Influence of regional fat masses assessed by dual X-ray absorptiometry (DXA) on insulin resistance and dyslipidaemia in obese subjects. Cross sectional - and weight loss studies

by

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1. Acknowledgments

The present study was carried out, while being a practitioner in clinical endocrinology in close connection with the Osteoporosis Clinic at the former Betanien Hospital during 2000-2004, later at Spesialistsenteret Pilestredet Park in Oslo. While dual X-ray absorptiometry has been used for measurement of bone density at the Osteoporosis Clinic for a long time, the technique was expanded to the study of body fat at the Overweight Clinic around 2000. Today, DXA has probably become the method of choice for the study of body composition in overweight/obese patients in clinical practice. It has also been shown to be very valuable for follow-ups during weight loss.

Above all, I am deeply indebted to my supervisor dr.med. Johan Halse, founder and daily head of the Osteoporosis Clinic for giving me the incentive and inspiration for this thesis. I am deeply grateful for his ever present availability and readiness for discussions, his valuable critical comments, and good advice for the project based on a superior knowledge in endocrinology. I am especially grateful for his faith in my ability to fulfil this project in awareness that without his contribution and excellent facilities provided by the use of DXA instruments situated in joint localities, the present study had not been possible.

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Finally, I want to thank professor dr.med. Erik Fink Eriksen for critical comments.
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## 2. Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CFM</td>
<td>Central fat mass</td>
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<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<td>DXA</td>
<td>Dual X-ray absorptiometry</td>
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<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FM</td>
<td>Fat mass</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis model assessment</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
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<tr>
<td>IVGTT</td>
<td>Intravenous glucose tolerance test</td>
</tr>
<tr>
<td>MCRestOGTT</td>
<td>Metabolic clearance rate estimated OGTT</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>OGGT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>QUICKI</td>
<td>Quantitative insulin check index</td>
</tr>
<tr>
<td>SAAT</td>
<td>Subcutaneous abdominal adipose tissue</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral abdominal tissue</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very light density lipoprotein</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-hip ratio</td>
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3. List of papers


These papers are referred to by their roman numerals in the text.
4. Introduction

The present study was undertaken to increase our knowledge of the effect about regional fat masses assessed by DXA (dual X-ray absorptiometry) in a cross sectional manner, and the effect of weight loss on the metabolic aberrations related to insulin resistance and dyslipidaemia in obese women and men.

4.1. Epidemiology of obesity

The epidemic of obesity took off from 1980 and has been increasing in almost all countries ever since. Only in 1997 did WHO accept that the consequences of overweight and obesity constituted a major global health problem (WHO) (1), (James 2008) (2). By then (1995), BMI (body weight (kg)/ height (m²)) was accepted as the method of choice for crudely assessing degrees of under/overweight (James 2008) (2). In the First National Health and Nutrition Examination Survey (NHANES) (1960-62), an estimated 31.6% of adult men and women were overweight (BMI 25.0-29.9 kg/m²), and 13.4% were obese. By the same survey (NHANES) (1999-2000), the proportion of overweight adults had increased only slightly, while the proportion of obese adults had risen dramatically approaching roughly 35% for women and 30 % for men (Calle et al. 1999) (3), (Manson et al. 2004) (4). Although highest in the US, the prevalence of obesity varies with ethnic origin approaching 20% in Europeans, following a similar trend by a lag period of about 10yrs, and 5% in the Japanese (James 2008) (2). In Norway, cross sectional surveys of the prevalence of obesity in the county of Nord-Trøndelag of all inhabitants aged > 20 years from 1984-1986 (n = 85100), and from 1995-1997 (n= 92434) exhibited an increase from 7.5 to 14% in non-diabetic men, and from 13 to 18% in non diabetic-women. The increase was particularly great in men < 60 years of age; and in women < 50 years of age (Midthjell et al.1999) (5).
4.2. Disease and mortality risk

BMI in itself is a strong predictor of overall mortality both above and below an optimum of about 22.5-25 kg/m² (J-curve) (Calle et al.1999) (3), (6). The progressive excess mortality above this range is due mainly to cardiovascular diseases. At BMI 30-35 kg/m² median survival is reduced by 2-4 years; at 40-45 kg/m² it is reduced by 8-10 years, which is comparable to smoking (6). The risk of type 2 diabetes and cardiovascular disease increase through all levels of BMI (Rudermann et al.1998) (7), as well as other obesity related diseases: cancer, gall bladder disease, fatty liver disease, pancreatitis, phlebitis, gout, dermatologic problems, polycystic ovary syndrome and infertility. The risk of death from all causes increases throughout the range of moderate overweight to severe obesity, assessed by BMI, for both men and women in all age groups as demonstrated in a prospective study of more than one million adults in the US (Calle et al.1999) (3). Even in developing countries, the adverse health consequences of overweight and obesity have begun to replace undernourishment and infection as major causes of early death and disability.

4.3. The metabolic syndrome

Initially described by Reaven in 1988 (8) the syndrome-X, consisted of cardiovascular and metabolic risk factor clustering with insulin resistance, glucose intolerance, hyperinsulinaemia, dyslipidaemia (decreased HDL-cholesterol, increased very-low-density lipoprotein triglyceride) and hypertension. This concept, today known as the metabolic syndrome, has been subject to numerous modifications, and now includes abdominal obesity (National Cholesterol Education program Adult Treatment Panel III 2006) (9) (table 1), (WHO 1999) (10), (Haffner 2006) (11), (International Diabetes Federation) (1999) (12).
Table 1. Adult Treatment Panel III: individual risk factors for the metabolic syndrome.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Defining level</th>
</tr>
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<tbody>
<tr>
<td>Abdominal obesity (waist circumference)</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>&gt; 102 cm</td>
</tr>
<tr>
<td>Women</td>
<td>&gt; 88 cm</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&gt; 1.7 mmol/l</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>&lt; 1.0 mmol/l</td>
</tr>
<tr>
<td>Women</td>
<td>&lt; 1.3 mmol/l</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>&gt; 130/ &gt; 85 mmHg</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>&gt; 5.6 mmol/l</td>
</tr>
</tbody>
</table>

Diagnosis is established when at least three of these risk factors are present. Adapted from Haffner (ref 11)

There is, however, debate as to the ability of each component of the syndrome to predict the metabolic syndrome. The prediction of morbidity and mortality also differs between the various definitions (Hunt et al. San Antonio heart study 2004) (13). Norberg et al. (2007) (14) suggested that the metabolic syndrome is a distinct pathophysiological entity with abdominal obesity as the central feature of the syndrome (figure 1). However, no consensus on a single unifying pathophysiological mechanism has been reached. Critics of the use of the metabolic syndrome in the clinical setting have argued that the constellation of risk factors for the metabolic syndrome does not offer more than the sum of its parts in term of diagnosis and management (Kahn et al. 2005) (15). In fact, a recent report of WHO Expert Consultation (Simmons et al. 2010) (16), emphasized that the metabolic syndrome is a premorbid condition rather than a clinical diagnosis, and should thus exclude individuals with established diabetes or known cardiovascular disease.
4.4. Treatment.

