing the blood sample and to avoid strenuous physical activity the day before blood sampling. Also alcohol ingestion was to be avoided for 48 hours before drawing the blood sample. In the case of acute infection, blood samples were taken after the symptoms had disappeared and the patient was well. All these general recommendations were considered before the measurements of fat distribution, body composition and the activity of cardiac vagal tone.

In study III, venous blood samples were drawn without stasis. The first three millilitres of blood were not used. Specimens were collected into two 4.5-ml vacuum Becton Dickson siliconated tubes containing 0.5 ml 0.129 M sodium citrate using a 0.9 x 40 mm needles (type H1004 VMS). Tubes were inverted gently 7 times. Specimens were centrifuged at 1400 x g for 30 minutes at room temperature within an hour. Plasma of the two tubes was combined into a G 12.4 x 85 mm propylene Mekalasi tube (Milian AIL-137 stopper) and inverted again 7 times. After that plasma was divided into 7 Eppendorf tubes (0.5 ml each) and immediately frozen in dry ice. The tubes were stored at - 70°C until shipped in batches to the central laboratory in Helsinki.

4.3.5. Measurements of serum glucose, insulin, lipids and plasma leptin

A three-hour oral glucose tolerance test (OGTT) was performed with 75 grams of glucose dissolved in 300 ml water (I). Blood samples for plasma glucose and insulin determinations were taken before and 30, 60, 90,120 and 180 min after the ingestion of the solution.

In studies I-IV, serum glucose was determined by a glucose dehydrogenase method (Barham et al 1972) and serum insulin by a radioimmunoassay with a double antibody-PEG technique CIS (CIS biointernational, B.P. 32, F-91192, Git-sur-Yvette Codex, France).

Serum and lipoprotein lipids were analysed after ultrasentrifugation and precipitation by enzymatic methods (Wahlefeld 1974, Siedel et al 1983). CHOD-PAP method was applied for cholesterol and HDL cholesterol and GPO-PAP method for triglycerides.

In study II, plasma leptin was measured by a human leptin radioimmunoassay (Human Leptin RIA kit, Linco Research Inc., St. Charles, MO) (Ma et al. 1996).

4.3.6. Haemostatic and haemodynamic studies

Haemostatic studies

Blood samples were analysed at the Department of Haemostasis at the Finnish Red Cross Blood Transfusion Service.

Fibrinogen was measured with ACL coagulometer (Instrumentation Laboratory, Milan, Italy) with the derived method (Rossi et al. 1988) (IL Test™ PT-Fibrinogen, Instrumentation Laboratory). A single lot of calibration plasma was used as a standard. The interassay precision was 1.9 %.

FVIIc was measured with the one-stage method using rabbit brain thromboplastin (Thromboplastin IS, Baxter Diagnostics Inc., Deerfield, USA) and human immunodepleted FVII deficient plasma (Behring, Marburg, Germany). The assays were carried out with ACL coagulometer. A frozen plasma pool of 44 blood donors was taken as 100 %. The interassay precision was 2.3 %.

PAI-1 was measured with a chromogenic method (Coatest® PAI, Chromogenix AB, Molndal, Sweden) according to the manufacture. The interassay precision was 5.5 %.
The reference values of fibrinogen and FVIIc were obtained from a plasma pool of 100 blood donors (50 female: 50 male). The reference values of PAI-1 were determined from blood samples of 56 donors. The laboratory reference values were 1.8-4.0 g/l for fibrinogen, 63-135 % for FVIIc and <23.0 AU/ml for PAI-1.

Haemodynamic studies

Casual blood pressure was measured by a mercury sphygmomanometer after a five-minute rest from the right arm by a trained observer (III, IV). Korotkoff phase I and V sounds were used for SBP and DBP measurements. The average of two measurements was used for the analysis.

4.3.7. Cardiac parasympathetic activity

In study IV cardiac vagal tone, an index of parasympathetic activity, was recorded in supine position in a quiet room in the room temperature using the vagal tone monitor (Delta-Biometrics, Inc. Bethesda, Maryland 1989). The monitor detects the R waves from the electrocardiogram (standard lead II), times the sequential R-R intervals (i.e. heart periods) to the nearest millisecond and computes a measure of cardiac vagal tone by quantifying the amplitude of respiratory sinus arrhythmia from the heart rate pattern. The vagal tone index was estimated from the time series of sequential heart periods (Newlin et al. 1990). The method consisted of the following steps: (1) the heart periods were converted into time-based data by sampling successive 500 msec intervals, (2) the time-based data were detrended with a 21-point moving polynomial to remove the influence of nonstationarities and slow periodicities on the amplitude of respiratory sinus arrhythmia, (3) the detrended data were processed by a band-pass filter to remove sources of variance outside the frequency band characteristic of spontaneous breathing for adults (0.12-0.40 Hz or approximately 7.5 to 22 breathes per min), and (4) the natural logarithm of the band-passed variance was calculated and used as the measure of vagal tone index. To increase the stability of the vagal tone index, it was monitored for sequential 30-second epochs and the mean of these epochs for 5 minutes was calculated. In case of arrhytmic events, monitoring was continued until ten 30-second intact epochs were collected. Arrhytmic events were discarded from the data. Cardiac vagal tone was adjusted for age, since it was found to be related to age in study IV (correlation coefficient - 0.437, p = 0.001).

4.3.8. Menopausal status

In study II serum follicle stimulating hormone (FSH) levels of the women who were aged $\geq$ 45 before weight loss (n=21) were determined at baseline and at the end of the study using an immunofluorometric method (AutoDEFIA hFSH kit, Wallac Oy, Turku, Finland). Younger women with regular menses were considered to be premenopausal (n=17). Using the cut off point of 30 IU/l, nine women were considered to be postmenopausal, eight were premenopausal, and four were perimenopausal (FSH-values <30 IU/l at the beginning and $\geq$30 IU/l at the end). Thus altogether 25 women were premenopausal and nine women postmenopausal.

4.3.9. Statistical analyses

Statistical analyses were performed with SPSS/PC+ and SPSS for Windows (SPSS, Chicago, IL, USA) statistic programs in studies I-II and III-IV, respectively. The Kolmagorov-Smirnov test was used to check the normal distribution of the variables. Due to skewed distribution, a logarithmic transformation was performed on plasma fibrinogen, insulin and PAI-1 values.

The effects of orlistat or smoking on variables were analysed by a simple factorial ANOVA (II, IV) or by a general linear model for repeated measures with a simple contrast (III). Orlistat treatment did not have any independent effect on the changes in leptin, cardiovascular risk factors and cardiac parasympathetic activity (all p > 0.05).

Differences in plasma leptin concentrations between the groups of menopausal status were analysed by a one-way analysis of variance (II). Plasma leptin concentrations adjusted for body fat percentage did not differ significantly between the obese premenopausal and postmenopausal women ($p > 0.05$).

Changes within the groups were analysed by a paired samples t-test (II, IV), by a general linear model for repeated measures with the simple contrast (III) or by a Wilcoxon-test for 2 related groups using the exact Monte Carlo-test (III). Bonferroni correction was used for multiple comparison (III).

The interaction between the variables was assessed by stepwise, linear regression (III, IV). Bivariate or partial Pearson correlation coefficients were calculated to analyse the association between the variables (I-IV). Correlation coefficients were compared by a test based on z-transformed correlation coefficients (I) (Zar 1984).

