INSULIN RESISTANCE IN OBESITY AND HYPERTENSION

An important link with cardiovascular disease

by

Kerstin Landin-Wilhelmsen

Göteborg 1990

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I. Landin K, Krotkiewski M, Smith U. Importance of obesity for the metabolic abnormalities associated with an abdominal fat distribution. Metabolism 1989; 38: 572-576

II. Landin K, Lönnroth P, Krotkiewski M, Holm G, Smith U. Increased insulin resistance and fat cell lipolysis in obese but not lean women with a high waist/hip ratio. Eur J Clin Invest 1990; 20: 530-535

III. Landin K, Stigendal L, Eriksson E, Krotkiewski M, Risberg B, Tengborn L, Smith U. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. Metabolism 1990; 39: 1044-1048

IV. Landin K, Tengborn L, Smith U. Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. J Intern Med 1990; 227: 273-278

V. Landin K, Tengborn L, Smith U. Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. Submitted.

VI. Landin K, Tengborn L, Chmielewska J, von Schenck H, Smith U. The acute effect of insulin on tissue plasminogen activator and plasminogen activator inhibitor in man. Submitted.

ABSTRACT

Landin-Wilhelmsen K. Insulin resistance in obesity and hypertension An important link with cardiovascular disease.

Hyperinsulinemia and insulin resistance in the skeletal muscle and adipose tissue are common features in type II diabetes, obesity and hypertension. These states are often linked and present in the same subject. The aim of this study was to separately assess the effect of obesity for the metabolic abnormalities associated with a high waist/hip ratio (WHR) as well as to study the glucose and lipid metabolism and fibrinolytic factors in non-obese hypertensive and normotensive men.

Higher blood pressure, increased insulin resistance and higher lipolytic activity in the adipose tissue were found in obese but not in non-obese postmenopausal women irrespective of WHR. Lean women with a high WHR had a lower risk factor profile than obese women with a low WHR.

Tissue plasminogen activator (t-PA) activity was lower and plasminogen activator inhibitor of endothelial cell type (PAI-1) activity as well as fibrinogen and triglycerides higher in obese women with a high WHR as compared to weight-matched women with a low WHR. No differences in blood pressure, glucose and lipid metabolism, lipolytic activity in the adipose tissue, skeletal muscle fibre composition, PAI-1 or fibrinogen were seen between the two groups of weight-matched, non-obese women with different WHR.

Lean men with mild, untreated hypertension had higher fasting plasma insulin, cholesterol, triglyceride, fibrinogen and PAI-1 levels than weight-matched men with normal blood pressure.

A strong positive correlation was seen between plasma insulin and PAI-1 levels in the obese and the hypertensive subjects. PAI-1 was also correlated to blood pressure, blood lipid levels and degree of insulin resistance.

Metformin increased the peripheral glucose uptake and lowered cholesterol and triglyceride levels in the hypertensive men. Blood pressure decreased markedly, whereas less effect was seen on the fibrinolytic variables.

During a hyperinsulinemic clamp with maintained euglycemia the t-PA activity increased rapidly and t-PA antigen, PAI-1 activity and antigen simultaneously decreased.

Long-term hyperinsulinemia and insulin resistance are thus associated with a decreased fibrinolytic activity. Short periods of hyperinsulinemia acted in the opposite direction, possibly due to acute sympatho-adrenal activation.

In conclusion, obesity, especially abdominal obesity, and hypertension are associated with hyperinsulinemia, insulin resistance, elevated blood lipids, PAI-1 and fibrinogen levels. Non-obese women, irrespective of WHR, did not show these abnormalities. Treating insulin resistance in hypertension improved the risk factor profile including the blood pressure. Insulin resistance is related to atherosclerosis and the present results also show a link with thrombogenic factors.

Key words: Obesity, waist/hip ratio, hypertension, insulin resistance, fibrinolysis, plasminogen activator inhibitor, metformin

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TITLES OF PUBLICATIONS INCLUDED.

This thesis is based on the publications listed below, which will be referred to in the following by their Roman numerals I-VI.

I. Landin K, Krotkiewski M, Smith U. Importance of obesity for the metabolic abnormalities associated with an abdominal fat distribution. Metabolism 1989; 38: 572-576

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CONTENTS

INTRODUCTION

Insulin action

Insulin is the most important anabolic and anti-catabolic hormone in the body. In addition, insulin has been shown to influence the electrolyte balance across the cell membranes.

The main target tissues for the metabolic effects of insulin are the skeletal muscles, liver and adipose tissue. Glucose transport and uptake in the skeletal muscles and adipose tissue are facilitated by the ability of insulin to increase the number of specific glucose carriers in the plasma membrane (139). This is a major action of insulin which is critical for the regulation of the ambient glucose concentration. Insulin also increases glycogen storage and suppresses hepatic glucose production.

The protein and lipid stores are regulated by insulin through both an increased synthesis and an inhibited degradation. Although all these effects can be seen in the target organs their quantitative contribution to over-all metabolism is obviously different. Glucose uptake in the postabsorptive state occurs mainly in the skeletal muscles. The liver is the main organ for glucose production, the skeletal muscles the major protein depot and the adipose tissue the main lipid depot in the body.

In order to elicit its effect insulin binds to specific membrane receptors (Figure 1), which are heterodimers consisting of two α- and two β-subunits linked together with disulfide bonds (62). The cytoplasmic domain of the β-subunit contains tyrosine kinase activity which becomes activated by the binding of insulin to the insulin receptor. Activation of the tyrosine kinase seems to be critical for the insulin effect (62).

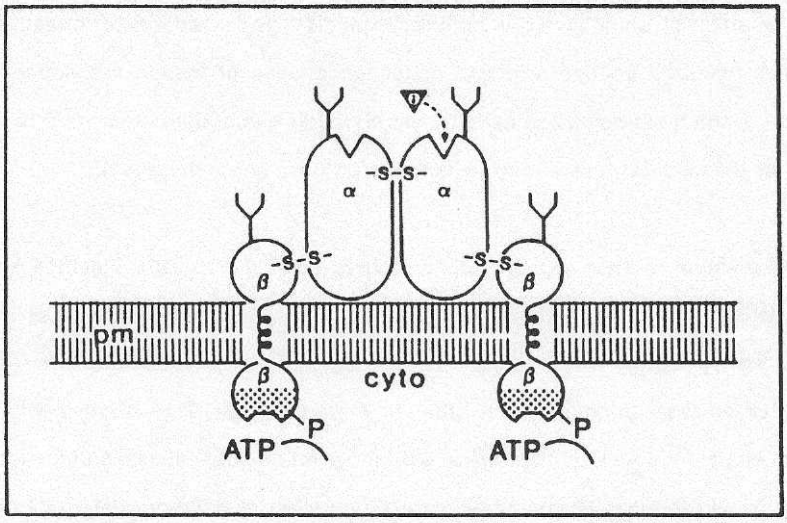

Figure 1. Schematic representation of the insulin receptor in the plasma cell membrane (pm). Binding of insulin (i) leads to a phosphorylation (P) and activation of the tyrosine kinase (dotted area).

Insulin resistance

In general, insulin resistance can be attributed to a receptor or a postreceptor perturbation (Figure 1).

Classically, a receptor change such as a reduced number of cellular binding sites should only shift the dose-response curve for insulin to the right (increase EC_{50} for insulin) without changing the maximal responsiveness. A more pronounced degree of insulin resistance can be elicited by a postreceptor defect where EC_{50} may be increased and the maximal responsiveness decreased.

Insulin resistance is a common disorder which may well have different pathogenetic mechanisms. It has been clearly established that cAMP is a potent antagonist to insulin action, the effect of which includes both receptor (127) and postreceptor changes. Current research is focused on two principal molecular causes of insulin resistance; impaired activation of the tyrosine kinase activity and impaired glucose transport due to a reduced number of glucose carriers and/or an attenuated intrinsic activity (61).

Insulin resistance is seen in common disorders such as obesity, diabetes and hypertension. It is not clear whether the insulin resistance in these conditions is mainly the result of environmental factors (such as obesity common in both diabetes and hypertension), or whether genetic factors play an important role. This is obviously a critical question since intervention measures would be much less successful in the face of defective gene products. Since insulin exerts a number of different effects it is clear that an impaired insulin action can influence both glucose and lipid metabolism as well as the electrolyte balance and, consequently, blood pressure regulation.

Insulin resistance in obesity

Obesity is associated with insulin resistance of varying degree (65, 73, 104). The molecular mechanism seems to be mainly an impaired glucose transport and uptake since the insulin receptor tyrosine kinase activity seems to be essentially normal (70). In support of this, the antilipolytic effect in human fat cells is also normally sensitive to insulin (58).

Insulin resistance in Pima Indians seems to be, at least in part, an inherited disorder (69). Thus, obesity per se may not be required but rather a genetic trait associated with it. The observation that the distribution of the adipose tissue is of importance supports this concept since it has been demonstrated that the adipose tissue distribution pattern (abdominal vs gluteo-femoral distribution) is genetically determined (24, 132).

Thus, it may well be that insulin resistance in obesity preceeds obesity and is linked to

a genetically determined predisposition. On the other hand, this concept is not supported by the observation that the insulin resistance in obesity becomes alleviated following weight reduction (63, 75, 126).

Another possibility is that the expanded adipose tissue in obesity plays an important pathogenetic role in eliciting the insulin resistance. Elevated levels of free fatty acids (FFA) could provide a possible link between obesity and insulin resistance (109). Recent studies from our laboratory have provided direct evidence for the ability of FFA to impair insulin binding and action in isolated hepatocytes (130). Furthermore, the concept that the adipose tissue may play an important role was suggested several years ago following studies in lipoatrophic diabetes (125).