Sufficient information is available from numerous observational studies and small or short-term randomized clinical trials, that weight reduction and physical activity offer substantial health benefits (National Institutes of Health, National Heart, Lung, and Blood Institute, Obesity Education Initiative 2003) (17), (Halbert et al. 1997) (18), (Stefanick 1999) (19). It has been shown that a moderate weight loss of 5-10% (Manson et al. 2004) (4) (17) is associated with improvement in risk factors for cardiovascular disease i.e. hypertension, insulin resistance, glucose tolerance and lipid profile. As the prevalence of obesity, and especially extreme obesity, has increased dramatically, so has the usage of bariatric surgery (most often the Roux en Y gastric by pass surgery). In Norway subjects are qualified for surgery with BMI > 40kg/m², or BMI > 35kg/m² if at least associated with one obesity-related comorbidity. The recent study by Hofso et al. 2010 (20) is the first controlled clinical trial,
comparing bariatric surgery with conventional therapy of one-year duration. The resolution of diabetes and cardiovascular risk factors in obese subjects were found to be improved after both treatment strategies. However, the effect was greatest in those treated with gastric bypass surgery. The study showed that weight reduction and not treatment of choice, predicted improvement in glycemic control and systolic blood pressure. Furthermore, most of the beneficial effects were observed after a weight reduction of > 10%. In contrast, liposuction has not shown improvement in indices of insulin resistance or serum lipids at 10 or 208 weeks after surgery (Mohammed et al. 2008) (21).

**4.5. Why assess body fat distribution?**

Although abdominal adipose tissue has for many years been known as the most important risk factor for diabetes and cardiovascular disease, leg subcutaneous adipose tissue (SAT) has been shown to have important modulating effects on insulin resistance and serum lipids in overweight/obese patients. Vague 1947 (22) was the first to put forward the hypothesis, that obesity is not a homogeneous condition. Vague described “android or male type obesity” as more often associated with mortality and risk for diabetes, hyperlipidaemia, hypertension, and atherosclerosis of coronary, cerebral and peripheral vessels, than the “gynoid” lower body or gluteofemoral, female type of fat distribution (anthropometric studies). A large number of studies have during the last two decades demonstrated that the detrimental influence of abdominal obesity on metabolic processes, is mediated by the intraabdominal fat depot: The visceral abdominal adipose tissue (VAT) was initially found to correlate with glucose intolerance in the presence of hyperinsulinaemia during an oral glucose tolerance test (OGTT), suggesting an insulin resistant state (Deprès J-P et al. 1987) (23), (Pouliot M-C et al. 1992) (24), an effect that was independent from total adiposity and subcutaneous abdominal adipose tissue (SAAT). However, this view has been challenged by Abate et al. 1995 (25). They found that truncal (thorax + abdomen) obesity, determined by skinfold thickness and
MRI of the abdomen, reflected at least as strong a correlate of insulin sensitivity (evaluated by the euglycemic clamp) just as well as VAT in obese men, while subcutaneous adipose tissue of the extremities was less influential. Goodpaster et al. 1997 (26) found an unfavourable effect of thigh intramuscular and subfascial fat on insulin resistance in a combined population of overweight and obese men and women, while thigh SAT was not. This effect of thigh SAT on insulin resistance is in accordance with the known favourable effect of leg fat measured by DXA on lipid profile with a broad range of BMI, first published in 1997 (Williams et al.) (27). But it was not until 2003, that Tankö et al. (28) and van Pelt et al. (2002) (29) found a favourable effect of leg fat on insulin resistance in a combined population of normal/overweight/obese postmenopausal women.

5. Methods for assessment of body composition

5.1. Anthropometric indexes of regional FM distribution and abdominal FM.

5.1.1. BMI, waist-to-hip ratio (WHR), waist circumference, sagittal diameter

**BMI.** The most widely used index for definition of overweight (BMI > 25 kg/m²) and obesity (BMI > 30 kg/m²), is not an index of FM distribution. (See sections 4.1, 4.2 and 5.3). Several other anthropometric indexes have been clinically useful for assessment of regional FM distribution and abdominal FM, and have been widely used in larger clinical trials. They can be summarised as follows:

**WHR (Waist-to-hip ratio).** The WHR has been the most widely used index of regional adipose tissue distribution. In a standing position, waist circumference is measured at the mid level between the iliac crest and the lower lateral costal margin, with the patient fully exhaled. The hip circumference is measured as the maximal distance around the hip, again applying tight pressure on the measuring tape. Current guidelines propose cut-off points of 1.0 in men and 0.85 in women to define abdominal obesity (Expert panel 1998) (30), (Pischon et al.)
WHR has been shown to be associated, albeit moderately, with the amount of VAT measured by CT or MRI (Ashwell et al. 1985) (32). WHR has been used to investigate the relation between regional adipose tissue distribution and metabolic profile and predicted subsequent diabetes in men (Ohlson et al. 1985) (33), and coronary disease in both women and men (Larsson et al. 1984) (34), (Lapidus et al.1984) (35). WHR was more predictive than BMI and skinfold thickness, and its effect was found to be independent of the level of total obesity.

**Waist circumference.** Current guidelines propose cut-off values of 102 cm for men and 88 cm for women to define abdominal obesity (Expert panel 1998) (30), (Pischon et al. 2008) (31). The use of waist circumference has been predominantly proposed in the past decade, largely because waist circumference is easier to measure and to interpret than WHR. Clinical categories of BMI, waist circumference and WHR have been shown to be useful to estimate overall metabolic risk in the general population, cardiovascular morbidity as acute myocardial infarction in the INTERHEART study (Yusuf et al. 2004) (36), and mortality in relation to abdominal obesity (Pischon et al. 2008) (31).

**Sagittal diameter.** Kvist et al. (1988) (37) was the first to demonstrate that the sagittal diameter measured on a CT scan was closely related to the volume of visceral fat ($r > 0.90$) in men and women over a wide range of BMI. Although lower correlations in obese men and women (van der Kooy et al. 1993) (38), correlations between sagittal diameter and waist circumference are quite high ($r = 0.84$) in obese men and ($r = 0.76$) in obese women (Van der Kooy et al.1993) (38). Not much difference between VAT and the correlations with the waist circumference ($r = 0.82$) and the sagittal diameter ($r = 0.85$) in men covering a wide range of fatness was found by Després et al.1991 (39). Because this measurement requires appropriate equipment and skilled personnel, the use of waist circumference is the preferred anthropometric measure in obese subjects (Seidell et al. 1996) (40).
5.1.2. Comparison between the anthropometric measurements.

A study from Pouliot et al. 1994 (41) evaluated three anthropometric indexes and their association with VAT and SAAT measured by CT. Briefly, as can be seen in table 2, there was a strong association between waist girth and body fat mass, and somewhat less between body fat mass and WHR.

Table 2. Correlations (r-values) between the anthropometric indexes and body fat mass, VAT and SAAT in 81 men and 70 women.

<table>
<thead>
<tr>
<th></th>
<th>Body FM</th>
<th>VAT</th>
<th>SAAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>WHR</td>
<td>0.70</td>
<td>0.55</td>
<td>0.71</td>
</tr>
<tr>
<td>Waist circumf, cm</td>
<td>0.93</td>
<td>0.94</td>
<td>0.77</td>
</tr>
<tr>
<td>Sagittal diameter, cm</td>
<td>0.87</td>
<td>0.95</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Adapted from Pouliot et al. (ref 41)

5.1.3. Limitation of anthropometric measures

BMI may be misleading as measure of obesity because neither weight nor height, take into account individual differences of fat distribution or lean body mass. Also, BMI does not display the large individual differences in fat mass distribution. Individual differences in VAT remain considerable, even when subjects with relatively similar BMI and percent body fat are investigated (Bouchard et al. 1993) (42). In fact, VAT is about twice the amount in men than in premenopausal women for a given amount of total body fat (Lemieux et al. 1993) (43).