The results are expressed as mean $\pm$ standard error of mean (SEM) (III-IV) and a value $p < 0.05$ was used a criterion for statistical significance. The 2-tailed significance was used.

5. RESULTS

5.1. The association of fat distribution examined by different methods with cardiovascular risk factors (I)

Waist circumference, WHR, waist-to-height-ratio (WHTR), sagittal and transverse diameter and sagittal-to-transverse-ratio (STR) correlated inversely with HDL cholesterol, r = from - 0.31 (p < 0.05) to - 0.53 (p < 0.01) after adjustment for age and BMI (**Table 5**). Waist circumference, sagittal and transverse diameters related to LDL cholesterol (r = 0.34 - 0.36, all p < 0.05). WHR and WHTR correlated with triglycerides, r = 0.38 and r = 0.40, respectively (all p < 0.05). In addition, all the measurements except transverse diameter associated with fasting insulin, r = from 0.32 (p < 0.05) to 0.50 (p < 0.01) and fasting glucose, r = 0.39 (p < 0.05) to r = 0.52 (p < 0.01). WHR was the only measurement that also correlated with the two-hour glucose concentration (r = 0.34, p < 0.05). The correlation coefficients given in **Table 5** did not differ from each other in the z-transformed correlation coefficient test (p = NS).

Table 5 Partial Pearson correlation coefficients between the cardiovascular risk factors and anthropometric measurements adjusted for age and BMI in study I (n=43).

Variable	Waist	WHR	WHTR	Sagitt.dm.	Trans.dm.	STR
S-HDL cholesterol	-0.46 **	-0.53 **	-0.39 *	-0.48 **	-0.31 *	-0.36 *
S-LDL cholesterol	0.36 *	0.28	0.22	0.34 *	0.35 *	0.16
S-Triglycerides	0.20	0.40 *	0.38 *	0.00	0.10	-0.06
S-Insulin	0.36 *	0.39 *	0.32	0.42 **	0.03	0.50 **
S-Glucose	0.41 **	0.49 **	0.52 **	0.39 *	0.04	0.45 **
2h-Glucose	0.14	0.34 *	0.29	0.11	-0.00	0.13

2h-Glucose, 2 h glucose concentration during oral glucose tolerance test; waist, waist circumference; WHR, waist-to-hip-ratio; WHTR, waist-to-height-ratio; sagitt. dm., sagittal diameter; trans. dm., transverse diameter; STR, sagittal-to-transverse-ratio; * p < 0.05; ** p < 0.01; *** p < 0.001.

DXA measurements correlated only with some cardiovascular risk factors after adjustment for age and BMI (**Table 6**). Accordingly, 'hip fat' and the 'lower lumbar fat-to-hip fat ratio' correlated negatively with HDL cholesterol (r = - 0.39, p < 0.05 and r=-0.52, p < 0.01, respectively) and 'hip fat' with triglycerides (r = - 0.42, p < 0.01). 'Abdominal fat', 'upper lumbar fat' and the 'lower lumbar fat-to-hip fat-ratio' correlated with fasting serum insulin, r = from 0.32 (p < 0.05) to 0.47 (p < 0.01). The latter two regions also correlated with fasting glucose (r = from 0.32 to 0.37, all p < 0.05).

Table 6 Partial Pearson correlation coefficients between the cardiovascular risk factors and DXA measurements adjusted for age and BMI in study I (n=43).

Variable	Abdominal fat	Upper lumbar fat	Lower lumbar fat	Hip fat	Lower lumbar fat:hip fat
S-HDL cholesterol	0.11	-0.29	-0.10	-0.39 *	-0.52 **
S-LDL cholesterol	0.13	0.28	0.10	0.09	0.16
S-Triglycerides	-0.04	-0.04	-0.09	-0.42 **	0.30
S-Insulin	0.32 *	0.47 **	0.22	-0.18	0.45 **
S-Glucose	0.18	0.37 *	0.02	-0.30	0.32 *

* p < 0.05; ** p < 0.01

5.2. The effect of weight loss on plasma leptin, cardiovascular risk factors and cardiac parasympathetic activity (II-IV)

Most of the weight loss, occurred at three months (**Table 7**). Thereafter, some additional weight was lost, amounting a total loss of 8.2 to 9.5 kg (9.0 - 9.5 %) at six months. From six to twelve months the average weight loss was 0.5 kg.

The mean plasma leptin concentrations decreased by 22 % during six months (p < 0.001) (**Table 7**). The concentrations of leptin expressed per kg of body fat did not change significantly with weight loss.

Table 7 Body weight, body fat and plasma leptin at the entry and changes during the studies (II, n=38; III, n=51; IV, n=52).

Variables	Study	Entry	Change at 3 months	Change at 6 months	Change at 12 months
Weight (kg)	II	93.1 (2.1)	ND	-8.2 (0.7) ***	ND
	III	96.5 (1.7)	-7.6 (0.5)***	-9.5 (0.7) ***	-10.0 (1.1) ***
	IV	93.4 (1.7)	ND	-8.7 (0.6) ***	ND
Fat mass (kg)	II	35.9 (1.2)	ND	-5.6 (0.6) ***	ND
	III	38.6 (1.1)	-5.2 (0.5) ***	-5.9 (0.7) ***	-6.9 (1.0) ***
	IV	36.1 (1.0)	ND	-5.4 (0.7) ***	ND
Leptin (ng/ml)	II	32.5 (2.1)	ND	-6.8 (1.6) ***	ND
Leptin/fat mass	II	0.89	ND	-0.09	ND
($ng \times ml^{-1} \times kg^{-1}$)		(0.04)		(0.04)	

Values are means (standard error of mean). ND, not done. Difference from the value at the entry. *** p < 0.001.

The mean serum insulin concentrations decreased with weight loss (p < 0.001), but the mean serum glucose concentrations remained unchanged (**Table 8**). Total and LDL cholesterol

concentrations decreased during the first three months ($p < 0.001$). LDL cholesterol decreased more with greater weight loss (**Figure 6**). However, despite a continuous decline of the mean weight during three to twelve months, total and LDL cholesterol showed no further decline, but instead rebounded (**Table 8**). No significant change was found in serum HDL cholesterol and triglycerides at any time point (**Table 8**).

Diastolic and systolic blood pressure also fell during the first three months ($p < 0.001$) (**Table 8, Figure 6**). Thereafter, blood pressure did not fall further, but showed a tendency to increase after six months (**Table 8**). Especially, the mean systolic blood pressure increased significantly from six to twelve months ($p < 0.05$).

Table 8 Cardiovascular risk factors at the entry and changes during the study III (n=51).