Taken together, it is likely that the insulin resistance in obesity leads to a small increase in the ambient glucose levels which, in turn, stimulates insulin release from the pancreas. The elevated insulin levels can then lead to increased effects of insulin on other factors which may be less insulin resistant. These could, for instance, include renal sodium handling which does not seem to become insulin resistant in obesity (35). Abdominal obesity is associated with a more marked degree of insulin resistance than that found in equally obese individuals with a gluteo-femoral obesity. Furthermore, abdominal obesity is a risk factor for cardiovascular disease and diabetes in both men and women (80, 84, 103). The large adipocytes in the expanded abdominal region in abdominal obesity have an increased metabolic rate including lipolysis (19, 112, 123) which leads to a high efflux of FFA which, in turn, can impair insulin action in the target tissues (109). Thus, the association between the metabolic aberrations and adipose tissue distribution may well be reconciled by the importance of the adipose tissue per se although parallel phenomena of a common genetic cause can not be excluded.

Insulin resistance in hypertension

Positive correlations between blood pressure and blood glucose as well as insulin levels

even after controlling for body weight have been reported (14). Resistance to the effect of insulin on glucose uptake has been demonstrated in hypertension (42, 118). However, there was no evidence for insulin resistance in the liver as the endogenous glucose production was normal and the liver was also normally sensitive to insulin. The fasting FFA and potassium concentrations were also normal in non-obese hypertensives as compared to weight-matched controls. These studies suggest that the major site for the insulin resistance is the skeletal muscles with decreased glucose utilization and impaired glycolysis.

Insulin resistance and the fibrinolytic process

The normal fibrinolytic process exerts an important protective effect against thrombosis formation. It is commonly accepted that fibrinolysis influences the growth, ultimate size and dissolution of the thrombus. The process is schematically outlined in Figure 2.

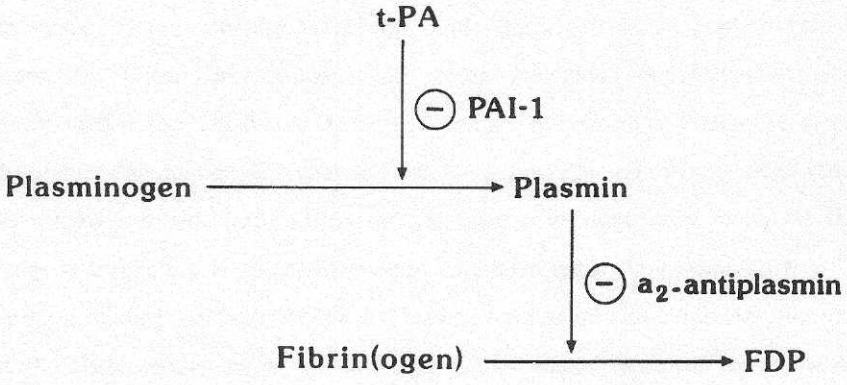

Figure 2. Scheme of the fibrinolytic process. FDP = fibrin degradation products. Other abbreviations are given in the text. ⊖ = inhibitory effect.

The plasminogen activator inhibitor of endothelial cell type (PAI-1) is a rapid inhibitor (30) of the tissue plasminogen activator (t-PA) a key factor for the fibrinolytic system. t-PA, with a molecular weight of 67 kDa, is taken up from the circulation mainly by the liver even though extrahepatic clearance also exists (101). An important feature of t-PA secretion is the rapid release of t-PA in response to certain systemic or local stimulus.

PAI-1 binds irreversably to t-PA to form a t-PA-PAI-1 complex which is degraded (30). PAI-1 is a glucoprotein with a molecular weight of 52 kDa and consists of 349 amino - acids. It is synthesized mainly in endothelial cells, platelets and hepatocytes. PAI-1 synthesis is stimulated by a variety of factors including endothelial damage, inflammation, tumor necrosis factor, interleukins etc. PAI-1 has considerable homology with a_2-antiplasmin and antithrombin III and is a member of the serine protease inhibitor gene family (115).

Increased levels of PAI-1 would act in a thrombogenic direction. Decreased t-PA and high levels of PAI-1 have been reported in obesity and type II diabetes (4, 5, 7, 60, 134) as well as in patients with recurrent deep vein thrombosis (99) which may explain their defective fibrinolysis. Interestingly, patients suffering a myocardial infarction as well as those with a recurrent event have elevated PAI-1 levels (8, 51, 52). In these disorders increased levels of fibrinogen and factor VIII have also been demonstrated as well as an increased platelet adhesiveness (71, 93, 117, 141).

Previous studies have not been able to separately assess the effect of adipose tissue distribution, obesity, diabetes and hypertension on metabolism and fibrinolysis as these abnormalities often are linked - "the metabolic syndrome" or the "syndrome X" - and present in the same subject (110). This interaction is difficult to disregard even when multivariate analyses are used.

AIMS OF STUDY

The aims of the present studies were to:

- study the importance of obesity per se for the metabolic abnormalities associated with abdominal obesity (publication I);

- study the lipolysis in adipose tissue from different regions in obese and lean women and the relationship with insulin sensitivity (publication II);

- study the fibrinolytic system in obese and lean women in relation to body fat and fat distribution (publication III);

- study glucose metabolism and the fibrinolytic system in non-obese hypertensive men (publication IV);

- study the effect of improving insulin sensitivity in mildly hypertensive men (publication V);

- study the fibrinolytic activity during a hyperinsulinemic, euglycemic glucose clamp (publication VI).

In order to separately study the importance of the adipose tissue distribution with and without obesity four groups of women were investigated. They were divided into groups with a high or a low waist over hip circumference ratio (WHR). The obese and non-obese postmenopausal women were carefully matched with regard to body weight, lean body mass and body fat in studies I-III.

In order to study glucose metabolism in hypertension excluding obesity as a confounding

factor, non-obese middle-aged men with untreated mild hypertension were matched for both body weight and adipose tissue distribution (WHR) with normotensive controls in study IV.

In an attempt to elucidate the importance of insulin resistance for the "metabolic syndrome" in hypertension metformin was given in study V to the hypertensive men from study IV. Metformin decreases insulin resistance, and the effects on blood lipids, fibrinogen, PAI-1 and blood pressure were studied.

To directly investigate the acute effect of insulin on fibrinolytic factors, blood samples for t-PA and PAI-1 were drawn repeatedly during an insulin infusion for 2 hours with maintained euglycemia in study VI.

SUBJECTS

Table 1 presents the clinical characteristics of the subjects included in the different studies.

Table 1. Characteristics of the subjects in studies I-VI.

Study	Group	N	Sex M/F	Age years	Body mass index, kg/m^2	Waist/hip ratio (WHR)
I	Obese with high WHR	10	F	54.2±2.3	36.0±6.5	0.87±0.05
	Obese with low WHR	10	F	54.8±4.3	32.4±2.9	0.75±0.04
	Non-obese with high WHR	10	F	55.1±2.3	25.9±3.4	0.86±0.05
	Non-obese with low WHR	10	F	56.0±3.1	26.2±4.5	0.75±0.04
II+III	Obese with high WHR	10	F	49.7±3.8	36.3±3.3	0.92±0.02
	Obese with low WHR	10	F	50.2±2.5	33.7±3.0	0.74±0.01
	Non-obese with high WHR	10	F	55.1±2.3	25.9±3.4	0.86±0.05
	Non-obese with low WHR	10	F	56.0±3.1	26.2±4.5	0.75±0.04
IV	Non-obese with hypertension	11	M	52.8±5.3	25.1±2.1	0.93±0.03
	Non-obese without hypertension	11	M	52.1±5.5	25.0±1.4	0.91±0.02
V+VI	Non-obese with hypertension	9	M	56.3±4.9	26.4±3.1	0.96±0.05

Values are means ± SD

Non-obese women (studies I-III)

The non-obese women in studies I, II, III were recruited by means of an advertisement in a local newspaper. Of 145 screened women only 13 (9%) had a WHR ≥ 0.80. This cut-off point was used because it was the median in a female, random population sample in Göteborg (80).

Ten of these women with a WHR ≥ 0.80 (mean height 164.0±7.3 cm and weight 69.4±7.4

kg) were matched against ten women with a WHR < 0.80 (mean height 163.1±4.4 cm and weight 69.3±9.5 kg) for both body fat and lean body mass. In the group with a high WHR mean body mass index (BMI) was 25.9±3.4 kg x m^{-2}, i.e. 113%±14% relative body weight (89) and in the group with a low WHR, BMI was 26.2±4.5 kg x m^{-2} and relative body weight 114%±8%. The mean age for the non-obese women was 55.5±2.7 years. They were all postmenopausal.

Obese women (studies I-III)

Twenty middle-aged, postmenopausal obese women, mean age 54.5±3.3 years, were recruited from the Obesity Outpatient Clinic of the Sahlgrenska Hospital. The obese women were matched for age, body fat, and lean body mass and were divided into two groups according to their WHR, i.e., ≥0.80 (n=10) and <0.80 (n=10). The BMI was 36.0±6.5 kg x m^{-2} and relative body weight 158%±9% in the group with a high WHR and 32.4±2.9 kg x m^{-2} and 150%±6%, respectively, in the group with a low WHR. Mean height was 167.0±4.5 cm and mean body weight 95.6±13.1 kg for the obese women. The obese women in study II and III were partly the same as in study I. The matching procedure was the same and the two obese groups showed similar values for BMI and WHR in the three studies.

All non-obese and obese women were healthy, with normal blood pressure levels and without drug treatment. They had sedentary work, either half- or full-time, and did not exercise regularly. All had a moderate alcohol consumption; mean recalled consumption was 1 L wine/month. Three obese and two lean women were smokers. The mean duration of the obesity, based on individual recall, was 28±6 years. All obese women had previously tried several times to reduce their body weight.

Non-obese men (studies IV-VI)

The non-obese men in study IV were recruited by means of an advertisement in a local newspaper. Their mean age was 52.2±6.1 years. They were selected on the basis of

elevated blood pressure, with three measurements on two different occasions yielding values $\geq$ 160 mm Hg systolic and/or $\geq$ 95 mm Hg diastolic phase V (n=11), or normal blood pressure (n=11) according to the WHO criteria (143). The two groups of men were carefully matched for body weight (mean 79.1±7.9 kg and 79.0±6.8 kg, respectively), BMI (mean 25.1±2.1 and 25.0±1.4 kg x m^{-2}, respectively), lean body mass, body fat and WHR (Table 1).