Pouliot et al.1994 (41), in a study using a large sample of men and women, showed that the use of WHR as a single anthropometric index of cardiovascular risk, is limited by the fact that for a given WHR value, there may be large variations in the level of total body fat and in the level of VAT, that are likely to be associated with important variations in the metabolic profile. Waist circumference has been reported to be more closely correlated with the level of
VAT and associated metabolic variables than the WHR in both sexes (Pouliot et al. 1994) (41). However, nearly one-quarter of obese individuals or individuals with a large waist do not have elevated VAT, whereas 10% of women and 20% of men with a normal waist circumference have high VAT, suggesting that misclassification exists within clinically useful adiposity categories (Pou KM et al. 2009) (44). Additionally, there may be individuals that develop cardiometabolic complications related to adiposity, but without BMI or waist circumference in high risk zone. Particularly in the aging population, discordance between SAAT and VAT tissue can be found, so that less SAAT and higher VAT may be associated with lower BMI and waist circumference (Pou et al. 2009) (44). Studies have shown that there is a need to develop sex-specific cut-off points appropriate for different populations, to assess risk for cardiovascular disease. For example South Asians, living in urban societies, have a higher incidence of abdominal fat distribution and cardiovascular complications for a given level of BMI than Europeans (Mc Keigue 1996) (45). Abdominal obesity has also been shown to be less strongly associated with risk factors for cardiovascular disease and type 2 diabetes in black than in white women (Dowling et al.1993) (46).

5.2. Compartment models

Earlier methods, such as hydrodensitometry by underwater weighing or air displacement plethysmography, represent two-compartment models in which the body is divided into two parts. One consists of body fat that is determined indirectly by subtraction of all the remaining tissues representing fat-free mass, from total body weight. A three-compartment model extends fat free mass to be divided into solids (mainly protein and minerals) and water. It also requires a measure of total body water. Four-compartment models based on the former models, would need an accurate measure of the protein/mineral compartments by neutron activating analysis for body protein and DXA for bone mineral content in addition to that of total body water (Ellis 2000) (47). There is good agreement between bone mass, fat mass and
fat-free mass measured by DXA and a multicompartiment model based on neutron activating analysis (Heymsfield et al.1991) (48). Alternatives to the four-compartment model, not involving under water weighing, that have been developed for estimating fat free-mass as a three-compartment model: the sum of body cell mass, extracellular volume and extracellular solids by using radioactive K isotopes and dilution techniques (Ellis 2000) (47). In clinical studies, these methods have been largely substituted by CT, MRI and DXA. All these methods have been evaluated with each other, and despite differences in precision, the correlations between these methods are good (Ellis 2000) (47). It has also been shown that CT provides a more accurate measurement of VAT than MRI (Desprès et al.1996) (49), (Seidell et al.1990) (50). Unfortunately, the major disadvantage with CT is the high radiation dose required for imaging. The standard procedure is consequently to take only one image slice as representative of the abdominal fat mass.

5.3. DXA, CT and MRI

Due to the use of two different wave-lengths of X-ray radiation, the DXA method distinguishes between fat mass and lean mass in soft tissues, whereas the CT provides muscle tissue volume and adipose tissue volume. Adipose tissue by CT consists of 80% fat and a lean compartment comprising, water, proteins and minerals of 20% (Snijder et al. 2002) (51) This lean compartment within adipose tissue is measured as lean tissue by DXA, and for comparison of FM between the methods, this lean tissue has to be subtracted.

CT has been demonstrated to be an accurate and precise technique for measuring soft tissue composition. It permits differentiation between VAT and SAT in a cross-section of the body (Van der Kooy et al. 1993) (52), (Plourde et al. 1997) (53). MRI compares well with CT-measured fat, and both techniques have a similar accuracy in comparison with chemical analysis (Mitsiopoulos et al.1999) (54), (Abate et al. 1994) (55), (Rossner et al. 1990) (56). To our knowledge no corresponding studies have been performed with DXA.
5.4. Assessment of visceral fat

Use of CT as reference model. In normal weight males, Busetto et al. (1992) (57) found significant correlations between waist circumference and visceral fat ($r = 0.70$) and WHR ($r = 0.67$), while in obese men, these correlations were not significant ($r = 0.50$) and ($r = 0.41$). In normal women, waist circumference was correlated ($r = 0.65$), but not WHR, while in obese females, neither WHR nor waist circumference were correlated with visceral fat. Snijder et al. (2002) (51) reported high correlations between CT and DXA ($r = 0.679$ and $0.835$ for men and women, respectively), but data from strict obese subjects were not provided.

Use of MRI as reference model. In normal weight men, WHR, waist circumference and DXA were equally useful for assessment of visceral fat ($r = 0.90$, $r = 0.89$, $r = 0.87$, respectively) (Kamel et al. 1999) (58). In normal weight women, DXA was slightly more useful ($r = 0.88$-$0.90$) than waist circumference measurement ($r = 0.77$), whereas WHR was without association to VAT. In strict obese women (BMI > 30 kg/m²), WHR, waist circumference and DXA were as useful as MR ($R = 0.70$-$0.75$). In obese men, however, DXA was moderately ($R = 0.46$), while waist circumference or WHR was not correlated to MRI (Kamel et al. 2000) (59).

6. DXA

6.1. Description

DXA, originally developed for measurement of bone density, is based on the division of the body mass in three compartments with different density: Fat mass, lean mass and bone mass. The X-ray consists of two energies that are attenuated by passage through tissues, and the degree of attenuation is related to thickness, chemical composition and density of the tissue. The mathematical calculation of body composition is based on the differences in these properties, and that the passage from one tissue to another will be recorded as a change in X-ray attenuation.
The three DXA devices we used were:

- Lunar Expert (software version 1.92) (Expert)
- Lunar Prodigy (version Encore) (Prodigy)
- Hologic Delphi W (version 11.1) (Delphi)

They differ in beam-angle that is narrow (4°) for Prodigy, wide (30°) for Delphi and intermediate (12°) for Expert. The X-ray direction of the Lunar instrument is opposite of the Hologic and the number of scintillators differs between the systems as well as differences in software algorithms (i.e. bone detection and distribution of soft tissue above bone). All instruments are subject to daily quality controls with the use of a whole body phantom simulating fat tissue, fat-free tissue and bone.

**DXA measurements**

A conventional body composition and regional fat masses are measured as follows: The trunk region includes the chest, separated from the head by the chin and the abdominal/pelvic area. The legs are separated from the pelvis: From a point at the extension of the horizontal line formed by the superior border of the iliac bone intercepted laterally by a line from both humeroscapular joints separating the arm, a line through the femoral neck is drawn that intercepts between the legs (Figure 2).
6.2. DXA in obesity

Most body composition analysis by DXA has been performed on subjects with BMI < 30 kg/m², showing influence of age, gender, and ethnicity on body composition (Gallagher et al. 1996) (60). Only few studies have been performed on obese subjects. This may be related to the necessity for restriction to those with weight < 125 kg and a body form that suits the examination table. In obese patients, it may well be that the DXA method is less precise because of deviation of the X-ray beam during the traverse of the obese body. Therefore, we investigated the relationship between body composition assessed by DXA fan-beam instruments and anthropometry performed on 136 obese men and women. This study reported excellent correlation between scale-weight and estimated weight by DXA (r = 0.993 (SEE 1.3 kg)) for the 3 different fan-beam instruments. Fat masses correlated highly to weight, BMI and waist circumference in postmenopausal women (r = 0.57 to 0.88) and to a lesser, but...
highly significant degree, in premenopausal women and men. WHR showed little or no relationship to fat masses. BMI explained 43 to 70 % of the variance in total FM (Table 3). Consequently, we postulated that DXA may be a very valuable instrument for further exploration of the relationship between fat mass and its distribution, and risk factors for diabetes and cardiovascular disease, also in strict obese subjects.
Table 3. Pearson's correlation coefficients between anthropometric variables and DXA-measurements of body composition in obese men and women.