Variable	Entry	Change at 3 months	Change at 6 months	Change at 12 months
S-Insulin (mU/l)	15.0 (0.9)	ND	-3.6 (0.9)***	-3.4 (0.8)***
S-Glucose (mmol/l)	5.8 (0.1)	-0.2 (0.1)	-0.3(0.1)	-0.3 (0.1)
S-Total cholesterol (mmol/l)	5.5 (0.1)	-0.4 (0.1)***	-0.2 (0.1)	-0.1 (0.1)
S-LDL cholesterol (mmol/l)	3.7 (0.1)	-0.4 (0.1)***	-0.3 (0.1)	-0.2 (0.1)
S-HDL cholesterol (mmol/l)	1.3 (0.0)	-0.1 (0.0)	-0.0 (0.0)	-0.0 (0.0)
S-Triglycerides (mmol/l)	1.4 (0.1)	-0.1 (0.1)	-0.0 (0.1)	-0.1 (0.1)
Diastolic blood pressure (mmHg)	88.0 (1.0)	-7.7 (1.1) ***	-8.1 (1.3) ***	-7.1 (1.3) ***
Systolic blood pressure (mmHg)	135.1 (2.0)	-10.5 (1.7) ***	-10.0 (1.9) ***	-7.3 (1.9) ***

Values are means (standard error of mean). ND, not done. Difference from the value at the entry. ***, $p<0.001$,

The average PAI-1 activity declined throughout the study ($p < 0.001$) (**Table 9**). The mean FVIIc declined insignificantly during the first three months and reached the baseline activity at twelve months (**Table 9**). From the baseline to three months women with weight loss less than 5 % had an increase in both activities, while a greater weight loss decreased the activities, and statistical significance was reached by at least 10 % weight reduction in both factors (**Figure 6**). The concentration of fibrinogen remained unchanged throughout the study (**Table 9**).

Weight reduction was accompanied by an increase in cardiac parasympathetic activity ($p < 0.05$) (**Table 9**).

Figure 6 Changes in activities of plasma PAI-1 and FVII, serum LDL cholesterol, systolic and diastolic blood pressure in relation to percentage weight loss from the baseline to month 3. Values are mean (standard error of mean). *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Table 9 Plasma activities of PAI-1 and FVII and concentration of fibrinogen at the baseline (study III; n=51) and cardiac vagal tone at the entry (Study IV; n=52) and their changes during the studies.

Variable	Baseline	Change at 3 months	Change at 6 months	Change at 12 months
PAI-1 (AU/ml)	23.9 (2.7)	-6.2 (2.5)	-6.1 (2.2) *	-9.0 (2.0) ***
Factor VIIc (%)	113.0 (2)	-2.5 (1.5)	0.3 (1.5)	2.3 (2.1)
Firbrinogen (g/l)	4.2 (0.2)	0.1 (0.1)	0.0 (0.1)	0.1 (0.1)
Vagal tone (log units)	5.0 (0.2)	ND	0.3 (0.1) *	ND

Values are means (standard error of mean). ND, not done. Difference from the value at the baseline and at the entry. *, $p < 0.05$; ***, $p < 0.001$.

5.3. The effect of weight change after weight loss on cardiovascular risk factors (III)

Between months six and twelve, 24 % of women had stable weight (within 1%), 35 % regained weight, and 41 % continued to lose weight.

Serum insulin concentrations increased in weight regainers ($p < 0.001$), whereas weight maintainers and losers sustained their serum insulin concentrations below the six-month levels (p = NS) (**Figure 7**). Serum LDL cholesterol concentrations rose in all women (mean±SEM), 0.2 mmol/l (0.2) in weight regainers, 0.1 mmol/l (0.5) in weight maintainers and 0.2 mmol/l (0.1) in weight losers (p = NS) (data only in this text).

The activities of PAI-1 and FVII remained below the six month values if the weight loss was sustained or continued, but only the activity of PAI-1 in weight losers was significantly lower than the six month value ($p < 0.001$) (**Figure 7**). In the regainers group the activities of PAI-1 and FVII raised insignificantly by an average 14.1 % and 7.7 %, respectively.

Both systolic (**Figure 7**) and diastolic blood pressure (data only in this text) increased in all weight change subgroups (mean±SEM). Diastolic blood pressure rose 0.5 mmHg (1.3) in weight regainers, 2.5 mmHg (1.8) in weight maintainers and 0.4 mmHg (1.5) in weight losers (p = NS) (data only in this text).
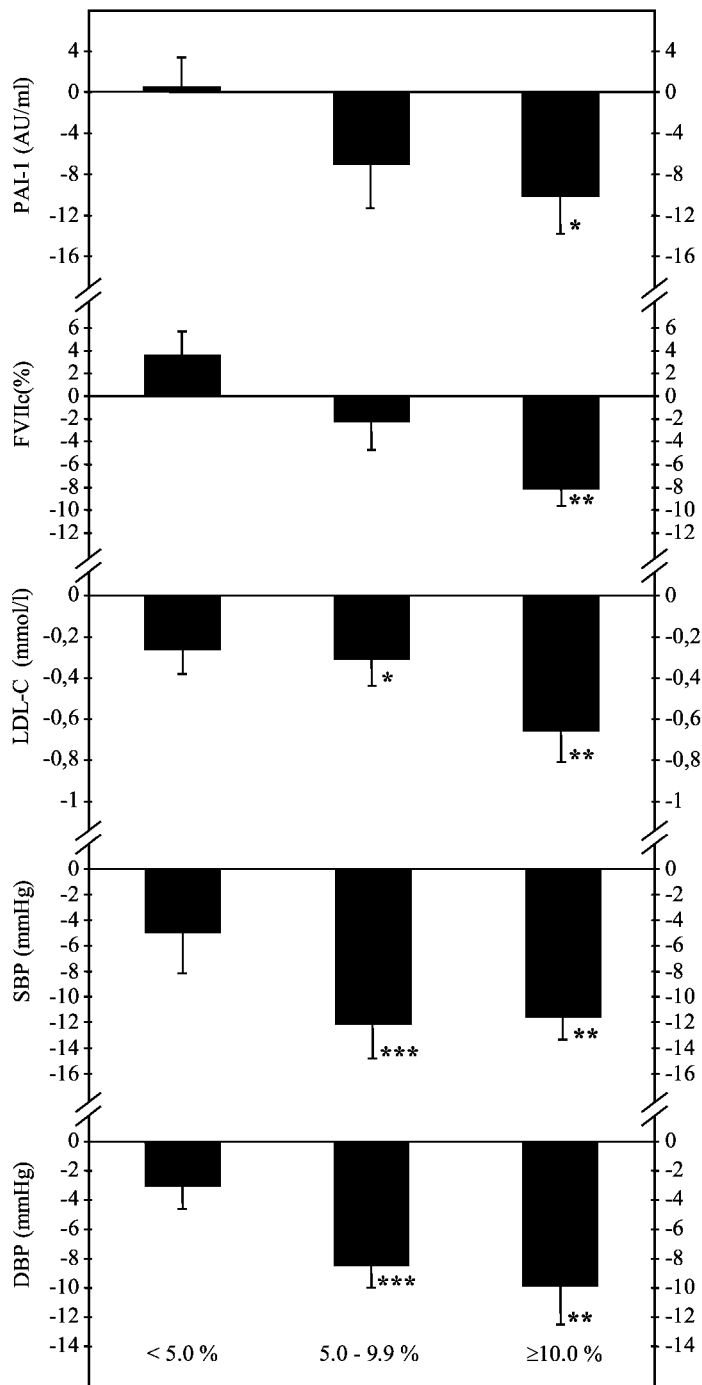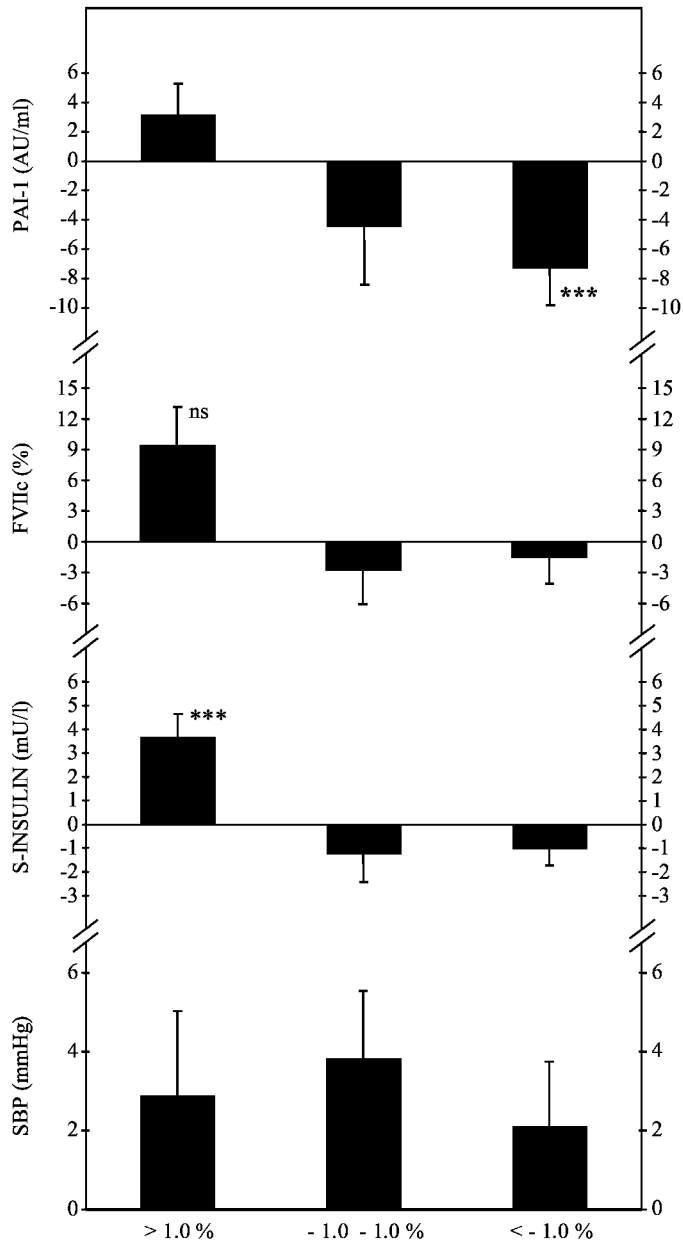
Figure 7 Changes in activities of plasma PAI-1 and FVII, serum insulin and systolic blood pressure in relation to percentage weight change from month six to month twelve. Values are mean (standard error of mean). ***, $p < 0.001$.