No medication was taken on a regular basis. Two men in the hypertensive group and three men in the control group were smokers during study IV. None of the subjects exercised regularly.

The nine men in studies V and VI were volunteers from the hypertensive group in study IV. They had now become 56.3±4.9 years, had a BMI of 26.4±3.1 kg x m^2 and a WHR of 0.96±0.05. These men had been regularly checked concerning their blood pressure during the three years after study IV. Besides, they had participated in a fibre diet study in the meantime. Basal characteristics, blood pressure, blood lipids and glucose disposal during this time period are given in Table 2. As seen, the blood pressure for the hypertensive men had been stable during the years. Nobody was on antihypertensive treatment. The two hypertensive smokers in study IV had stopped smoking when studies V and VI were performed.

Ethical aspects

All studies were approved by the Ethical Committee of the University of Göteborg and all participants gave their consent.

Table 2. Body mass index, metabolic data and blood pressures in men of studies IV-VI from 1986-1990.

Variable	Study IV, 1986		Placebo, 1987		Fibre suppl., 1987		Metformin, 1989-90	
	Normotens. n=11	Hypertens. n=11	Normotens. n=11	Hypertens. n=11	Normotens. n=11	Hypertens. n=11	Hypertens. n=9	
							before	after
Body mass index, kg/m^2	25±1	25±2	25±1	25±2	25±1	25±2	26±3	26±3
Fasting plasma insulin, mU/l	6±2	10±4	6±2	10±4	6±2	11±4	14±7	9±5
Glucose disposal mg/kg BW x min	12±3[1]	10±3[1]	12±3[1]	10±3[1]	13±3[1]	10±3[1]	4±2[2]	5±2[2]
Cholesterol, mmol/l	5.0±0.6	5.8±0.9	5.1±0.6	5.4±0.6	4.8±0.7	5.4±0.7	6.3±1.1	5.4±0.8
Triglycerides, mmol/l	0.9±0.5	1.7±1.2	1.0±0.5	1.5±1.3	0.9±0.5	1.5±0.7	2.1±0.9	1.7±1.0
Systolic blood pressure, mm Hg	137±3	164±17	123±3	155±14	119±6	143±14	155±13	116±10
Diastolic blood pressure, mm Hg	86±4	97±2	80±6	92±7	79±5	90±6	94±4	70±5

Values are means ± SD

1) Insulin infusion rate 0.12 mU/kg BW x min^{-1}
2) Insulin infusion rate 0.08 mU/kg BW x min^{-1}

METHODS

Body height, body weight and body mass index

Body height and weight were measured with the subjects in underwear and without shoes. Height was measured to the nearest cm and weight to the nearest 0.1 kg. The measurements were performed in the morning after an overnight fast.

Body mass index (BMI) was calculated as body weight in kg divided by height^2 (131). Relative body weight was calculated as actual divided by ideal body weight x 100 (89).

Waist and hip circumference measurements

Waist circumference was measured with a soft tape in the midaxillary line, midway between the lowest rib margin and the iliac crest according to WHO criteria (145). This level is similar to the smallest girth above the umbilicus in women. Hip circumference was measured over the widest part of the gluteal region. Both waist and hip circumferences were measured in the standing position after an overnight fast. All measurements were performed by a single observer (the author) standing in front of the subject. The waist/hip circumference ratio (WHR) was also calculated (73).

Total body potassium, lean body mass and body fat

Total body potassium was determined in a whole body counter detecting natural ^{40}K (Nuclear Enterprise, Edinburgh, England) and expressed in mmol (122). Lean body mass was estimated according to the method of Forbes et al (43) in which 1 kg of lean body mass equals 68.1 mmol of potassium (Lean body mass = Total body potassium/68.1). Body fat was calculated by subtracting the lean body mass from the body weight.

Blood pressure measurements

Blood pressure was calculated as the mean of three measurements on the right arm after 10 min in the supine position at room temperature ~ 20° C. Diastolic pressure was measured as Korotkoff phase V (143). A random zero sphygmomanometer (Hawksley &

Sons, Lancing, England) was used. A cuff size corresponding to the circumference of the right arm was chosen (92). Blood pressure was also measured on the left arm with similar results. The measurements in each study were performed by the same observer.

Blood glucose and lipid analyses

Venous blood samples were drawn from an antecubital vein in the morning after an overnight fast. Glucose in studies I-IV was analysed with a glucose oxidase method (Kabi, Stockholm, Sweden). In studies V and VI glucose was assayed with the glucose-6-phosphate dehydrogenase method (Beckman Instruments, Fullerton, CA, USA). An oral glucose tolerance test, 100 g glucose in lemon-flavoured water, was performed in the morning after an overnight fast. Fasting blood glucose values below 6.7 mmol/l were considered normal (144).

Insulin and C-peptide were determined with radioimmunoassay techniques using Phadiaseph insulin-kit (Pharmacia, Uppsala, Sweden and NOVO, Copenhagen, Denmark) in studies I-IV. C-peptide in studies V-VI was assayed with an in-house RIA method with commercially available reagents with iodinated C-peptide (NOVO, Bagsvaerd, Denmark) and antiserum (Diagnostica, Falkenberg, Sweden).

Cholesterol and triglycerides were determined enzymatically (Boehringer Mannheim, West Germany) (119, 137) by the Department of Clinical Chemistry, Sahlgrenska Hospital. High density lipoprotein (HDL) was analysed according to Seigler et al (116) and the low density lipoprotein (LDL) calculated according to Friedewald's formula where LDL cholesterol (mmol/l) = total cholesterol - HDL-cholesterol - 0.45 x triglycerides (45). Free fatty acids were determined according to Dole et al (37).

Glucose clamp technique

A hyperinsulinemic, euglycemic clamp was performed essentially as described by DeFronzo et al (36). After an overnight fast, an indwelling catheter (Venflon^{R}, Viggo,

Helsingborg, Sweden), was inserted into an antecubital vein for glucose, insulin and potassium administration. A second catheter was placed into a handvein of the contralateral arm for blood sampling. Heating pads were used to give arterialized blood with an oxygen saturation of 93±1% (10). After 10 min infusion of a priming dose of insulin, a solution of 0.5 IU/ml of porcine (studies II and III) or human (studies IV-VI) insulin (Actrapid, NOVO, Copenhagen, Denmark), dissolved in isotonic saline, was infused at a constant rate with an infusion pump (IMED 922H, San Diego, USA).

In studies II, III, V and VI an insulin infusion rate of 0.08 IU/kg body weight x min^{-1} was used, giving plasma insulin levels ~100 mU/l. In study IV the insulin infusion rate was 0.12 IU/kg body weight x min^{-1} giving a plasma insulin concentration of ~200 mU/l. Blood glucose levels were kept constant at 4.9-5.0 mmol/l by the continuous venous infusion of glucose, 10% weight/volume, in studies II, III, IV, and 20% weight/volume in studies V and VI. Potassium (0.1 mmol/ml) was infused at a rate of 50 ml/h. Blood for measurements of the on site glucose levels was drawn every 5 min and rapidly determined with BM-test, Glycemie 1-44 (Boehringer Mannheim, West Germany). The coefficient of variation (CV) was 5.5% for the repeatedly drawn blood glucose samples. Blood samples for the subsequent chemical determinations were also drawn every 10 min during the clamp. Glucose analyses with the reflectometer and the glucose oxidase and/or glucose-6-phosphate dehydrogenase were closely correlated with a CV of 4.5%. These techniques gave similar results (r=0.95).

The clamp was performed for 2 hours and the glucose disposal calculated from the steady-state glucose infusion rate over the last 30 min. During this plateau the glucose infused equals the glucose metabolized (36). The glucose disposal rate during the clamp was expressed as the amount of glucose infused per kg body weight and per kg lean body mass. It is important to express glucose uptake as a function of muscle mass since the skeletal muscles are the major determinants of glucose elimination in response to insulin.

Fibrinogen, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) antigen and activity

Venous blood samples (4.5 ml) for fibrinogen determinations were drawn after an overnight fast into 5 ml Vacutainer tubes containing 0.5 ml of 0.13 mol/l trisodium citrate and 50 mg ε-aminocaproic acid and performed as described by Nilsson and Olow (100) using a syneresis method.

Samples for t-PA antigen and PAI-1 activity and antigen were drawn in the fasting state into precooled 5 ml Vacutainer tubes containing 0.5 ml of 0.13 mol/l trisodium citrate. The t-PA antigen and PAI-1 antigen were assessed using kits from Biopool (Umeå, Sweden). Blood samples for PAI-1 antigen were also drawn in Diatube tubes to avoid release of PAI-1 antigen derived from platelets. Results of PAI-1 antigen from the two different tubes did not differ in this work. PAI-1 activity was measured as described by Eriksson et al (39) in study III using a two stage method with reagents from Biopool, and in studies IV-VI kits from Biopool (Umeå, Sweden). One unit of PAI-1 was defined as the amount of inhibitory activity that neutralized one unit of t-PA at saturating concentrations of t-PA.

For t-PA activity determinations (study V and VI) blood was collected directly into the mixture of 0.13 mol/l trisodium citrate and 0.2 mol/l sodium acetate buffer pH 3.9 and assayed with kits from Kabi Diagnostica (Mölndal, Sweden). All tubes were centrifuged at 2000 x g and +4° C for 20 min. In study III, the t-PA activity was determined according to Gyzander et al (50) and it was analysed both before and after stimulation with 20 min venous occlusion using a sphygmomanometer with blood pressure halfway between the systolic and diastolic pressures. The blood samples were drawn before the venous occlusion and immediately before the deflation.

All samples were analysed in duplicate and the mean values calculated. The inter-assay CV for PAI-1 activity was 5.4% and the intra-assay CV was 1.7% in study III. The CV for

PAI-1 activity was 6% at normal levels and 14% at levels above 18 U/ml in study IV. CV was 17% for PAI-1 activity equal to 6 U/ml and 9% for PAI-1 activity equal to 17 U/ml in studies V and VI. The CV was 4% for PAI-1 antigen, 4% for t-PA activity and 17% for t-PA antigen.