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 46</td>
<td>n = 57</td>
<td>n = 33</td>
</tr>
<tr>
<td><strong>Trunk FM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.696‡</td>
<td>0.887‡</td>
<td>0.591‡</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.477‡</td>
<td>0.849‡</td>
<td>0.673‡</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>0.674‡</td>
<td>0.847‡</td>
<td>0.796‡</td>
</tr>
<tr>
<td>WHR (a)</td>
<td>0.25</td>
<td>0.335†</td>
<td>0.111</td>
</tr>
<tr>
<td><strong>Leg fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.724‡</td>
<td>0.822‡</td>
<td>0.442†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.654‡</td>
<td>0.789‡</td>
<td>0.535‡</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>0.348*</td>
<td>0.573‡</td>
<td>0.677‡</td>
</tr>
<tr>
<td><strong>Total fat mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.828‡</td>
<td>0.887‡</td>
<td>0.593‡</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.656‡</td>
<td>0.837‡</td>
<td>0.691‡</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>0.591‡</td>
<td>0.778‡</td>
<td>0.838‡</td>
</tr>
<tr>
<td><strong>Lean body mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.576‡</td>
<td>0.502‡</td>
<td>0.694‡</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.402†</td>
<td>0.239</td>
<td>0.168</td>
</tr>
</tbody>
</table>

* = p < 0.05, † = p < 0.01, ‡ = p < 0.001, (a) = no correlations between waist- to- hip-ratio and other fat masses.

6.3. DXA in weight loss.

Measurement of body composition changes with weight loss during energy restriction in postmenopausal women was examined by Mahon (2008) (61), who found that on an
individual basis (a four-compartment model being the reference model), the DXA may be used to obtain an estimate for the changes in body composition for a postmenopausal woman (BMI 29.0 ± 2.9 kg/m²). Further support for DXA, as a reliable method, is provided by Ritz et al. (2007) (62), who found that compartment changes induced by weight loss, were accurately evaluated by DXA.

6.4. Why DXA?

DXA is presently the preferred method for measuring body composition. Owing to high precision, low radiation dose and high scan speed, fan-beam instruments are increasingly in use in clinical practice. Trunk fat and total abdominal fat by DXA, have been shown to be highly correlated with risk factors for cardiovascular disease (van Pelt et al. 2002) (27), (Paradisi et al.1999) (63).


7.1. Surrogate measures vs. euglycemic-hyperinsulemic clamp technique.

The euglycemic-hyperinsulinemic clamp technique is the gold standard method for estimation insulin sensitivity because it directly measures insulin action on glucose utilization under steady-state conditions (de Fronzo et al. 1979) (64), (Matsuda et al. 1999) (65), (Monzillo et al. 2003) (66). However, this method is time consuming and thus not suitable for large scale trials (Rabasa- Lhoret et al. 2001) (67).

A number of surrogate indexes have been derived from fasting glucose levels to evaluate insulin resistance and sensitivity, such as HOMA-IR (Homeostasis model assessment) and QUICKI (Quantitative insulin check index) (Hansson et al. 2000) (68), (Bastard et al. 2003) (69). These two surrogate indexes are mathematically related; i.e. QUICKI is proportional to 1/log HOMA-IR. Their major advantage is their simplicity and their validation vs. the euglycemic- hyperinsulinemic clamp technique (Wallace et al. 2004) (70), (Hanley et al.
HOMA-IR is defined as by the product of fasting glucose and fasting insulin divided by a constant (glucose mmol/l x insulin pmol/l)/135. This constant or denominator is a normalizing factor i.e. the product of normal fasting plasma glucose of 4.5 mmol/l typical for a “normal” healthy individual and insulin of 30 pmol/l (Muniyappa et al. 2007) (76). Multiple independent studies reported excellent linear correlations between QUICKI and glucose clamp estimates of insulin sensitivity in healthy subjects, obesity, diabetes, hypertension, and many other insulin resistant states (Katz et al. 2000) (73), (Mather et al. 2001) (77), (Chen et al. 2003) (78), (Bastard et al. 2001) (79). Over a wide range of insulin sensitivity/resistance levels, QUICKI has been found to have a substantial better linear correlation with insulin sensitivity measured by the hyperinsulinemic glucose-clamp (r = 0.80-0.90) than measures derived from the HOMA-IR. (Munyiappa et al. 2008 (76), Yokoyama et al. 2003) (80), (Mather et al. 2001) (77), (Katsuki et al. 2002) (81), (Skrha et al. 2004) (82). However, log HOMA-IR was found to be roughly comparable to QUICKI in this regard. Other studies have shown similar high correlations (r = 0.83-0.89) for HOMA-IR (Matthews et al. 1985) (83), and HOMA-IR or log HOMA-IR have been used extensively in large epidemiological studies, prospective clinical trials and clinical research studies (Munyiappa et al. 2008) (76).

7.1.1. HOMA-IR in obesity vs. other insulin resistant states.

In 88 overweight and obese (BMI ~32.5 kg/m²) postmenopausal women, Malita et al. 2006 (85) found that correlations between insulin sensitivity/resistance indices (QUICKI, fasting insulin, HOMA-IR) vs. insulin sensitivity derived from the hyperinsulinemic euglycemic clamp method, were similar (r = 0.40-0.49). No substantial differences were found between age, genders (r = -0.80 for men and women), obese (r = -0.765) vs. non obese (r = -0.80), non-diabetic (r = -0.754) vs. diabetic (r = -0.695) (Bonora et al. 2000) (75). Good correlations
were observed for log HOMA-IR with insulin sensitivity derived from the clamp method in 
obese subjects with $r = 0.46$ at different clamp levels, and $r = 0.35$ in lean subjects. Thus, in 
subjects with severely impaired or absent beta-cell function, HOMA-IR may not give 
appropriate results, and transformation to log HOMA-IR is useful for evaluation of insulin 
resistance in individuals with glucose intolerance, mild to moderate diabetes, and other insulin
resistant states (Munyiappa et al. 2008) (76).

7.1.2. QUICKI in obesity vs. other insulin resistant states.
Quantitative insulin sensitivity check index (Katz et al. 2000) (73), (Yokohama et al. 2003) 
(80) has shown high correlations with the hyperinsulinemic euglycemic clamp method ($r = 
0.89$) for obese subjects. Mather et al. (2001) (77) reported data from 256 clamp studies in 
152 subjects, lean (n = 69), obese (n = 72) and type 2 diabetes (n = 11). Good correlations 
were observed between QUICKI and insulin sensitivity derived from the clamp method in 
obese subjects ($r = 0.73$ at different clamp levels) and $r = 0.40$ in lean subjects.