5.4. The association of cardiovascular risk factors with leptin, blood coagulation and fibrinolytic factors and cardiac parasympathetic activity (II-IV)

The associations between the changes in plasma leptin (Study II), activities of PAI-1 and FVII (Study III) and cardiac parasympathetic activity (Study IV) with the changes in cardiovascular risk factors from the beginning to the end of the studies were also studied. The correlations were controlled for total body fat mass (kg) and also for age in the change of cardiac PSA. Most of the associations were weak (data only in this text). Significant correlations were detected between the changes in serum insulin concentrations and the changes in plasma leptin (r = 0.40, p < 0.01), activities of PAI-1 (r = 0.34, p < 0.05 and r = 0.32, p < 0.05, respectively, at six and twelve months) and FVII (r = 0.40, p < 0.01 at six months). The change in serum insulin was not correlated with the change in cardiac parasympathetic activity (r= -0.28, p = NS). The change in serum glucose was associated with changes in the activity of PAI-1 (r = 0.44, p < 0.01) and cardiac parasympathetic activity (r = - 0.30, p < 0.05). The change in triglycerides was associated with the change in FVII (r = 0.40, p < 0.01 and r = 0.41, p < 0.01, respectively, at six and twelve months).

5.5. The association of fat distribution with plasma leptin and cardiac parasympathetic activity (II, IV)

Hip circumference correlated with plasma leptin levels before weight reduction (r = 0.57, p < 0.001), while waist circumference, visceral fat and abdominal subcutaneous fat did not (all p = NS) (**Figure 8**). All the above correlation coefficients were adjusted for fat percentage. Hip circumference and waist circumference reduced with weight loss on an average by 5.8 % and 6.9 %, respectively (all p < 0.001). The amounts of abdominal subcutaneous fat and visceral fat declined by 17.4% and 18.7 %, respectively (all p < 0.001). After weight loss hip and waist circumference correlated with plasma leptin, r = 0.41 (p < 0.01), r = 0.57 (p < 0.001), respectively (**Figure 8**). Also both visceral fat and abdominal subcutaneous fat correlated with plasma leptin: r = 0.39 (p < 0.05), r = 0.43 (p < 0.01), respectively (**Figure 8**). Waist-to-hip-ratio was not related to plasma leptin levels either before or after weight loss (p=NS).
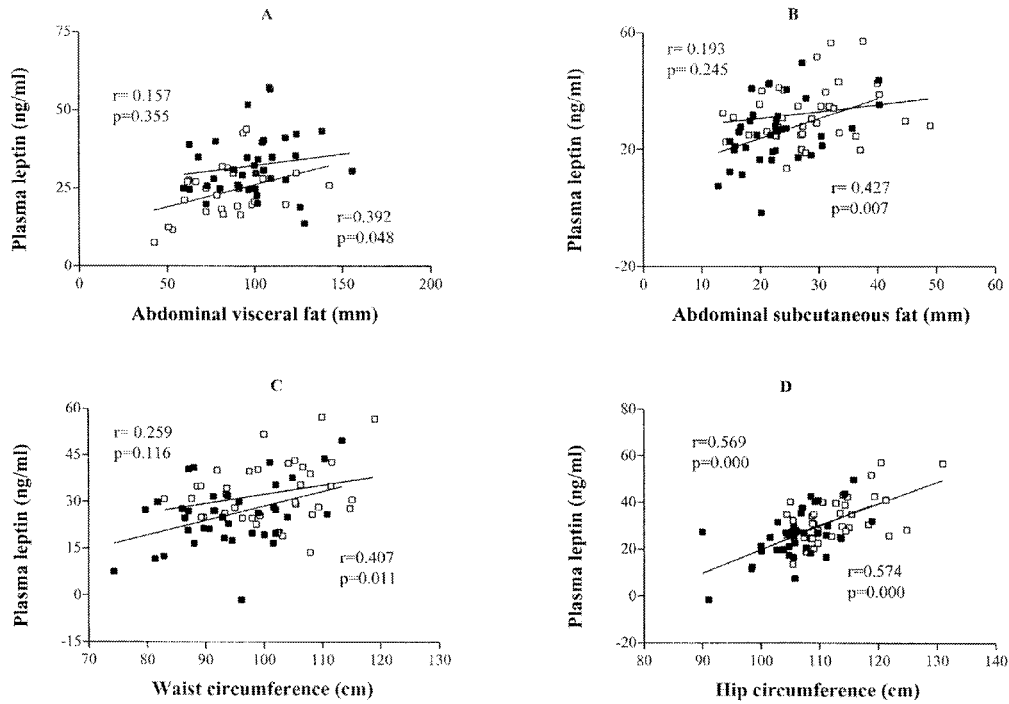
Figure 8 The correlation of plasma leptin with visceral fat (panel A), with abdominal subcutaneous fat (panel B), with waist circumference (panel C) and with hip circumference (panel D) adjusted for body fat percentage. The upper r- and p-values describe the situation before weight reduction (■) and the lower values after the weight reduction (▢).