The reference range for PAI-1 activity tested by the Coagulation Laboratory of the hospital was 3-18 U/ml (subjects with BMI above 25 kg/m^{-2} excluded). The reference range and the reproducibility are in accordance with previous studies (8, 30, 34).

Fibrinogen, t-PA and PAI-1 in studies V and VI were analysed on both venous and arterialised blood in connection with the glucose clamp. No differences in results were seen between the two samples.

Fat biopsy technique and adipose tissue incubations

After an overnight fast, a subcutaneous needle biopsy was taken with a 2.0 x 100 mm needle (Terumo Europe N.V., Belgium) and a 20 ml syringe in local anaesthesia (Carbocain^{R}, Astra, Södertälje, Sweden). The biopsies, weighing about 300 mg, were taken from the abdominal region lateral to the umbilicus and from the femoral region one third of the distance between the patella and the superior, anterior iliac spine. In some individuals with insufficient femoral fat the biopsies were taken from the gluteal part. This region was denoted gluteo-femoral. Care was taken not to infiltrate the tissue to be excised with the local anaesthetic agent. The biopsies were immediately placed in medium 199 (Statens Bakteriologiska Lab., Stockholm, Sweden) with 40 mg/ml albumin and the fat cells isolated from the stroma with collagenase (Sigma Chemical Co., St Louis, MO). The incubation techniques have been described in detail and have been extensively used in the laboratory (123, 126). After the incubations at 37°C and pH 7.4, cells and medium were transferred to soft plastic tubes and centrifuged through silicone oil to separate the cells and the incubation medium as described earlier (47). The glycerol content of the medium was analyzed enzymatically (85) and was taken as an index of lipolysis.

Skeletal muscle biopsy technique and muscle analyses

Skeletal muscle biopsies were taken with a conchotome (Wisex AB, Mölndal, Sweden) under local anaesthesia, (Carbocain^{R}, Astra, Södertälje, Sweden) from the left lateral vastus muscle. The muscle biopsies, weighing about 10-20 mg, were immediately trimmed, mounted, and frozen in isopentane and liquid nitrogen. They were stored at -80°C for the subsequent analyses. Serial cross sections (10μm) were cut with a cryotome and stained for myofibrillar adenosine triphosphatase. The reactions were carried out at pH 9.4 following alkaline preincubation at pH 10.3 (105). Thereafter, fibre classification into slow twitch fibres (ST, type I) and fast twitch fibres (FT, type II) were performed. The FT fibres were further classified into FT_a and FT_b types by preincubations at pH 4.6 and 4.4 (113). Approximately 600 fibres were counted per sample, and their fibre type distribution was calculated. Both the inter- and the intraindividual CV were 5%.

Statistical analyses

Mean values, standard deviations, univariate and multivariate linear regressions were calculated with conventional methods. In studies I, II and III the differences between groups were tested with Pitman's non-parametric, unpaired test and, when applicable, tests for paired data (25). Two-sided analysis of variance (ANOVA) with interaction was also used. In study IV, Student's t-test for unparied data and in studies V and VI Student's t-test for paired data were used. Values of $P<0.05$ (two-tailed tests) were considered significant.

Comments on obesity, fat distribution and body composition

Obesity

Obesity is a relative term which refers to an individual's weight in comparison with certain reference weights. The reference values for normal body weight are based on population studies where weight and height are measured. Different indices have been used but BMI is probably the most widely recognized. BMI also correlates reasonably well

to body fatness (145). A BMI $\geq$ 30 kg x m^{-2} is considered to signify obesity (26, 131, 145) while normal weight is around 25 kg x m^{-2}. Mean values in a population are not necessarily equal to the reference or ideal body weight. This can be exemplified with the Pima Indians who have a mean BMI around 30 kg x m^{-2} (69). Mean BMI was 26.7 kg x m^{-2} for women aged 50-60 years in Norway (136) and 25.2 kg x m^{-2} for age-matched women in Göteborg (102). The median relative body weight was 120%, corresponding to 27.5 kg x m^{-2}, for women aged 50-60 years in the Framingham Study (57). Mean values for men of the same age was 25.3 kg x m^{-2} in Norway (136) and 25.0 kg x m^{-2} in Göteborg (84). The median relative body weight was 115% (95) or about 26 kg x m^{-2} for men in the USA (57).

The obese women in studies I-III had a mean BMI of 34$\pm$4 kg x m^{-2} and the non-obese women had 26$\pm$3 kg x m^{-2}. Hence, the obese women were not extremely obese and the non-obese not extremely lean but can be considered as having normal weight for that age group. Similarly, the men in studies IV-VI can also be considered as normal-weight with a BMI of 25$\pm$2 kg x m^{-2} (study IV) and 26$\pm$3 kg x m^{-2} (studies V-VI).

Fat distribution

Not only the amount of body fat but also the localization of the adipose tissue is important to consider. In fact, it has been suggested that the amount of body fat is not an important consideration but that the risk profile is exclusively related to the fat distribution (73, 84, 103). Fat preponderance in the abdominal region is associated with an increased risk for diabetes, hyperlipidemia and hypertension as well as cardiovascular disease (1, 53, 66, 73, 80, 84, 103, 133). This type of fat distribution is typical for males and has been called abdominal, android, central or "apple-shaped" distribution. However, suggestion has been made to call this fat distribution for abdominal obesity (18). It is most frequently seen in men presumably due to constitutional factors and the sex hormone profile (66, 82, 132). Abdominal fat distribution is also seen in women and the prevalence increases with increasing body weight (53, 80).

The typical female fat distribution is the so called gluteo-femoral, femoral, gynoid, peripheral or "pear-shaped" fat distribution (132). However, it has been suggested to consistently use the term gluteo-femoral distribution (18). This distribution is seen in the majority of women (~90%) (53, 80). Although the fat distribution can be expressed in different ways the most widely used measurement is the waist/hip circumference ratio (WHR) (73). Epidemiological studies have also shown, that abdominal obesity, defined as WHR$\geq$1.0 in men and $\geq$0.8 in women, is associated with an increased morbidity and mortality (80, 84). An abdominal fat distribution is rare among non-obese women. Of 145 non-obese women who were screened in study I, only 13 women (9%) had a WHR $\geq$ 0.80. This prevalence is similar to that reported in other studies (53, 80).

In studies I-III both the obese and the non-obese women were divided in two groups depending on a WHR $\geq$ 0.80 or < 0.80. In this way we could separately study the importance of WHR independent of degree of obesity. However, the mean WHR in the obese group with abdominal fat distribution was higher than in the corresponding lean group supporting the relationship between WHR and amount of body fat (124). Furthermore, it was difficult to find non-obese women with a high WHR. The value of WHR in this group may not only reflect the fat distribution but muscular or skeletal factors as well as gastrointestinal bloating may play a role. The men in study IV had a mean WHR of 0.92$\pm$0.02 and the two groups of hypertensive and normotensive men did not differ from each other.

Body composition

Lean body mass (LBM) was higher in the obese women of studies I-III compared to all non-obese women irrespective of WHR. LBM was also higher in non-obese men than in obese women ($p<0.001$) showing a sex difference. No difference was seen between the two groups of men in study IV due to the matching procedure. Total body potassium was also measured before and after the metformin treatment period to verify that there was no change in LBM and body fat.

The body consists of about 80% LBM and 20% fat. LBM consists of about 60% skeletal muscle and visceral organs in addition to skeletal tissue. LBM was calculated from naturally occuring ^{40}K which gives a measure of total body potassium. The potassium content in adipose tissue and bone is negligible and, hence, total body potassium mirrors the LBM (32).

Obesity is associated with an increased cellular number as well as increased cell size in visceral organs leading to a 25% increase of total organ weight in obesity (98). This may, in part, explain the higher total body potassium uptake over the abdominal region and, together with increased skeletal muscle mass, the increased total body potassium in obesity.

Forbes' formula was originally based on measurements on four cadavers (44). Many authors have questionned this formula and described other ways of estimating LBM based on extracellular water volume, total body water, densitometry, anthropometric values, skinfold measurements, computed tomography, and muscle biopsies with potassium determinations (9, 15, 22, 27, 28, 38, 54, 97, 120, 138).

Total body potassium is lower in women than in men, decreases with age and increases with increasing body weight (32, 76, 106). Figure 3 illustrates this relation in 139 healthy subjects screened in studies I-IV.

Total body potassium seems to plateau at around 4500 mmol in men and around 3000 mmol in women (Figure 3), indicating that the relationship between body weight and total body potassium is not a continuous linear function. Thus, with increasing body weight the relative amount of body fat increases (see also 76, 77 and study I). This is consistent with the computed tomography findings of increased intracorporeal fat in different locations in conjunction with increasing body weight (9, 120). As a consequence, total body potassium per kg body weight is lower in obese than lean subjects, especially in

women (76, 77). In addition, the potassium content in biopsies of skeletal muscle is lower in obese as compared to lean subjects (76).

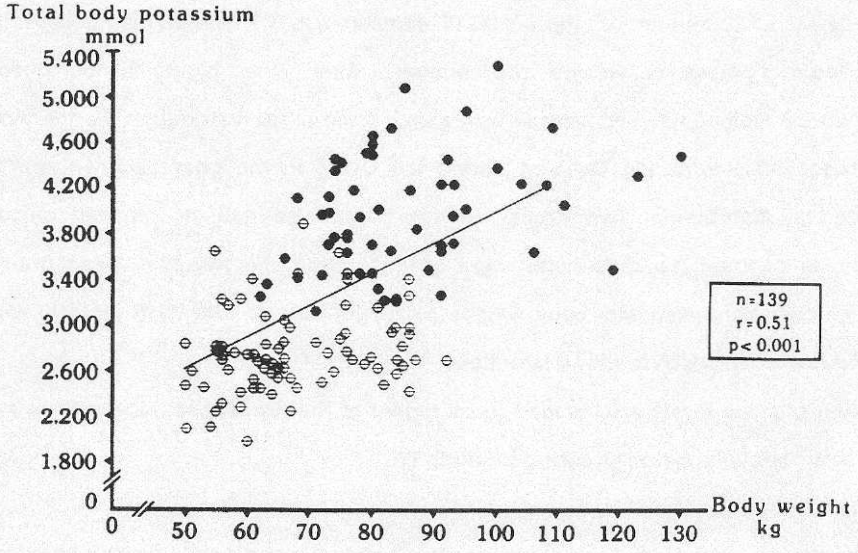

Figure 3. Total body potassium in relation to body weight in middle-aged men ● and women o.