7.2. Surrogate measures vs. insulin suppression test
It was not until 2004 that Kim et al. (85) (table 4) reported on surrogate measures for insulin 
resistance and sensitivity, comparing normal, overweight and obese subjects by using insulin
suppression test as “gold standard” reference model (Greenfield et al.1981) (86). Fasting 
plasma insulin, HOMA-IR and QUICKI were found to provide comparable information about 
insulin mediated glucose uptake, but did not explain more than 13 % of the variability of 
insulin action in normal weight, 13% in the overweight and 37% in obese subjects. In this 
study the best surrogate measure was Insulin-AUC (Areal Under Curve) with $R^2 = 0.48$ in 
obese subjects (Table 4).
Table 4. Pearson's correlation coefficients between steady state plasma glucose by insulin suppression test and surrogate measures of insulin resistance by degree of obesity.

<table>
<thead>
<tr>
<th></th>
<th>Normal weight</th>
<th>Overweight</th>
<th>Obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose</td>
<td>0.20 *</td>
<td>0.19 †</td>
<td>0.40</td>
</tr>
<tr>
<td>Fasting Insulin (log)</td>
<td>0.33</td>
<td>0.55</td>
<td>0.56</td>
</tr>
<tr>
<td>HOMA-IR (log)</td>
<td>0.36</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td>QUICKI</td>
<td>-0.33</td>
<td>-0.54</td>
<td>-0.61</td>
</tr>
<tr>
<td>I-AUC (log)</td>
<td>0.69</td>
<td>0.72</td>
<td>0.69</td>
</tr>
</tbody>
</table>

* P < 0.01, † P < 0.01, all other P-values are < 0.001.

Adapted from Kim et al. (ref 85)

7.3. Methods for assessment of insulin sensitivity based on glucose tolerance tests.

7.3.1. Intravenous glucose tolerance test.

The frequently sampled intravenous glucose tolerance test (FSIVGTT) (Bergman 1989) (87), measures insulin sensitivity by computerized analysis of glucose and insulin measurements of 180 minutes duration. Although reasonable correlations of estimates of insulin sensitivity from this method, compared to glucose clamp measurements in healthy subjects, have been obtained, correlations are weaker in insulin resistant populations (Beard 1986) (88). This method is nearly as labour intensive as the clamp method which represents an obstacle to its clinical usefulness.

7.3.2. Oral glucose tolerance test (OGTT)

Estimates of insulin sensitivity derived from OGTT, predict the development of type 2 diabetes in epidemiological studies (Hanley et al. 2003) (68). Furthermore, insulin sensitivity derived from OGTT correlates well with hyperinsulinemic-euglycemic clamp measures (Saad et al. 1994) (89), (Dalla Man et al. 2005) (90), (Munyiappa et al. 2008) (76). The advantage of
surrogates based on dynamic testing, is that information about insulin secretion can be obtained at the same time as information of insulin action. These tests (Schianca et al. 2003) (91) are based on changes in glucose and insulin values during a 2 h OGTT and have been shown to be highly correlated ($r = 0.80$) with insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp technique in non-diabetic and obese subjects (Stumvoll et al. 2000) (92). One such test, the MCRest OGTT (metabolic clearance rate estimated OGTT) (Stumvoll et al. 2000) (92), have been shown to be correlated with the hyperinsulinemic-euglycemic clamp only in obese subjects ($r = 0.61$, $P < 0.0001$) (Mari et al. 2001) (93). We therefore chose this test as an additional index of insulin sensitivity.

7.4. Beta-cell function

The relationship between glucose and insulin measurement in the basal state, may be explained as the balance between hepatic glucose output and insulin secretion, which is maintained by a feed back loop between the liver and beta-cells (Turner et al. 1979) (94). Basal plasma insulin levels are assumed to provide a measure of the degree to which basal plasma glucose are due to insulin resistance. The ratio of fasting insulin to glucose reflects beta-cell function and is calculated as HOMAsecr (Matthews et al. 1985) (83). (See also section 9.3.2). If the plasma insulin level is increased two-fold, the beta cells have to function at twice the normal rate just to overcome insulin resistance (Turner et al. 1979) (94). HOMAsecr declines with deteriorating beta-cell function.

Although HOMAsecr and the hyperinsulinemic euglycemic clamp measure insulin secretion and sensitivity in two different states, i.e. the basal and maximally stimulated state, respectively, they have been shown to be highly correlated ($r = 0.69$, $p < 0.01$) (Matthews et al. 1985) (83). The IVGTT and the OGTT yield measures of dynamic non-steady state insulin secretion and insulin sensitivity over the middle of the physiological range (Wallace et al. 2004) (70). (See also section 7.3.2). In obese subjects beta cell function, as measured by
OGTT, and when related to underlying insulin resistance, has been found to be similar to measures in lean subjects within different categories of glucose tolerance (Gastaldelli et al. 2001) (95). However, data from Japanese subjects indicate that obesity causes a decrease in insulin secretion, especially during the late phase of OGTT, even if glucose tolerance remains normal (Akehi et al. 2008) (96). Most importantly, beta cell function has been reported to be deteriorated with age in most studies (Chen et al. 1985) (97), (Fritsche et al. 2002) (98) (Chang et al. 2006) (99).

8. Mechanism of insulin resistance in obesity

8.1. Relation with lipid metabolism

The mechanism or mechanisms that explain the relationship between obesity and insulin-mediated glucose uptake is/are complex and to date largely unresolved (Kim et al. 2010 (100). Perhaps the most important mechanism is that insulin resistance in obesity is related to the failure to normally suppress free fatty acids (FFAs) in response to the increase in insulin or an increase of insulin during meal ingestion. The mechanism underlying this resistance to the antilipolytic effect of insulin is not clear (Jensen et al. 2008) (101).

Some central features underlying the resistance to the antilipolytic effect of insulin can be summarized:

1. Visceral obesity has been considered as the initial event that leads to insulin resistance by the increase in FFA (free fatty acid) levels antedating the components of the metabolic syndrome (Ohlson et al. 1985) (34), (Larsson et al. 1984) (35). Because of the higher metabolic activity with greater sensitivity to the lipolytic effects of catecholamines than SAT, VAT has been suggested to be the key factor predisposing to complications of obesity (Kissebah 1997) (102), (Björntorp 1991) (103). Because VAT is drained by the portal venous
system, visceral adiposity has been suggested to flood the liver and the systemic circulation
with FFA (Björntorp 1990) (104), (Kissebah 1989) (105).

2. This view has been challenged by Jensen et al 2008 (101). Human in vivo studies have also
shown that upper body SAT delivers a considerable amount of FFA to the systemic
circulation under basal and insulin suppressed conditions, probably greater than visceral fat.
In contrast, leg SAT lipolysis is the most sensitive to insulin suppression of FFA-release
(Jensen 2008) (101).

3. Another focus for understanding the link between obesity and insulin resistance has
recently been put on the importance of differences in fat-cell size within SAT. Briefly,
Kursawe et al. 2010 (106) found that adolescents with higher visceral fat were more insulin
resistant and had a greater proportion of small to large cells in SAT. They propose that
impaired adipose differentiation and lipogenesis decrease fat storage capacity in SAT,
necessitating displacement of fat to organs such as the liver and muscle. This ectopic fat
deposition has been suggested to lead to organ dysfunction and insulin resistance (Kursawe et
al. 2010) (106). Thus, the primary role of visceral fat may be contested (Kim et al. 2010)
(100).