Changes in hip and waist circumferences correlated with the changes in leptin concentrations after controlling for initial fat percentage, r = 0.38 (p < 0.05), r = 0.45 (p < 0.01), respectively. Similarly, changes in abdominal subcutaneous fat correlated with plasma leptin (r = 0.37, p < 0.05). Changes in plasma leptin concentrations had a stronger correlation with the changes in the measures of total body fat than fat distribution; BMI (r = 0.65), body weight (r = 0.67) and body fat (r = 0.59) after adjusting for initial body fat percentage (all p < 0.001).

Age- and fat mass-controlled cardiac vagal tone did not correlate with waist (r= 0.09, r=0.04) or hip circumference (r=-0.16, r=-0.20) before or after weight loss, respectively (all p = NS). Neither was the change in cardiac vagal tone correlated with the changes in waist (r=-0.28) or hip circumference (r=0.04) (all p = NS).

6. DISCUSSION

6.1. Methodological aspects

6.1.1. Subjects and study design

Three European multicentre weight reduction trials of orlistat offered a large enough number of obese Finnish women to study both clinically and statistically significant changes in different risk factors with weight loss (Sjöström et al. 1998, van Gaal et al. 1998, Rössner et al. 2000). Omitting men may have weakened the association of abdominal fat with cardiovascular risk factors in study I, since in general, the correlation coefficients for body fatness and cardiovascular risk factors are higher in men than in women (Smith et al. 2001). As the average age of women in the present study was 44 years, almost all of them were premenopausal. Natural estrogen might have provided some protection against the mechanisms that link body fatness and cardiovascular risk factors. In addition, due to exclusion criteria applied in orlistat studies (Sjöström et al. 1998, van Gaal et al. 1998, Rössner et al. 2000), female subjects were rather healthy, which might have decreased the response of cardiovascular risk factors to weight loss. Thus, our results can be generalised to middle-aged, healthy, obese women.

Compliance to study protocol was good, since only three and four subjects from studies II and III, respectively, were excluded from the analysis due to missing some measurements during the study visits. In study IV the subjects of two trials of orlistat were combined and women with vagal tone measurements both before and after a 6-month weight loss were included. Combining the subjects of two studies had no effect on the results, because the study designs of the trials were similar except for the doses of orlistat. However, orlistat did not have any independent effect on cardiac PSA.

The first blood samples of FVII, PAI-1 and fibrinogen were drawn one month after the beginning of weight loss. The average weight loss of 3.3 kg may have improved fibrinolysis and coagulation already at the first examination and lead to an underestimation of the change in FVII, PAI-1 and fibrinogen at 3 months. However, it is likely to be of minor significance in those women who lost less than 5 % of initial body weight at 3 months because they lost weight only 1.8 kg during the first study month (data not shown).

6.1.2. Measurement of fat mass

Total body fat mass was determined by BIA in studies II-IV. Impedance measurements both in Helsinki and Kuopio were converted into an estimation of fat mass by using a prediction equation provided by the manufacture. Since the absolute accuracy of impedance depends on the prediction equation employed (Elia 1992), the validity of our measurement would have been increased if the most appropriate prediction equation for these obese women could have been used.

We used ultrasonography to measure abdominal subcutaneous and intra-abdominal fat. Ultrasound measured intra-abdominal fat includes, in addition to omental and mesenteric fat, intestinal loops and blood vessels and also the identification of structures may be difficult in obese subjects (Armellini 1991, Tornaghi et al. 1994). Therefore, the validity of the

measurement is questioned. According to our unpublished data, the correlation coefficient between the ultrasound and CT measured intra-abdominal fat was 0.77, showing moderate validity for the ultrasound measured visceral fat. Thus, the method used was more valid than anthropometric measurements, but less valid than imaging methods like CT and MRI.

6.1.3. Cardiac PSA

The evaluation of PSA using respiratory sinus arrhythmia has some caveats. Respiratory sinus arrhythmia fails to capture the tonic component of PSA and there is great interindividual variability in the coupling of respiratory sinus arrhythmia to total PSA with advancing age (Pfeifer et al. 1983, Porges 1986). In addition, respiratory sinus arrhythmia is modified by respiratory kinetics and tidal volume (Eckberg 1983), which may change by weight loss due to the decrease of abdominal fat. The confounding effect of age was controlled by adjusting our results for age.

6.1.4. Statistical analysis

The independent association of total abdominal fat, abdominal fat distribution and peripheral fat with cardiovascular risk factors (I), leptin (II) and cardiac PSA (IV) was investigated. The associations were controlled for BMI (I), fat percentage (II) and fat mass (kg) (IV). In general, the problem with this adjustment procedure might be that the measures of abdominal fat were present in the measures of total body fat. This makes the inferences drawn from the statistical analysis less robust and subject to criticism. As BMI is a very crude measure of obesity and contains both fat and lean mass, it is possible that our results were underadjusted for total body fat. In study II the measures of abdominal fat distribution could be treated as independent measures, since both were measures of distances and expressed in millimeters.

6.2. The effect of weight loss on plasma leptin, cardiovascular risk factors and cardiac parasympathetic activity (II-IV)

Leptin levels remained proportional to the amount of total body fat also after weight loss which confirmed the observation that the decrease of leptin is proportional to the decline of actual amount of adipose tissue when energy intake deficit is modest (Nicklas et al. 1997). In the short term, a strict energy restriction can have a stronger effect on leptin concentration than on body weight. A six-week low-energy diet of 4.2 MJ/day reduced serum leptin concentration by 55 % which was 10 times more than the body weight reduction (Wadden et al. 1998). In the future it might be interesting to know whether the change of leptin in relation to the amount of fat stores and energy intake influence the maintenance of weight loss result. It could be hypothesised that obese subjects that show the most marked reductions in leptin levels during the strict energy intake would be at a greater risk for weight regain. Strongly decreased leptin levels could stimulate the excretion of hypothalamic neuropeptides and consequently increase sensation of hunger and food intake (Stephens et al. 1995, Havel 2000, Schwartz et al. 2000).

In addition to the decrease in plasma leptin concentrations, modest weight loss could

significantly improve most cardiovascular risk factors in obese women. As expected, serum insulin, total and LDL cholesterol, the activities of PAI-1 and FVII in plasma and SBP and DBP decreased. Intentional weight loss has previously been shown to decrease serum insulin, total and LDL cholesterol as well as SBP and DBP (Dattilo and Kris-Etherton 1992, Wing et al. 1995a, Ditschuneit et al. 1999, Tuomilehto et al. 2001). Also the activities of FVII and PAI-1 have been shown to decrease with weight loss (Primrose et al. 1992, Folsom et al. 1993, Svendsen et al. 1996, Hankey et al. 1997, Lindahl et al. 1998, Marckmann et al. 1998a, Kockx et al. 1999, Mavri et al. 1999).