LBM increases with physical exercise due to increasing skeletal muscle mass. However, in extreme athletes as in canoeists (3), total body potassium values of 5000 mmol were achieved at a body weight of 70 kg which gives an impossibly high calculated LBM of 73.5 kg according to Forbes' formula. Taken together, Forbes' formula is probably valid under most conditions but may yield erroneous results in individuals with the greatest divergence from a "normal" body composition, i.e., massive obesity or extreme athletes.

RESULTS AND DISCUSSION

A. Insulin resistance in relation to obesity, fat distribution and hypertension

Glucose and insulin levels: All obese women had higher fasting glucose levels and glucose levels at 30 minutes during an OGTT compared to the lean women irrespective of WHR (study I). However, women with abdominal obesity had higher fasting glucose levels than the weight-matched women with gluteo-femoral fat distribution. Furthermore, the glucose levels remained elevated during the OGTT in the obese women with an abdominal fat distribution. Two women in this group also had an impaired glucose tolerance. In contrast, no differences were seen between the two non-obese groups. Fasting glucose correlated with body weight ($p=0.0006$) but not with WHR ($p=0.19$) when tested by two-sided ANOVA with interaction.

The fasting glucose levels also tended to be higher in the non-obese hypertensive men than in their carefully matched controls (study IV).

The fasting plasma insulin levels were higher in women with abdominal obesity compared to all other women (studies I-III). Obese women with low WHR had higher insulin levels than their lean counterparts. No differences were seen between the two non-obese groups. Insulin correlated to body weight ($p=0.003$) as well as to WHR ($p=0.006$) and these effects were additive ($p=0.05$) (ANOVA with interaction).

The fasting insulin levels were also higher in the non-obese, mildly hypertensive men than in the weight-matched controls (study IV). The insulin levels for all groups are summarized in Figure 4.

These results show that in postmenopausal women hyperinsulinemia and the associated aberrations in glucose metabolism are more strongly related to obesity per se than to WHR. These findings also document the importance of obesity for the metabolic abnormalities associated with an abdominal body fat distribution (53, 66, 73, 103, 128). Furthermore, hypertension in non-obese individuals is associated with hyperinsulinemia even

when fat distribution (WHR) is taken into account.

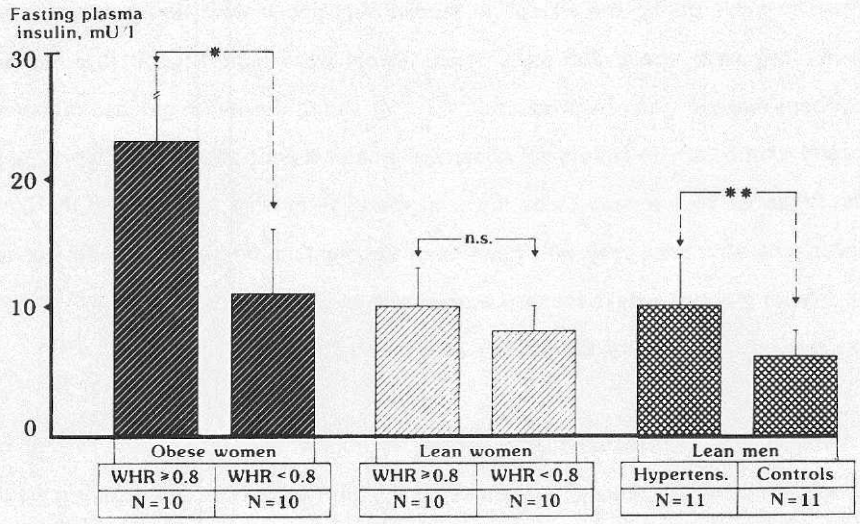

Figure 4. Fasting plasma insulin levels in middle-aged obese and lean women with different WHR and in hypertensive and normotensive men. Means ± SD are shown. * = $p<0.05$, ** = $p<0.01$.

Glucose clamp studies (studies II-V): Insulin sensitivity was directly assessed with the euglycemic clamp technique in studies II-V. Glucose disposal rate, expressed per kg body weight, was lower in obese women compared to non-obese women, irrespective of fat distribution pattern. However, obese women with an abdominal fat distribution had a lower glucose disposal rate than equally obese women with a gluteo-femoral distribution. When the glucose disposal rate was expressed per kg LBM, the most appropriate reference, a difference was seen between obese and non-obese women with a high WHR. No differences were found between the two non-obese groups of women (studies II-III). Glucose disposal rate correlated negatively with body weight ($p=0.0001$) and with WHR ($p=0.007$) but there was no additive effect of the two (ANOVA with interaction).

The glucose disposal rate, expressed per kg LBM, tended to be lower in the non-obese

men with untreated hypertension compared to the weight-matched controls (study IV).

The insulin levels during the clamps in studies II-III and V were above 100 mU/l and in study IV they were above 200 mU/l. These levels were high enough to suppress the endogenous hepatic glucose production (36, 74). Thus, the lower glucose disposal rate in obesity, particularly in abdominal obesity, indicates a peripheral insulin resistance. The insulin response also tended to be lower in the hypertensive men than in the controls. However, this difference may well have been underestimated since the effect of insulin on peripheral glucose uptake reaches a maximum at ~300 mU/l. Thus, the insulin-glucose uptake relationship is relatively flat at ~200 mU/l.

The reason for insulin resistance in obesity is unclear. One possibility is that only a subgroup of obese individuals, viz., those with a high WHR (66), are insulin resistant. In fact, current focus has mainly been on the fat distribution rather than on obesity. Since the fat distribution seems, at least in part, to be genetically determined one possibility is that a common cause leads to both a high WHR and the metabolic disturbances, including insulin resistance. If this were the case, the metabolic abnormalities would be found in non-obese individuals with a high WHR. The current results are not in agreement with this concept. In fact, the data clearly underline the importance of obesity per se. The fat distribution also plays an important role but only when combined with obesity. This finding underlines the importance of detecting and treating obesity! However, it should be emphasized that these studies were carried out on postmenopausal women and the results need to be confirmed in other populations.

The association between the metabolic abnormalities and obesity support an important role of the adipose tissue. One link could be the increased mass of lipolytically active fat cells leading to elevated FFA-levels. FFA can impair glucose uptake in peripheral tissues (109). Furthermore, FFA seems to directly impair both insulin binding and action in isolated liver cells (130).

Insulin resistance in some cases of hypertension and an association with impaired glucose tolerance (14) is now well-established although the mechanism is unknown. It is unlikely that the hypertension per se induces insulin resistance since lowering the blood pressure with β-blocking agents and/or diuretics, if anything, may enhance the insulin resistance (13, 108, 121). One possibility is that an increased sympathetic nervous system activity plays a role since catecholamines are powerful antagonists to insulin action (127). The data in study IV as well as those of others (42, 107) show the insulin resistance in hypertension is not mediated through an association with obesity or an altered fat distribution.

Effect of metformin on insulin sensitivity: Metformin improved the glucose disposal rate during the euglycemic clamp in the hypertensive men (study V). The fasting plasma insulin and C-peptide levels also decreased as well as the fasting glucose levels. Withdrawing metformin for 2 months essentially restored the levels to the pre-treatment situation. Taken together, these data show that the insulin sensitivity was improved during the treatment with metformin. This effect was not due to any change in body weight, fat distribution or body composition during the treatment period (study V).

Metformin has previously been shown to increase insulin action on peripheral glucose uptake and hepatic glucose production in obesity and type II diabetes (56). However, the mechanism of action of metformin at the cellular level is not clear. It enhances insulin action on glucose uptake (31), possibly through an effect on the glucose transporting proteins (67). Whether the insulin receptor tyrosine kinase activity also becomes altered is conjectural (12).

B. Blood pressure in relation to obesity and fat distribution

Systolic blood pressure was higher in obese women with a high WHR compared to non-obese women irrespective of body fat distribution There were no clear differences in systolic blood pressure within the two groups of obese or within the non-obese women

(study I). Diastolic blood pressure was higher in all obese compared to the non-obese women irrespective of WHR. The same results were obtained in studies II and III. Body weight correlated with systolic (p=0.0008) and diastolic (p=0.0003) blood pressure, whereas WHR did not (p=0.55 and p=0.71, respectively; two-sided ANOVA with interaction).

A link between hypertension on one hand and obesity, insulin resistance and glucose intolerance on the other is well-established as discussed previously (96) and was further supported by the data in study I. However, an important conclusion from study I is that obesity per se seems to be the major determinant for blood pressure elevation rather than the fat distribution. A similar conclusion could be drawn from the relationship between obesity and abnormalities in glucose and lipid metabolism as well as in the fibrinolytic process (see studies II-III).

Effect of metformin on blood pressure: Treatment with metformin (850 mg b.i.d.) in an attempt to enhance the insulin responsiveness and lower the plasma insulin levels markedly decreased the blood pressure in the mildly hypertensive men (study V). There was a 40±19 mm Hg decrease in systolic and 24±5 mm Hg decrease in diastolic blood pressure after the 6 weeks' treatment period. Both systolic and diastolic blood pressure increased when metformin was withdrawn for 2 months.