4. LPL activity is an important first step in plasma triglyceride clearance and FFA delivery to
the adipocyte for deposit as triglycerides, particularly in the postprandial state (Fielding et al.
1998) (107). Insulin and glucose have been shown to stimulate adipose tissue lipoprotein
lipase (LPL) activity and to reduce LPL activity in the muscle, implying a preferential
postprandial partitioning of FFA toward adipose tissue and away from muscle (Farese et al.
1991) (108). In type 2 diabetes and obesity, LPL activity in adipose tissue is delayed, and LPL
activity in skeletal muscle is increased instead of decreased by hyperinsulinemia (Yost et al.
1995) (109), (Sadur et al. 1984) (110). This leads to an increase in FFA uptake and storage as
triglycerides in skeletal muscle.
5. FFAs impair glucose metabolism in insulin sensitive tissues, such as muscle, which is the most relevant site of insulin resistance (Boden 1995) (111), and liver (Schulman 2000) (112). Studies have also demonstrated that FFAs induce insulin resistance by initial inhibition of glucose transport (Kelley et al. 1996) (113) in skeletal muscle.

6. Inflammatory adipokines, secreted by enlarged visceral fat cells in obesity, have recently emerged as a possible link between obesity and insulin resistance (Fontana et al. 2007 (114), (Fried et al. 1998) (115), (Skurk et al. 2007) (116). These adipokines are associated with increased risk for cardiovascular disease (Gustafson et al. 2007) (117), (Trøseid et al. 2009) (118), as well as the inflammatory marker high sensitive CRP (Haffner 2006) (119) in the metabolic syndrome. It is therefore proposed that the addition of a marker of inflammation in a future definition of the metabolic syndrome will provide a more optimal prediction of cardiovascular disease and diabetes (Haffner 2006) (119).

8.2. Relation with hormones

Some central features are briefly described.

**Cortisol**

Cushing syndrome is characterized by a change in distribution of fat from peripheral to central parts of the body, mainly the abdominal region, but the clinical distinction between primary obesity and Cushing syndrome is not always easy. However, hypercortisolemic subjects have been found to have a significantly greater VAT area, than primary obese subjects (Wajchenberg et al. 1995 (120). Although plasma glucocorticoid levels are not elevated in obesity, the activity of the enzyme 11 beta-HSD1, which regenerates active glucocorticoids from inactive forms, is commonly elevated in omental adipose tissue, but not subcutaneous adipose tissue (Bujalska et al. 1997) (121), (Mazusaki et al. 2003) (122). This enzyme is suggested to play an important role in promoting visceral obesity (Mazusaki et al. 2003) (122). Further, the density of the glucocorticoid receptors is higher in visceral than in
other adipose tissues without decline after excess cortisol exposure (Björntorp 1997) (123). Studies of the hypothalamic pituitary axis in visceral obesity have also revealed alterations: Abnormalities of ACTH pulsatile secretion (Pasquali 1998) (124) with hyperresponsiveness of the hypothalamic-pituitary adrenal axis resulting in increased cortisol secretion have been invoked. In the presence of hyperinsulinaemia, this would tend to increase LPL activity and decrease lipolytic activity resulting in lipid accumulation (Björntorp 1997) (123).

**Testosterone**

A recent meta-analysis supports the presence of a sex-dependent association between testosterone and the metabolic syndrome: Testosterone and free testosterone levels are lower in men, but higher in women with the metabolic syndrome. In both men and women, the metabolic syndrome is associated with lower SHBG levels (Brand et al. 2010) (125). However, the relative influence of hormonal factors vs. fat mass, on insulin resistance and dyslipidaemia in obesity still remains poorly elucidated.

In men visceral fat mass is strongly and negatively correlated to plasma total and free testosterone and sex-hormone binding globulin (SHBG) concentrations (Seidell et al. 1990 (126), (Haffner et al. 1993) (127), (Haffner et al. 1994) (128), (Oh et al. 2002 (129). Low SHBG is proposed to be due to hyperinsulinemia (Haffner et al. 1988) (130). In moderately obese men, testosterone levels are decreased because of the low SHBG-binding capacity, but free testosterone levels are normal as are LH levels indicating a normal pituitary-gonadal axis. However, in morbidly obese men (BMI > 40kg/m2), total and free testosterone and FSH and LH levels are decreased suggesting a hypogonadotropic hypogonadism syndrome (Kley et al. 1981) (131), (Giagulli et al. 1994) (132). Changes in SHBG and bioavalable testosterone are all associated with insulin resistance and glucose levels, and proposed as being independent of adiposity (Phillips et al. 1993) (133). But the matter is controversial, as evidence suggest that VAT may be the principal factor in men, linking with risk factors as defined in the metabolic
syndrome (Phillips et al. 2003) (134). Also Vikan et al. 2010 (135) found that men with lower total testosterone and SHBG exhibited an increased risk of diabetes, and this risk seemed to be dependent on the degree of obesity.

In women visceral obesity is associated with elevated levels of total testosterone, free testosterone and a reduction in SHBG (Glass et al. 1989) (136). Studies in premenopausal women suggest that obesity through changes in SHBG levels leads to insulin resistance (Tschernof et al. 1999) (137), or that hyperandrogenicity by itself may be an additional determinant of hyperinsulinemia in obese women (Krotkiewski et al. 1990) (138). In postmenopausal women, a significant association between androgenicity and insulin sensitivity that is independent of obesity and central obesity has been found (Lee et al. 2004) (139), (Kalish et al. 2003) (140), (Oh et al. 2002) (129), (Ding et al. 2006) (141), (Khaw et al. 1991) (142). Philips 2008 (143) hypothesized that in women, free testosterone caused preferential accumulation of VAT and induced insulin resistance both directly and via VAT accumulation. He proposed that sex hormone alterations may cause VAT accumulation and thus underlie the “metabolic syndrome” with insulin resistance as a component of it both in men and women. This view is in accordance with Björntorp 1997 (144) who proposed that multiple endocrine abnormalities as elevated cortisol and low sex-steroid and growth hormone cause enlargement of visceral fat depots.

**Oestrogens**

In men oestradiol is suggested to play a more important role in the relationship between sex hormone and insulin resistance than has generally been considered (Phillips et al. 2003) (134). Both testosterone and the oestradiol-to-testosterone ratio (E/T ratio) have been found to correlate with fasting insulin, but after controlling for VAT only the E/T ratio correlation with insulin remained significant. On the other hand insulin levels were significantly associated with E and T independently of VAT and age. In line with this, a large study by Vikan et al.
2010 (135) showed that men with higher levels of oestradiol had an increased risk of later diabetes independent of obesity.

In women it has long been recognized that an increased production of oestrogens in obesity seems to protect postmenopausal women from visceral fat accumulation (Haarbo et al. 1991) (145). This has been explained by downregulation of the density of androgen receptors by oestrogens in female adipose tissue (Bjøntorp 1997) (123). The female distribution of body fat tend to disappear, at least partially, with the menopause, inasmuch as women tend to accumulate visceral fat that can be prevented by hormone replacement therapy (HRT) (Haarbo 1991) (145). However, Sites et al. 2001 (146) reported that HRT in non-obese postmenopausal women did not alter visceral fat accrual over a 2-year period. They also found a reduction in insulin sensitivity, which was explained by the use of progestin in addition to oestrogen in the HRT compound (see section below). Recent metaanalysis suggest that when abdominal fat is reduced by the use of oestrogens, insulin resistance is reduced (Salpeter et al. 2006) (147).