The present results suggest that less weight loss is needed to improve the traditional cardiovascular risk factors than to decrease the activities of PAI-1 and FVII in moderately obese healthy women. However, no percentage estimate for weight loss can be given, because the first blood samples of FVII and PAI-1 were drawn after a 1 month run-in period, during which a mean weight reduction of 3.3 kg which may have influenced the activities of these factors. The results of the Finnish Diabetes Prevention Study demonstrate that a weight loss of only five percent can decrease serum insulin, glucose and lipid levels as well as blood pressure in subjects with impaired glucose tolerance (Tuomilehto et al. 2001). More weight reduction was required (5 - 10 %) to decrease the activity or antigen of PAI-1 in obese men, supporting the results of our study (Folsom et al. 1993).

Weight loss did not affect serum glucose, HDL cholesterol, triglycerides and plasma fibrinogen in our obese women. All these concentrations were within the reference values already before weight loss, even though the women were markedly obese, also abdominally. It is probable that most of the abdominal fat was subcutaneous fat and not visceral adipose tissue. High amounts of visceral fat measured by CT have been shown to be related to elevated serum glucose and triglyceride concentrations and low HDL cholesterol concentrations both in men and women as the study subjects were matched for total body fat and visceral fat (Desprès et al. 1989, Pouliot et al. 1992).

The concentration of fibrinogen before weight loss was not known, since the first blood samples were taken one month after the beginning of weight loss. Therefore, we cannot be sure whether the concentration of fibrinogen changed during the rapid weight loss. However, it is unlikely that weight loss affected plasma fibrinogen. Firstly, changes in fibrinogen were not related to changes in BMI in a prospective study and weight loss did not alter the concentrations of fibrinogen in a controlled, randomised weight reduction study (Svendsen et al. 1996, Folsom et al. 2000). Secondly, the increase in plasma fibrinogen has been associated with the markers of insulin resistance like low HDL cholesterol and elevated triglycerides, which were within reference limits in our study (Folsom et al. 2000). Seasonal variations may have influenced the results in studies that reported decreases in plasma fibrinogen with weight loss (Folsom et al. 1993, Marckmann et al. 1998b).

Our data on PSA confirmed the results from two studies that cardiac PSA increases with weight reduction (Aronne et al. 1995, Karason et al. 1999). In the latter studies data from men and women were analysed together, although gender is suggested to affect the HRV. Women have been reported to have greater vagal activity than men (Barnett et al. 1999). Studies that did not observe any association between cardiac PSA and weight change have had either a small sample size (Karason et al. 1999) or a modest weight loss (3.6 kg) in obese women combined with a large range of age (21-57 yr.) (Zahorska-Markiewicz et al. 1993). High-frequency R-R interval power may be less responsive to weight reduction in older persons

than in younger persons, because cardiac PSA decreases with advancing age (Pfeifer et al. 1983). The increase of cardiac PSA with weight loss suggests that low cardiac vagal tone is a consequence of the obese state, which has also been proposed by Karason et al. (1999). Additional studies are needed to evaluate the effect of weight maintenance on cardiac PSA.

6.3. The effect of weight change after weight loss on cardiovascular risk factors (III)

Weight loss stopped in most of women by six months. Thereafter, 41 % of the women continued to lose weight, 24 % had stable weight and 35 % regained weight by twelve months. Independently of the changes in weight, LDL cholesterol, SBP and DBP rose in all weight change subgroups. Serum insulin and the activities of PAI-1 and FVII rose with weight regain, but the decreased concentration and activities were sustained if the weight loss was maintained or continued.

Weight regain has been shown to elevate LDL cholesterol concentrations in other studies, too (Wing et al. 1995b, Fogelholm et al. 2000). The improvements in LDL cholesterol concentrations has been sustained if more than 4.5 kg weight was lost and weight loss was maintained within ± 2.3 kg at 12 and 18 months (Wing et al. 1995a). If weight maintenance is partial, subjects who maintained losses more than 10 % of the initial body weight had significantly greater reduction in LDL cholesterol than those who maintained losses of 5 to 10 % (Wadden et al. 1999). Body cholesterol pools receive up to two thirds of their input from de novo synthesis (Rudney and Sexton 1986). In obese subjects cholesterol synthesis rates are greater than those in nonobese subjects (Miettinen 1971, Stålberg et al. 1997). Even modest weight loss (7 %) has suppressed cholesterol synthesis contributing to a decline in circulating total and LDL concentrations (Di Buono et al. 1999). It might be that elevated synthesis is induced by a certain amount of weight regain. However, it is not known how much weight regain is needed to trigger it. Our study suggests that only a few kilograms are required, since the average weight regain was only 2.8 kg. As long-term (more than 6 months) weight loss was also associated with an increase in LDL cholesterol concentrations, factors other than weight loss per se might be involved. These may include changes in diet and exercise, eg. changes in the intake of saturated fatty acids affect LDL cholesterol levels (Mensink et al. 1992). Thus the compliance with diet might have lessened during the study.

Weight loss decreases blood pressure in both hypertensive and nonhypertensive obese subjects (Mertens and van Gaal 2000). Our results suggest, that the beneficial effect of weight loss on blood pressure is not sustained in the long run, even when the weight loss is maintained. However, despite some rebound, blood pressure levels remained lower than the initial values before weight loss. This rebound effect, reported also in some other studies (Wing et al. 1995b, Fogelholm et al. 2000, Sjöström et al 2000, Stevens et al. 2001), does not seem to be connected with the initial body weight, or the amount of lost or maintained weight. Subjects in most of the studies, including our study, were markedly obese, but those in the SOS study were severely obese (Wing et al. 1995b, Fogelholm et al. 2000, Sjöström et al. 2000, Stevens et al. 2001). The amount of weight loss has varied from 4.4 to 31 kg (Wing et al. 1995b, Fogelholm et al. 2000, Sjöström et al. 2000, Stevens et al. 2001), and weight loss maintenance has varied between all lost weight (our study) to 20 kg out of 31 kg (Wing et al. 1995b, Fogelholm et al. 2000, Sjöström et al 2000, Stevens et al. 2001). The blood pressure rise in the obese may be caused by increased activity of the sympathetic nervous system (Tuck

1994), hyperinsulinaemia (Spraul et al. 1994, Grassi et al. 1995, Vaz et al. 1997, Salonen et al. 1998) and perhaps by other factors (Bergholm et al. 2001). Energy restriction and weight loss decrease sympathetic nervous system activity and thereby lower blood pressure (Jung et al. 1978, Landsberg et al. 1978). Further research is needed to determine what factors affect the rebound of blood pressure.

Unlike LDL cholesterol and blood pressure, the activities of PAI-1 and FVII paralleled body weight changes after weight loss in the long term. Even a minor weight regain had adverse effect on the activities, while weight maintenance and continuance sustained the activities at the level which was attained in weight loss. A significant rebound of PAI-1 activity has also been observed after an average body weight regain of only two kg in six months in the randomised and controlled study (Svendsen et al. 1996). Weight maintenance sustained the improved coagulation and fibrinolysis during half a year follow-up (Marckmann et al. 1998b). Further weight loss averaging from two to ten kg decreased more PAI-1 activities in six to twelve months follow-ups (Primrose et al. 1992, Marckmann et al. 1998b). For a sustained decrease in the activity of PAI-1 and FVII, weight maintenance should be emphasised after weight loss. This requires the development of effective weight maintenance programmes.