No changes were seen in body weight or body composition during the metformin treatment period and blood pressure as well as the various metabolic variables returned to pre-treatment levels when metformin was withdrawn for 2 months. It is not likely that the blood pressure reduction was due to "regression to the mean" since the subjects had maintained a similar blood pressure for an observation period of 3 years. Furthermore, a placebo-controlled fibre treatment period for 3 months 3 years prior to the present study only produced a small change in mean arterial blood pressure (from 113 to 108 mm Hg, Table 2).

The reason(s) for this effect of metformin remains to be clarified. Metformin improved

insulin resistance as discussed above and lowered the insulin levels. One possibility is, then, that the effects of insulin on variables of importance for blood pressure regulation were altered by the improved insulin sensitivity and the concomitant reduction in the ambient insulin concentrations. Another possibility is a direct effect of metformin on the blood pressure, for instance, via an attenuated activity of the sympathetic nervous system. However, there was no decrease in the resting pulse rate during the treatment period (69±9 vs 69±11 beats/min).

C. Blood lipids in relation to obesity, fat distribution and hypertension

Serum cholesterol levels were high (mean level range 5.9-6.8 mmol/l) in all women in studies I and III. Cholesterol was higher in women with abdominal compared to gluteo-femoral obesity. The non-obese groups did not differ from each other.
Cholesterol was also higher in the non-obese hypertensive men compared with the controls (study IV).

Serum triglycerides were highest in women with abdominal obesity compared to all non-obese women (studies I and III) and compared to the obese women with a low WHR (study III). There were no differences between the two non-obese groups. Triglycerides correlated with body weight ($p=0.01$) but not with WHR ($p=0.29$) when two-sided ANOVA was used. The fasting free fatty acid (FFA) levels did not differ between the two obese or lean groups of women irrespective of WHR (studies I and II).
The non-obese hypertensive men in study IV had higher triglyceride levels than healthy weight-matched controls.

Cholesterol and triglycerides are risk factors for cardiovascular disease in both men and women (29, 64, 81, 140, 142). The positive relationship between blood pressure and serum cholesterol as well as triglyceride levels is also well established. A relationship between insulin resistance/hyperinsulinemia and elevated triglycerides is established as well. Insulin influences both VLDL-catabolism and -synthesis. However, fat cells from in-

dividuals with hypertriglyceridemia are resistant to insulin including to the antilipolytic effect of insulin (83). The importance of this finding for the in vivo situation was recently documented (146). Thus, a reason for the elevated triglycerides can be an increased FFA supply for VLDL-synthesis (146). Elevated triglyceride and insulin levels were seen in the women with abdominal obesity (studies I-III) in contrast to the women with gluteo-femoral obesity and all non-obese women. Non-obese men with mild untreated hypertension (study IV) also showed higher insulin and triglyceride levels when compared to weight-matched controls. Thus, the results support the concept that insulin resistance and hyperinsulinemia are related to aberrations in lipid metabolism. Furthermore, obesity plays an important role since high WHR in the absence of obesity was not related to elevated lipid levels. The FFA levels were not clearly elevated in obesity even though lipolysis was enhanced (study III). However, the turn-over rate is rapid and direct measurements with the tracer technique in vivo have shown an increased FFA turn-over rate in abdominal obesity even without distinct elevations in plasma levels (59).

Effects of metformin on blood lipids: Metformin significantly decreased total cholesterol with 0.96±0.8 mmol/l, LDL with 0.83±0.6 mmol/l and triglycerides with 0.49±0.36 mmol/l in the hypertensive men (study V). The fasting FFA levels were unaffected by metformin treatment. Body weight and body composition also remained unchanged. All blood lipids had returned to pre-treatment levels two months after the withdrawal of metformin.

These results illustrate a marked improvement of the blood lipid profile after metformin treatment, clearly in contrast to the effect of traditional antihypertensive treatment (108, 121). Thus, metformin not only seems to lower blood pressure but also to beneficially affect other components of the "metabolic syndrome". The reduction in total cholesterol was not accompanied by any significant change in the HDL-cholesterol (study V).

Metformin has been reported to improve the lipid profile in obesity (56). A likely reason for this is probably an increased insulin sensitivity in the liver, adipose and skeletal muscle tissue accompainied by decreased plasma insulin levels. The decrease in triglyce-

rides seen in study V was also related to the decrease in fasting plasma insulin (r=0.54).

Whether both the reductions in blood pressure and lipid levels during metformin treatment are the result of the reduced insulin resistance and lower ambient insulin concentrations is not known. However, the results are clearly compatible with such a hypothesis which should be the subject of further investigations.

D. Skeletal muscle fibre composition in relation to obesity and fat distribution

No differences were seen in the muscle fibre composition between the non-obese women with high or low WHR (study I). Differences in muscle fibre types have, however, been reported among obese women with different WHR (72, 88, Figure 5).

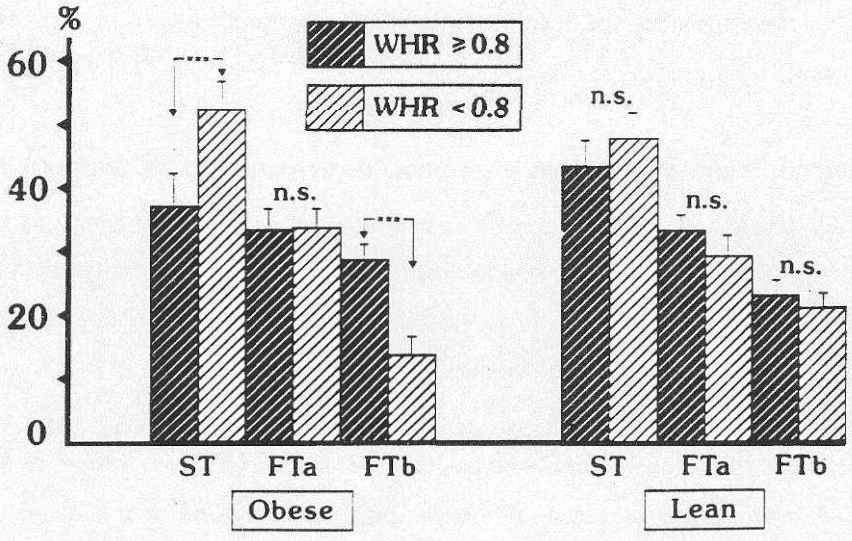

Figure 5. Relative skeletal muscle fibre composition in obese and lean women with different WHR. ST = slow twitch, type I fibres. FT_a and FT_b = fast twith, type IIa and IIb fibres, respectively. Means ±SD are shown. *** = p<0.001 (72 and publ I. Reproduced with permission).

An association has been seen between abdominal obesity and an increased number of fast twitch (type IIb), glycolytic muscle fibres (72, 88) Lillioja et al (88), also showed a negative correlation between slow twitch (type I), oxidative muscle fibres and WHR in obese individuals. A decreased insulin sensitivity in obesity, and preferentially abdominal obesity, is then related to a decreased number of type I and increased type IIb muscle fibres, decreased muscle enzyme activity, decreased muscle potassium, increased muscle sodium and increased muscle Na/K-ratio, increased muscle fat content and increased diffusion distance due to a decreased capillary density (3, 76, 77, 78, 88, 90). These alterations in muscle morphology, enzymatic activity, electrolytes and capillarization may have metabolic repercussions and influence glucose uptake and metabolism in response to insulin (40, 88). However, an important conclusion from the results in study I is that the environment, e.g. diet and physical activity, rather than genetic factors seems to play an important role. Muscle fibre composition is mainly genetically determined. However, the observation that the muscle fibre composition is changed in abdominal obesity but not in non-obese individuals with an increased WHR supports an important effect of the environment.

E. Adipose tissue metabolism in relation to obesity and fat distribution

Abdominal fat cells were larger in obese women with a high WHR when compared to all other women (study II). Mean fat cell size in the gluteo-femoral region was larger in both obese compared with the lean groups. Fat cell number was also higher in the obese women when compared to the lean women.

Irrespective of WHR, rates of basal and isoproterenol-stimulated lipolysis were markedly enhanced even in the presence of insulin, both in abdominal and gluteo-femoral adipocytes in obese when compared to lean women. No clear difference in lipolytic response was seen between the two non-obese groups. In addition, stimulated lipolysis was higher in abdominal adipocytes than in gluteo-femoral adipocytes in the obese women with an abdominal obesity. The results were the same if the lipolytic rate was

expressed per unit cell surface area. The lipolytic response in both regions and in all incubations correlated with body weight (p=0.0001) but not with WHR (p=0.29-0.87; two-sided ANOVA).

Obesity is associated with fat cell enlargement and an increased number of fat cells (17, 20). The regional changes in fat cell morphology are the result of a number of factors including gender and age (17, 20). In general, fat cell enlargement is associated with an increased cellular metabolic rate and triglyceride turn-over (21). This was also seen in study II in the enlarged fat cells from both obese groups. In addition, lipolysis was further enhanced in abdominal obesity due to both larger fat cells as well as an increased lipolytic rate per unit surface area, i.e., an increased "intrinsic activity". Previous studies have also shown that abdominal fat cells have a greater lipolytic responsiveness than gluteo-femoral adipocytes (126, 147).

Taken together, the data in study II clearly show the importance of obesity and fat cell size enlargement for the lipolytic response. At least for subcutaneous fat cells, the regional differences in metabolism seem to play a less important role for lipolysis than the effect of cellular enlargment. However, it is important to emphasize that we did not study the intra-abdominal fat cells which have an even greater responsiveness to catecholamines than the subcutaneous cells (111) and are less sensitive to the antilipolytic effect of insulin (23). These fat cells may play an important role for the over-all FFA production, not least since their venous blood is drained by the portal vein. FFA may impair insulin action in skeletal muscles, thereby influencing glucose uptake, as well as in hepatocytes (130). Thus, the relatively small difference in fat cell lipolysis seen between the obese women with abdominal vs gluteo-femoral obesity should not be taken as evidence against the importance of FFA for the insulin resistance and metabolic perturbations seen in abdominal obesity. Apart for strengthening the importance of obesity per se they suggest that the intra-abdominal fat cells may play an important role.