A positive association between endogenous oestradiol and insulin resistance was found by Goodman-Gruen et al. 2000) (148). In addition, a large study of 845 healthy postmenopausal women (mean BMI 25.9 kg/m², range 17-40 kg/m²) showed a surprisingly positive association between HOMA-IR and total and bioavailable oestradiol (Kalish et al. 2003 (141). The odds ratio of insulin resistance across each quartile of total oestradiol, bioavailable oestradiol, and bioavailable testosterone was significant and increased (all P < 0.001). Lower SHBG was associated with higher odds ratio of insulin resistance, independent of central adiposity as assessed by WHR. The results suggested that oestrogen may be equally or more important than testosterone for insulin resistance (Kalish et al. 2003) (141). The increased odds ratio for a greater insulin resistance observed among those with the highest quartiles of bioavailable oestradiol, and also bioavailable testosterone, is suggested to be a consequence of
decreased SHBG (Preziosi et al. 1993) (149). Ding et al. 2006 (142) concluded in a metaanalysis that endogenous sex hormones, may differentially modulate glycemic status and risk of type 2 diabetes in men and women, but with lower risk in men; - the inverse association of SHBG with risk was stronger in women than in men.

**Progestins**

Unfavourable effects of HRT on insulin sensitivity may be due to the use of progestin supplement that are known to antagonize the favourable oestrogen effect on insulin resistance (Gaspard et al. 2009) (150), (Demir et al. 2008) (151), (Fernandez et al. 2008) (152).

**Growth hormone-IGF-1.**

The involvement of growth hormone (GH) in the regulation of visceral fat mass in humans is demonstrated by the observation that in acromegaly there is a reduction in visceral adipose tissue (Brummer et al.1993) (153). GH is reduced in obesity, but levels of IGF-1 in obesity have been variously reported to be increased, normal, or decreased (Smith 1996) (154). However, hyperinsulinemia decreases IGFBP-1 which may account for the majority of studies demonstrating a decreased total IGF-1 level, while free IGF-1 is increased (more in men than in women) (Frystyk et al.1995) (155), and by feed-back GH decreases in obesity. Adipose tissue could be a source of IGF-1 in addition to that produced by the liver. The reversibility with weight loss suggests that the alterations in the GH-IGF-1 axis are secondary to the obese state and not causative (Frystyk et al. 1995) (155).

**Adipocytokines**

Adipose tissue is recognized as being very hormonally active, and an increasing number of hormones and cytokines involved in glucose metabolism, lipid metabolism, inflammation, blood pressure and feeding behaviour have been characterized (table 5).
Table 5.

<table>
<thead>
<tr>
<th>Effect on</th>
<th>Adipocytokine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake</td>
<td>Leptin.</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Adiponectin, Resistin, Visfatin, Omentin, Vaspin.</td>
</tr>
<tr>
<td>Vasodilatation</td>
<td>Apelin.</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>ETP (cholesteryl ester transfer protein), LP L (lipoprotein lipase), HSL (hormone sensitive lipase), A- FBP 4 (ap2) (Adipocyte fatty acid binding protein 4), perilipin, RBP (Retinol-binding protein), ASP (acylation stimulating protein).</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>AT II (angiotensin II), ACE (angiotenin converting enzyme), AGT (angiotensinogen).</td>
</tr>
<tr>
<td>Inflammation</td>
<td>TNF-α (tumor necrosis factor-α), IL-6 (Interleukin-6), CRP (C-reactive protein), Adipsin (Adipocyte trypsin/complement Factor D).</td>
</tr>
<tr>
<td>Macrophage activation</td>
<td>MCP -1 (Macrophage chemo attractant protein -1), ICAM -1 (Intercellular adhesion molecule -1).</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>PAI-1 (Plasminogen activator inhibitor-1).</td>
</tr>
</tbody>
</table>

Adapted from Hajer et al. European Heart Journal 2008;29:2959 -71

Plasma adipocytokine levels rise with an increase in adipose tissue and adipocyte volume, except adiponectin which is lower in obesity (Weyer et al. 2006) (156). Considerable interest has been devoted to this hormone that has been implicated in the relationship between subcutaneous adipose tissue and insulin resistance (Shand 2003) (157). A close positive relationship between adiponectin and insulin sensitivity has been found (Weyer et al. 2006)
Adiponectin is also negatively associated with fasting triglycerides and positively with HDL cholesterol (Tschritter 2003) (158). However, the positive relationship between adiponectin and insulin sensitivity has also been suggested to be dependent on the degree of adiposity (Kantartzis et al. 2005 (159). In fact, an inverse association between adiponectin and VAT has been found while SAT correlated positively with adiponectin (Hanley et al. 2007) (160). In obese men Buemann et al. (2005) (161) demonstrated a positive relationship between lower body fat mass and adiponectin, on the one hand, and lipid parameters and insulin sensitivity, on the other.

Madsen et al. 2008 (162) studied the effect of weight loss on serum adiponectin. They found that only after a weight loss of at least 10% of 3 years duration could an increase in serum adiponectin levels be observed. Previous studies have shown divergent results, and those showing no effect are characterized by a mean weight loss of < 10kg (Behre 2007) (163). Although its role is at present unclear, several arguments indicate that adiponectin act as a protective hormone in conditions of energy deprivation (Behre 2008) (164).

9. The study

9.1. Aims-Design

The purposes of the study were to examine

1. The usefulness, in a clinical setting, of assessment of body composition by dual X-ray absorptiometry (DXA) in women and men with a broad range of BMI values

2. In a cross sectional manner the influence of total and regional fat masses on parameters of insulin resistance and serum lipids on strictly obese women and men (BMI> 30 kg/m²).

3. During a one-year weight loss the effects of reduction of these fat masses on the same parameters of insulin resistance and serum lipids.

More specifically the study was designed to examine:
• The relationship between three different fan-beam systems and to establish conversion equations between these systems (paper I).
• The influence of regional fat masses on insulin resistance and serum lipids in overweight (BMI < 30 kg/m²) and obese postmenopausal women (paper II).
• The influence of regional fat masses on insulin resistance and serum lipids in obese women and men (paper III).
• The influence of age, menopausal stage and selected hormonal factors on insulin resistance and serum lipids in obese pre- and postmenopausal women (paper IV).
• The influence of weight loss with reduction of total and regional fat masses on insulin resistance and serum lipids during a one-year weight loss program in obese women (paper V) and men (paper VI).

9.2. Subjects

In study I, body composition data from 3 different DXA instruments were obtained from 21 healthy volunteers (8 males and 13 females) aged 30-84 years with BMI range 21.8 – 39.8 kg/m². Selection of subjects was based on the requirement to have a wide range of body weights.