6.4. The association of fat distribution examined by different methods with cardiovascular risk factors

6.4.1. Anthropometric measurements

In the present study, the abdominal fat accumulation was examined by both conventional (WHR, waist circumference) and more recently developed anthropometric indices (WHTR, sagittal diameter, transverse diameter, STR). All of them related significantly to serum lipids, insulin and glucose even after adjusting for age and BMI. The correlation coefficients of these anthropometric measurements with cardiovascular risk factors did not differ statistically significantly in the z-transformed correlation coefficient test. Thus, none of the above anthropometric measurements was superior to others in assessing the association between visceral fat and cardiovascular risk factors.

In other studies the associations of different anthropometric measurements with cardiovascular risk factors have been inconsistent. Waist circumference and sagittal diameter were suggested to be superior to WHR in some studies (Pouliot et al. 1994, Turcato et al. 2000, Öhrvall et al. 2000), while waist circumference and WHR related more closely to the cardiovascular risk factors than sagittal diameter in another study (van der Kooy et al. 1993). Richelsen and Pedersen (1995) found that sagittal diameter and the ratio of sagittal diameter to height (SDH) correlated more strongly with cardiovascular risk factors than WHR and waist circumference. The superiority of the methods have been based on the strength of correlations and even a slightly stronger correlation has been interpreted to be a sign of a better measurement technique of visceral fat (Pouliot et al. 1994, Richelsen and Pedersen 1995, Öhrvall et al. 2000). However, the conclusions of these studies might have been changed if the z-transformed correlation coefficient test would have been performed. The controversy of the results may also be explained by the different adjustment for age and body fat. Ageing is associated positively both with visceral fat and cardiovascular risk factors, and visceral fat with total body fat (Seidell and Bouchard 1997). Thus, lack of control for age and body fat

makes associations stronger than they really are (Pouliot et al. 1994, Richelsen and Pedersen 1995, Öhrvall et al. 2000). In fact, van der Kooy et al. (1993) controlled their results in female subjects for age and body fat mass and found that only waist circumference and WHR were associated with the metabolic risk factors. The different measures of total body fat may also be a plausible explanation for the inconsistencies in the results. We adjusted our results for BMI, while van der Kooy et al. (1993) used underwater weighing. Adjustment for BMI might be insufficient to properly control for the effects of total body fat on the relation between visceral fat and risk factors (Seidell and Bouchard 1997). Therefore, it is possible that our results might have been changed if we had controlled for body fat mass as measured by DXA.

It is difficult to compare our results to those of previous studies, since our measurement sites differed from those of other studies and thus, also the amount of visceral and subcutaneous fat was different in our study (Zamboni et al. 1992, Pouliot et al. 1994, Turcato et al. 2000, Öhrvall et al. 2000). Instead of skin landmarks, we used bone landmarks, the midway between the lower rib margin and the superior anterior iliac crest as the level for measuring waist circumference, transverse and sagittal diameters and the level of throchanters for measuring hip circumference. The amount of abdominal fat using these bone landmarks has been shown to correlate best with cardiovascular risk factors (Seidell et al. 1990). Skin landmarks, eg. the umbilicus level, is difficult to determine, since it varies among obese individuals. Furthermore, the results in the measurement of circumferences can differ greatly depending on which skin landmarks are used. The waist circumference at the level of umbilicus and the so-called minimal waist circumference can differ by up to 26 cm in the same individual (Weidner et al. 1995).

Our results indicate that all of the simple anthropometric measures estimating abdominal visceral fat are applicable in obese women. However, waist circumference and WHR are the only measurements that have cut-off points based on epidemiological studies (Han et al. 1995, Lean et al. 1995, Lean et al. 1996, Lean et al. 1998), and may therefore be easily introduced clinically. In the follow-up of weight loss, waist circumference has correlated better with the change of visceral adipose tissue than WHR (Lemieux et al. 1996b). Therefore, waist circumference may be superior in clinical use. The problem is that the cut-off points of waist circumference are based on populations in the Netherlands, not on Finnish populations (Han et al. 1995, Lean et al. 1995, Lean et al. 1998). National cut-off points are needed, because populations and ethnic groups differ in the risk of CVD and type 2 diabetes at given levels of waist circumference (Dowling and Pi-Sunyer 1993, Okosun et al. 2000). Thus, studies aiming to determine the Finnish cut-off points for waist circumference are needed. In the meanwhile, the yet unpublished Finnish *Current Care* guidelines on adult obesity proposes cut-off points (100 cm and 90 cm) that are close to the 88 cm in women and 102 cm men suggested by Lean and his group (Lean et al. 1995, Lean et al. 1998).

6.4.2. DXA measurements

Our DXA measurements showed that the area between the first and fourth lumbar vertebrae (upper lumbar fat) correlated with most cardiovascular risk factors. After adjustment for age and BMI, only the correlations with fasting insulin and glucose remained significant. These associations may be explained by the fact that 'upper lumbar fat' has been shown to contain the highest amount of visceral fat in abdominal area. (Jensen et al. 1995). Visceral fat, in turn,

has independently correlated with fasting insulin, glucose and glucose tolerance also in women (Desprès et al. 1989, Jensen et al. 1995) 'Abdominal fat' measured by DXA contained total abdominal fat from the dome of diaphragm to the top of femur and correlated significantly only with serum fasting insulin. The area contained a substantial amount of subcutaneous gluteal fat, which has not been shown to contribute to cardiovascular risk factors (Desprès et al. 1989, Carey et al. 1996). Even though abdominal subcutaneous fat and visceral fat could not be assessed separately, the importance of the chosen measurement area in relation to metabolic risk factors in our DXA measurements was confirmed.

As the abdominal region could be exactly defined in DXA measurements, we expected stronger associations between DXA measurements and cardiovascular risk factors than that was the case in our study. The limitations of the DXA method could in part weaken the putative associations. DXA can measure subjects with a limited range of thickness (e.g. 15-22 cm) (Snead et al. 1993), but sagittal diameter was as much as 28 cm in some of our patients. DXA also measures soft tissue composition directly in non-osseous pixels and assumes a similar composition in pixels containing bone (Snead et al. 1993). Abdominal fat area in our study contained more bone mineral than any other area and thus more of the fat tissue was assumed It has also been suggested that in individuals with increased upper body adipose tissue (obese, older individuals) DXA may underestimate abdominal fat (Snead et al. 1993, Svendsen et al. 1993b, Milliken et al. 1996). The small number of study subjects (n=38) might have weakened the associations with cardiovascular risk factors.

6.5. The association of fat distribution with leptin and cardiac PSA (II, IV)

Body fat distribution has been suggested to partly explain the wide variation in plasma leptin concentrations at a given level of body mass (Maffei et al. 1995). Our data indicated that total body fat as well as peripheral fat (femoral/gluteal region) and abdominal subcutaneous fat correlated with circulating leptin concentrations and with the changes in its concentrations. Visceral fat correlated with leptin concentrations after weight loss, but it was not correlated with the change in leptin levels. The association of plasma leptin concentrations with all abdominal fat depots (MRI, CT measured) and total body fat in other studies agrees well with our result suggesting that the amount of total body fat is a major determinat of circulating leptin concentration (Couillard et al. 1997, Rönnemaa et al. 1997, Gower et al. 2000). The association of plasma leptin with total body fat may be explained by the fact that subcutaneous adipose tissue is the major (about 85 percent) compartment of total body fat (Abate et al. 1995). In women subcutaneous fat cells are large both in abdominal and peripheral areas and they express high amounts of leptin mRNA compared to visceral adipocytes, thus elevating concentrations of circulating leptin (Lönnqvist et al. 1997a, van Harmelen et al. 1998, Lefebvre et al. 1998, Couillard et al. 2000). In addition, subcutaneous adipose tissue is sensitive to insulin, which has been shown to increase leptin mRNA expression in human abdominal subcutaneous adipose tissue and plasma leptin concentrations in healthy subjects and in patients with type 2 diabetes (Mauriege et al. 1995, Malmström et al. 1996, Saad et al. 1998, Pratley et al. 2000).