The finding that the increased fat cell lipolysis in obesity, particularly abdominal obesity,

was not paralleled by increased fasting FFA levels probably reflects the rapid FFA turn-over. Jensen et al (59) recently showed that abdominal obesity is associated with an increased FFA turn-over when compared to gluteo-femoral obesity even if the ambient plasma FFA levels were similar. An increased FFA turn-over rate has also been reported in obese Pima Indians (87). Furthermore, the FFA turn-over is inversely correlated with the glucose disposal rate (86).

F. Tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1) and fibrinogen in relation to obesity, fat distribution and hypertension

PAI-1 and t-PA in relation to insulin: PAI-1 activity was highest in the obese women with an abdominal obesity when compared to their weight-matched counterparts with low WHR as well as all non-obese women. No difference was seen between non-obese women irrespective of WHR (study III). Figure 6 shows the PAI-1 activity in all groups of subjects in studies III and IV.

t-PA activity stimulated by venous occlusion, tended to be lower in the obese women with an abdominal fat distribution compared to the obese women with a gluteo-femoral fat distribution.

A positive correlation was seen between PAI-1 activity and BMI, WHR and fasting blood glucose levels. PAI-1 activity levels were also positively correlated to fasting plasma insulin levels and inversely correlated to the glucose disposal rate when all individual data were used for the calculations. In multivariate regression analyses including BMI, WHR and fasting insulin as independent variables, only insulin was significant ($p=0.005$). When each of the four groups of women in study III was analysed separately the correlations between WHR and PAI-1 as well as between fasting plasma insulin and PAI-1 activity were only significant in the two obese groups.

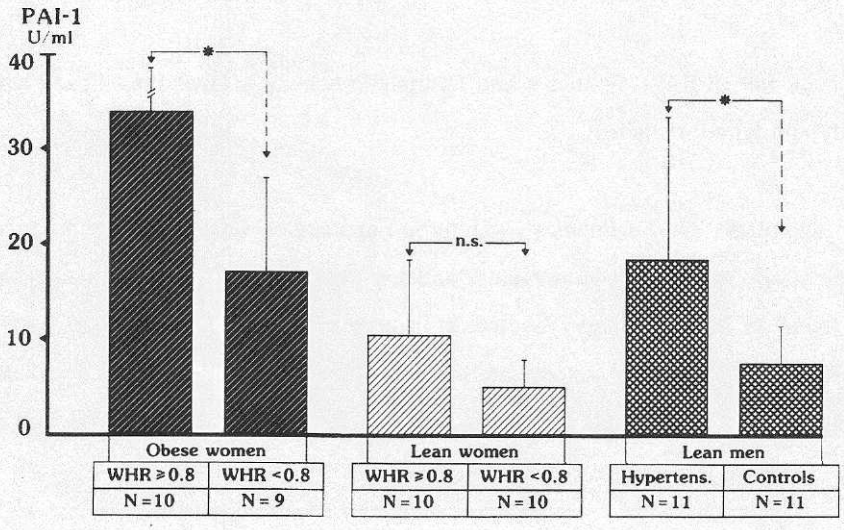

Figure 6. Plasminogen activator inhibitor (PAI-1) in middle-aged obese and lean women with different WHR and in hypertensive and normotensive men. Means ± SD are shown. * = $p<0.05$.

The present results are basically in agreement with previous findings of a decreased fibrinolytic activity in obesity (5, 134). However, the data in study III show that the impaired fibrinolytic activity, measured as t-PA and PAI-1 activity, is mainly seen in women with an abdominal obesity. In the absence of obesity a high WHR carries little importance in terms of metabolic risk for cardiovascular disease discussed previously as well as for the fibrinolytic system.

Fibrinolytic activity (global tests only) has been reported low in diabetic patients (4, 7, 41, 117). However, there seems to be a difference between type I and type II diabetics. PAI-1 levels are normal or low in type I, insulinopenic diabetics (4, 11, 48). This agrees with the hypothesis that insulin, in particular high portal insulin levels, plays a major role in stimulating the PAI-1 synthesis. However, in type I diabetes, with frequent insulinopenia as well as hyperinsulinemia due to exogenous insulin, the fibrinolytic activity may vary (41). Type II diabetics have markedly increased PAI-1 levels and low t-PA activity. These differences are more pronounced in obese than in non-obese type II diabetic subjects (7, 11, 60). PAI-1 is positively correlated with the plasma insulin but not consistently with the blood glucose levels (60). Thus, endogenous insulin may well be a

major regulator of PAI-1 synthesis and fibrinolytic activity in hyperinsulinemic subjects (obesity and type II diabetes).

Insulin stimulates PAI-1 synthesis in cultured hepatocytes where PAI-1 is produced, in addition to the vessel wall endothelium and the platelets (2). However, insulin has not been found to stimulate PAI-1 synthesis in endothelial cells in vitro (2). Endotoxin, septicemia, tumor necrosis factors, interleukins and damaged endothelium stimulate PAI-1 synthesis in the endothelial cells in vivo (Figure 7).

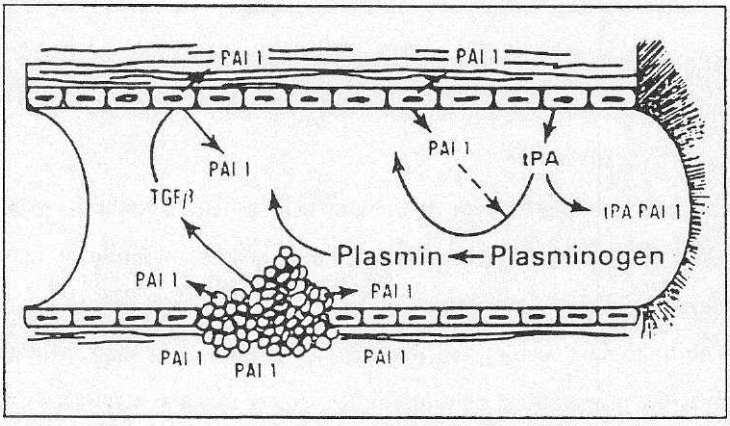

Figure 7. Fibrin-platelet thrombus. Schematic diagram depicting the potential role of PAI-1 in the regulation of vascular fibrinolysis. With permission from Haemostasis and the author (115). TGF = transforming growth factor. Other abbreviations are given in the text.

<u>PAI-1 in hypertension</u>: PAI-1 activity was higher in the non-obese men with mild, untreated hypertension compared with the weight- and WHR-matched controls (study IV). PAI-1 data from all participants in studies III-IV are shown in Figure 6.

The positive correlation between insulin and PAI-1 activity and the negative correlation between glucose disposal and PAI-1 in the women in study III were also seen in the non-obese men in study IV.

Furthermore, PAI-1 was positively correlated with the triglyceride levels as well as the mean arterial blood pressure (Figure 8).

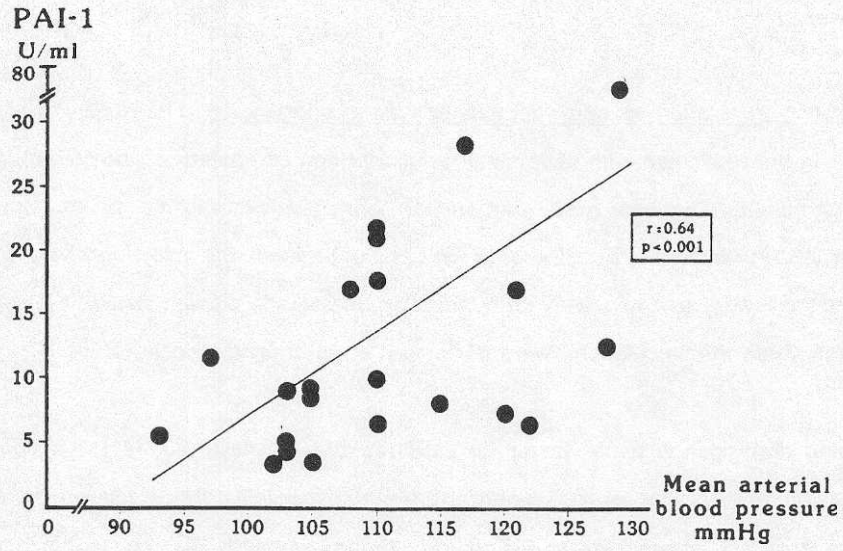

Figure 8. Plasminogen activator inhibitor (PAI-1) in relation to mean arterial blood pressure in hypertensive and normotensive men (n=22).

These results further support that PAI-1 (synthesis?) is related to the ambient insulin levels and that PAI-1 is elevated in hyperinsulinemic conditions irrespective of the cause of the insulin resistance, i.e. obesity, type II diabetes or hypertension. Furthermore, even a small increase in the plasma insulin level like that seen in the mildly hypertensive non-obese men in study IV, was associated with a rise in PAI-1.

The correlations between PAI-1 and blood pressure as well as between PAI-1 and triglyceride levels may well be secondary to the insulin resistance and hyperinsulinemia in hypertension. A positive correlation between PAI-1 and triglycerides when corrected for BMI has recently been demonstrated in a healthy population (129) as well as in subjects with coronary artery disease (94). High PAI-1 levels have also been found in patients with acute myocardial infarction (8, 51, 52, 94) where the level is of prognostic importance for

recurrence, as well (51).

Both the metabolic and the fibrinolytic abnormalities in the hypertensive individuals may be of profound importance for their increased risk for cardio- and cerebrovascular diseases.

Fibrinogen in relation to body composition and hypertension: Fibrinogen levels were higher in obese women with abdominal fat distribution compared to women with gluteo-femoral obesity. The latter group had similar fibrinogen levels to the non-obese women (study III). Furthermore, no difference was seen between the two non-obese groups. Fibrinogen levels were positively correlated with the fasting plasma insulin levels as well as WHR when the calculations were performed on all individual data (study III).