Recent studies have suggested that body fat distribution may also be associated with the variation in cardiac PSA (Gao et al. 1996, Gutin et al. 2000). In the present study abdominal

obesity (measured by waist circumference) did not correlate with cardiac vagal tone after adjustment for age and body fat mass. None of the above studies controlled for fat mass. Furthermore, the band for the high frequency peak used was not the one that recommended by cardiologists (Malik et al. 1996) (Gao et al. 1996, Gutin et al. 2000) Our results suggest that the change in cardiac PSA is related to total body fat mass, not fat distribution. Further studies are needed to find factors that are behind the great inter-individual variation in cardiac PSA.

6.6. The association of cardiovascular risk factors with leptin, blood coagulation and fibrinolytic factors and cardiac parasympathetic activity (II-IV)

The concentration of serum insulin was the most important cardiovascular risk factor that associated with the changes in plasma leptin and activity of PAI-1 throughout the study. This indicates that the decrease in chronic hyperinsulinaemia with weight loss can reduce plasma leptin and PAI-1 activity. On the contrary, the increase in cardiac PSA was not associated with serum insulin.

Insulin has been shown to increase both leptin mRNA expression in human abdominal subcutaneous adipose tissue and plasma leptin concentrations in healthy and type 2 diabetic subjects (Malmström et al. 1996, Saad et al. 1998, Pratley et al. 2000). The direct association between the changes in leptin mRNA, plasma leptin and insulin seems to be an insulin-sensitive process (Tuominen et al 1997, Saad et al. 1998, Pratley et al. 2000) During physiologic hyperinsulinaemia leptin mRNA was negatively related to glucose disposal rates (Pratley et al. 2000). Thus, individuals with higher glucose disposal values, who were more insulin sensitive, had lower leptin mRNA levels before the clamp and a greater increase during the clamp (Pratley et al. 2000). Plasma leptin response has also been higher in insulin-sensitive subjects compared to insulin-resistant subjects (Tuominen et al 1997, Saad et al. 1998). The decrease in plasma leptin concentration with weight loss in our study suggests that insulin sensitivity in adipocytes is improved.

Insulin is a potent inducer of PAI-1 synthesis in human hepatocytes (Alessi et al. 1988, Kooistra et al. 1989, Moragne et al. 1999), but in human adipose tissue the results have been inconclusive (Halleux et al. 1999, Morange et al. 1999). Also clinical data fail to support a direct acute contribution of insulin to the regulation of PAI-1 activity (Vuorinen-Markkola et al. 1992, Mykkänen et al. 1994, Nagi et al. 1996, Fendri et al. 1998). Conflicting results may be due to pooling the results of men and women, small sample sizes, different methods, diverse dosages of insulin and short duration of euglycaemic hyperinsulinaemic clamp studies. In our study the significant correlation between the change of PAI-1 activity with the change of serum insulin independently of fat mass supports the idea that chronic hyperinsulinaemia rather than acute hyperinsulinaemia affects PAI-1 activity.

Basal spectral powers were significantly lower in obese subjects compared to controls, and they further declined during hyperinsulinaemic clamp (Muscelli et al. 1998). However, recent studies have reported that only insulin-sensitive subjects respond to acute hyperinsulinaemia by increasing low frequency component and decreasing high frequency band (van de Borne et al. 1999, Paolisso et al. 2000, Bergholm et al. 2001). Insulin has failed to stimulate acutely the cardiac autonomic activity in insulin-resistant patients including obese, hypertensive and diabetic subjects (Paolisso et al. 2000). Our study indicated that chronic hyperinsulinaemia and its decrease during weight loss was not associated with the increase in cardiac PSA in

obese women. Thus, this result may mean that cardiac PSA is unresponsive to insulin during a modest weight loss. As hyperinsulinaemia and insulin resistance are strong predictors of type 2 diabetes and CVD, the unresponsiveness of HRV to insulin in insulin-resistant subjects could be a mechanism linking insulin resistance and CVD (Folsom et al. 1994, Salomaa et al. 1995).

7. SUMMARY AND CONCLUSIONS

1. The anthropometric methods used in the measurement of fat distribution did not affect the association of abdominal fat distribution with cardiovascular risk factors. Thus, any of the measurements can be used in obese women. However, waist circumference is recommended in clinical use, since it has been shown to correlate well with the change of visceral fat and weight loss, and it also has cut-off points. Unfortunately, the cut-off values based on the Finnish population studies are lacking. The area between the first and the fourth lumbar vertebrae ('upper lumbar fat') is recommended for the DXA measurements of abdominal fat.

2. Plasma leptin decreased proportionally with decreasing body fat during modest energy restriction. Weight loss was accompanied by significant decreases in serum insulin, and total and LDL cholesterol, activities of PAI-1 and FVII in plasma and SBP and DBP and by an increase in cardiac PSA. Serum glucose, HDL cholesterol, triglycerides and plasma fibrinogen remained unchanged. Therefore, modest weight loss can decrease most of the cardiovascular risk factors in markedly obese women.

3. Most women ceased to lose weight by six months. By 12 months 41 % continued to lose weight, 24 % had stable weight and 35 % had regained weight. Independently of the changes in weight, LDL cholesterol, SBP and DBP tended to rise in all women. Serum insulin and the activities of PAI-1 and FVII increased with weight regain, but the decreased concentration and activities were remained when weight loss was sustained or continued. The increase in LDL cholesterol and blood pressure independently of weight change suggests that other factors than weight loss per se might be involved, and further research is needed on factors affecting the rebound in blood pressure. The present results also suggest the importance of weight maintenance in sustaining the improved hyperinsulinaemia, coagulation and fibrinolysis in the long term. Development of effective weight maintenance programmes remains a challenge.

4. The decrease in chronic hyperinsulinaemia was associated with decreases in plasma leptin concentrations and PAI-1 activity, but not with cardiac PSA. This may indicate that cardiac PSA is unresponsive to insulin during a modest weight loss. The unresponsiveness of HRV to insulin in insulin-resistant subjects could be a mechanism linking insulin resistance and CVD.

5. Total body fat as well as peripheral fat (femoral/gluteal region) and abdominal subcutaneous fat correlated with circulating leptin concentrations and with the changes in its concentrations. This might mean that the amount of total body fat is a more important correlate of circulating leptin concentrations than fat distribution. Cardiac PSA was not associated with abdominal fat, suggesting the importance of total body fat mass in the change of cardiac PSA.

In conclusion, healthy, markedly obese women can benefit from weight loss via decrease in most of the cardiovascular risk factors and increase in cardiac PSA. Furthermore, the maintenance of a modest weight loss is associated with long-term benefits in the activities of PAI-1 and FVII and serum insulin concentration. The increase in LDL cholesterol and blood pressure independently of weight change suggests that also other factors than weight loss per se might be involved in the long-term changes. However, despite the increase in LDL

cholesterol and some increase in blood pressure, their values remained below the initial values, which can be considered to lower the long-term risk for CVD.

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