Elevated fibrinogen is a risk factor for cardiovascular disease (93, 141). A relationship also exists between tobacco smoking and fibrinogen levels (141). However, there were similar numbers of smokers in the various groups in study III. Thus, the increased fibrinogen levels in abdominal obesity could not be related to differences in smoking habits. Similar to PAI-1, the fibrinogen levels correlated with insulin.

The fibrinogen levels were also higher in the non-obese men with mild, untreated hypertension compared to the healthy controls (study IV). A positive correlation was also seen in this group with insulin and triglyceride levels. Furthermore, fibrinogen correlated inversely with glucose disposal. Two men in the hypertensive group and three in the control group were smokers. Thus, differences in smoking habits cannot explain the higher fibrinogen level in the hypertensive group. Taken together, these findings show that elevated fibrinogen is another factor associated with insulin resistance and hyperinsulinemia. It is likely that the described association between fibrinogen levels and cardiovascular disease (93, 141) reflects a much wider risk factor panorama than merely elevated fibrinogen levels. However, it is also possible that a high fibrinogen level may, by itself, contribute to an increased risk for thrombus formation.

Effect of metformin on fibrinolytic components and fibrinogen in hypertension : Apart from the improved insulin resistance, blood pressure and lipid profile during metformin treatment a small, but significant, increase in t-PA activity and a decrease in t-PA antigen was seen during metformin treatment (study V). t-PA activity decreased while t-PA antigen did not change after metformin had been withdrawn for 2 months. Fibrinogen and PAI-1 activity remained unchanged during metformin treatment.

Vague et al (135) found a decrease in PAI-1 levels in obese, hyperinsulinemic non-diabetic women during metformin treatment. Concomitantly, the plasma insulin levels decreased while body weight remained unchanged (135). The men in the present study V had only slightly elevated mean plasma insulin levels, 14 mU/l initially and 9 mU/l after metformin, compared to 25 mU/l initially vs 19 mU/l after metformin in the obese women studied by Vague et al. The difference in initial insulin levels between the present study and that by Vague et al and the change induced by metformin might be one explanation for the lack of effect on PAI-1 in study V. The effectiveness of metformin may vary in different groups, which might be an explanation for the variable effect of metformin on PAI-1.

The effect of sulphonylurea compounds on fibrinolysis has been debated. These drugs enhance insulin release, clearly an unwanted effect in non-diabetic subjects with hypertension. Furthermore, evidence has been presented that the fibrinolytic activity in diabetics decreased during treatment with certain sulphonylurea compounds as compared to dietary treatment or phenformin (6). However, this was not the case for gliclazide (49). These findings can probably be explained by the effect of insulin on PAI-1 and the fibrinolytic process as discussed above.

G. Fibrinolytic components during a hyperinsulinemic, euglycemic clamp

PAI-1 antigen and activity as well as t-PA antigen levels decreased and t-PA activity increased gradually during the 2 hours hyperinsulinemic, euglycemic clamp study (study VI). Both PAI-1 antigen and activity and t-PA activity tended to return to the initial levels

1 hour after the insulin infusion was stopped. The plasma insulin level was kept at 84±12 mU/l and the blood glucose level maintained at 4.9±0.6 mmol/l. C-peptide decreased during the clamp and increased to the initial level 1 hour after the insulin infusion was stopped. PAI-1 and t-PA antigen or activities were not affected by saline infusion for 2 hours.

Thus, unexpectedly in comparison with the in vitro experiments (2) fibrinolytic activity increased during the hyperinsulinemic, euglycemic clamp. As euglycemia was maintained, the increased fibrinolysis cannot be explained by a secondary hypoglycemia. When the insulin infusion was stopped, PAI-1 levels increased which is consonant with the short half-life of t-PA (91) and its rapid inhibition of PAI-1 (30). This unexpected finding could have several possible explanations which still are compatible with the concept that chronic hyperinsulinemia stimulates PAI-1. A previous study has shown an increased fibrinolysis, measured as diluted blood clot lysis time, during an insulin tolerance test and hypoglycemia (55). One explanation could be an increased elimination of PAI-1 via increased binding to the endothelium, the platelets or the hepatocytes. The ability of insulin to lower the plasma triglycerides and to inhibit FFA release from the fat cells may also play a role. A third, and probably the most likely possibility, is an activation of the sympatho-adrenal system during the hyperinsulinemic, euglycemic clamp (79). In fact, increased fibrinolytic activity has been shown after an injection of adrenaline and after physical exercise (16). Such effects are probably mediated via the β_2-receptors (46, 74).

A diurnal fluctuation of the fibrinolysis also exists with higher fibrinolytic activity in the afternoon than during the night and early morning (68). This pattern may be due to a diurnal fluctuation of the insulin antagonistic hormones (16).

CLINICAL CONSIDERATIONS

Importance of obesity and fat distributions

Studies I-III showed higher lipid levels, impaired glucose metabolism, decreased fibrinolytic activity and higher blood pressure in obese compared to non-obese subjects. Lean women with a high WHR had a lower risk factor profile than obese women with a low WHR. These findings show the importance of obesity for precipitating the risk factors associated with an abdominal fat distribution. There is evidence that fat distribution pattern may, at least in part, be genetically determined (24). Even if this is the case, it may be concluded from the present study that the high WHR in postmenopausal women plays a limited role in the absence of obesity. In contrast, when combined with obesity a high WHR clearly enhances the various metabolic and fibrinolytic aberrations. It is, thus, still important to identify risk individuals with obesity and institute therapy particularly when combined with a high WHR.

Importance of hypertension

Hypertension is a strong risk factor for cardiovascular disease. Hypertension, even of a mild degree and without obesity, was associated with elevated insulin, cholesterol, triglyceride, fibrinogen and PAI-1 levels in study IV. These findings link metabolism to thrombogenesis and may further increase the risk for cardiovascular events. However, it should be pointed out that all hypertensives may not be insulin resistant. There are patients with renal, hormonal or other causes of secondary hypertension who usually are excluded in studies of "normal" essential hypertension.

The hypertensive men in the present study represent healthy, middle-aged men with untreated, mild, uncomplicated hypertension living in a well developed and well nourished society. This population is by far the most common and growing population group and, hence, also important to study and treat. The present studies clearly support the concept that hypertension may be but one result of a much more ubiquitous abnormality and risk

factor complex. If hyperinsulinemia and insulin resistance indeed are the common denominators for the metabolic and thrombogenic factors, which the present findings suggest, they may be even more important to treat than a moderately elevated blood pressure per se.

Treatment of insulin resistance

Hyperinsulinemia and insulin resistance are found in a number of conditions which are associated with an increased risk for cardiovascular disease such as diabetes, abdominal obesity and hypertension. Common findings in these individuals are elevated lipid levels, blood pressure, plasma fibrinogen and PAI-1 level. The finding in this study support the concept that identifying and treating insulin resistance and hyperinsulinemia should be an important objective for society. Unfortunately, limited tools are at our disposal to achieve this goal. One important strategy must be to decrease body weight through an appropriate diet and increased physical activity. This strategy can also be widely recommended in the whole population considering the great number of overweight individuals.

A potential genetic part of the "metabolic syndrome" may not be easy to manage with non-pharmacologic treatment only. Current antihypertensive treatment has decreased the stroke mortality rate but has had less effect on the incidence of coronary heart disease (33). This may be due to the inability of the agents to normalize the various metabolic and fibrinolytic aberrations noted. In fact, both blood lipid levels and blood pressure have to be lowered to see any beneficial effect on coronary heart disease morbidity (114).

A promising possibility may be to treat hypertensive and other insulin-resistent individuals with an agent which enhances insulin action. In the present study, metformin was given to non-obese, non-diabetic men with mild untreated hypertension. Both lipid and blood pressure levels decreased markedly. However, no effect was seen on plasma fibrinogen or PAI-1 levels but a tendency for an increased fibrinolytic activity, due to an increased t-PA activity, was noted. Results in obese individuals also support a favourable effect of

metformin on the risk factor profile (12, 135). It seems important for the future to develop new safe efficacious agents with a similar or even more pronounced effect but without the undesirable side-effects of metformin.

SUMMARY AND CONCLUSIONS

The importance of obesity for the metabolic abnormalities associated with a high WHR was studied in postmenopausal women. Glucose and lipid metabolism as well as fibrinolytic factors were also studied in non-obese men with mild, untreated hypertension.

- Blood pressure, insulin resistance and lipolytic response in the adipose tissue were higher in obese than in non-obese women irrespective of WHR.

- t-PA activity was lower, PAI-1 activity, fibrinogen and triglycerides higher in obese women with a high WHR as compared to equally obese women with a low WHR. No differences in metabolic or fibrinolytic factors were seen between the two groups of non-obese women with different WHR.

- Lean women with a high WHR had a lower risk factor profile than obese women with a low WHR.

- Lean men with mild, untreated hypertension had higher plasma insulin, cholesterol, triglyceride, fibrinogen and PAI-1 levels than weight-matched men with normal blood pressure.

- A strong positive correlation was seen between plasma insulin and PAI-1 levels. PAI-1 was also correlated to body weight, blood pressure, blood lipids and degree of insulin resistance.

- Metformin increased peripheral glucose uptake, decreased cholesterol, triglyceride and insulin levels. Blood pressure was also markedly decreased while the effect on the fibrinolytic process was less pronounced.

- Acute hyperinsulinemia with maintained euglycemia increased the t-PA activity and decreased PAI-1 activity and antigen levels.

- The results indicate that insulin resistance and chronic hyperinsulinemia are associated with an increased thrombogenesis. Acute hyperinsulinemia acts in an opposite direction, possibly due to a concomitant activation of the sympatho-adrenal system by insulin.

- Obesity, especially abdominal obesity, and hypertension are associated with insulin resistance, hyperinsulinemia, elevated blood lipids, fibrinogen and PAI-1 levels. Non-obese women, irrespective of WHR, did not show these aberrations. Treatment of insulin resistance in hypertension improved the risk factor profile including the blood pressure.

- These findings support that insulin resistance is related to increased risk for atherosclerosis and thrombogenesis.

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