The clinical studies (II) (III) (IV) (V) (VI) comprised obese, healthy Caucasian Norwegian men and women, consecutively referred to our out-patient clinic and invited to participate in a one-year weight loss program. Inclusion was defined by a body mass index (BMI) equal to or exceeding 30 kg/m². For practical reasons, in connection with DXA measurements, an upper limit of 125 kg was imposed after weight and height measurements. Patients with known diabetes were excluded. Analysis comprised women (n = 109); 46 premenopausal (III), aged 26-55 years, 63 postmenopausal women, (II) (III), aged 46-75 years and men, (n = 113) (III), between 26 and 63 years of age. In paper II the obese women (n = 63) were compared with an overweight group (BMI 25-30 kg/m²) comprising 36 healthy
postmenopausal women, aged 55-64 years recruited from another clinical study (Gauiler J-M et al. 2004) (165). The postmenopausal women (II) (III) (n = 63), were all > 1 year past menopause. Current use of oestrogens, antihypertensives, thyroid medication and statins were continued (II) (III) (V) (VI), while the overweight group (n = 36) was not on any medication. Postmenopausal women, subject for fat mass matching (n = 19) with premenopausal subjects (n = 19), were selected as not current oestrogen users (IV), or had discontinued oestrogens at least one year prior to the study. Only subjects who completed the program with a weight loss of at least 4 kg were subject for analysis (35 women and 9 men) (V) (VI).

The study population for study II-VI comprised patients referred to our clinic for treatment of obesity. The first patients were included in January 2000 and the study was concluded at the end of 2002. Both oral and written informed consent to use anonymized data obtained during the study for scientific studies and publication were given by the participants. Before study start, we contacted the Regional Ethical Committee and explained the nature of the study. We were informed that given the individual and clinical patient-doctor profile of the study no formal recognition of the study by the committee was required. We have later (after study completion) become aware of a change in policy of the committee regarding such studies. For study I approval by the Regional Ethics Committee was obtained and written informed consent obtained from the participants.

9.3. Methods

9.3.1. Clinical procedures

In all subjects, weight to nearest 0.1 kg on a calibrated Seca-scale and height was measured on a calibrated Harpenden stadiometer to calculate BMI as weight (kg)/height (m²). After an overnight fast, venous blood was drawn for analysis. A 75 g oral glucose tolerance test (OGTT) was administered, and blood samples were obtained immediately, before, and 60 and
120 minutes after glucose ingestion for analysis of glucose, insulin and C-Peptide. Within an hour after weight and height measurements, a whole body scan was performed on each of the three DXA machines (paper I). In the clinical study (II) (III) (IV) (V) (VI), obese patients were assigned consecutively at random to an unoccupied DXA machine. Total and regional fat mass (FM) and lean mass were measured by three different DXA absorptiometers: Lunar Expert, Lunar Prodigy (both Lunar GE, Madison, MI) and Hologic Delphi W (Hologic, Waltham, MA). All DXA data were transformed to Prodigy values according to correction factors (I).

9.3.2. Laboratory methods

Glucose in venous blood was measured by a glucose dehydrogenase method (HemoCue B-glucose analyzer, Sweden), insulin and C-Peptide were determined in serum samples (Immuno 2000, DPC, Cal.). Indices of insulin resistance, sensitivity and beta cell function were calculated: HOMA-IR (fasting plasma glucose (mmol/l)*fasting serum insulin (pmol/l)/135), HOMA secr (fasting insulin (pmol/l)*3.33/(fasting glucose (mmol/l)-3.5), and QUICKI 1/(log Io+logGo) were calculated as well as MCRest OGTT (18.8-0.271*BMI-0.0052*Insulin120-0.27*glucose 120). HbA1C was measured by an inhibition of latex agglutination method, (DCA 2000, Bayer, Germany). Serum levels of total cholesterol, HDL cholesterol and triglycerides were measured by a Beckman Synchron analyzer (Beckman, LA, Cal.) (II, III, IV, V, VI). In 39 men (III) the laboratory analyses were done using the same procedures as in women, while 74 men recruited from another center were tested for total cholesterol, HDL cholesterol, triglycerides and glucose concentrations by using automated analyzer equipments (Hitachi 911; Hitachi Limited, Tokyo, Japan) and reagents from Boehringer Mannheim, Germany. HbA1C was measured by ion exchange chromatographic method (TOSOH G7, Japan). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (Friedewald et al. 1972) (166). Potential differences in normality
distribution or reference values for each of these parameters between the two centers are considered negligible according to NORIP (Nordic reference interval project).

Oestradiol, total testosterone, sex hormone binding globulin (SHBG), cortisol, free thyroxine, free triiodothyronine, human growth hormone (HGH), insulin like growth factor-1 (IGF-1) were measured by Imulite. In the event of oestradiol values below the detection limit 0.07 nmol/l in this assay, 0.069 nmol/l was chosen for statistical analysis. For DXA- measurements see section 6.

9.3.3. Diet counselling.

All subjects underwent diet counselling according to our weight loss (WL) program on an individual basis, supplied with an educational program for whole groups of patients also aimed at theoretical understanding. These introductory lectures were prepared and given by a physician. The individual WL program consists of frequent individual counselling according to the principles of life style behavioural modification therapy, dietary advice with emphasis on frequent meals (at least five meals a day) and a moderate caloric restriction suited for long-term adherence as well as advice on increased physical activity. No specific diet was imposed, but dietary changes based on repeated food diaries were encouraged to achieve a reduction of daily caloric intake of > 500 calories according to individual preferences. The counselling schedule was not fixed and the number of visits at the clinic would depend on the success in achieving a reasonable WL goal.

9.3.4. Statistical methods.

All data are presented as continuously distributed variables with means and standard deviations (SD). Group comparisons were performed using Student's unpaired T-test and ANOVA one way analysis, or the Mann-Whitney rank sum test and Kruskal-Wallis ANOVA on ranks in case of no normality (II and III). In papers IV,V,VI, group comparisons were performed using the Student's paired T-test or Wilcoxon signed rank sum test were used when
appropriate. To estimate associations between variables, either Pearson’s correlation coefficient, or in case of non-normality, Spearman rank order correlation was used. Linear regression analysis (IV,V,VI) and forward multiple stepwise regression analysis (II,III,IV,V,VI) were performed to assess the relative importance of trunk FM, leg FM and trunk/leg FM ratio to the indices of insulin resistance and lipid metabolism. Generally, P-values < 0.05 were considered statistically significant. Statistical estimates were performed using the Sigma Stat version 3.1 (Sys. Stat. LA, Cal, USA).

10. Results

10.1. Assessment of body composition by DXA (Paper I)

In this study we sought to establish conversion factors between the three fan-beam DXA-instruments in subjects with a broad range of BMI (21.8-39.8 kg/m²). There were no significant differences between scale weight and DXA measurements of total body mass, or between body mass measurements between the 3 DXA instruments. Correspondingly there were no significant differences in measurements of fat mass or lean mass between the DXA devices. Their respective Pearson correlations were overall > 0.95 (P < 0.0001). Bland Altman testing revealed no significant differences between means of differences between scale weight and total mass measured by the 3 DXA devices. However, a significant negative correlation between scale weight and total body mass estimated by Expert (r = - 0.52, P = 0.016); indicating a tendency for Expert to overestimate total body mass with increasing body mass. This was due to significant differences in means of differences of fat mass between the three DXA devices resulting from an overestimation of fat mass by Expert with increasing fat mass and body weight relative to Prodigy (r = - 0.69, P < 0.001) and to Delphi (r = - 0.79, P < 0.001). In spite of these minor differences between the DXA devices, conversion equations,
based on simple linear regression analysis, forcing all correlations through the origin could be established, and allowing for the use of one regression coefficient for conversion of each body composition parameter \( y = bx \) between the DXA-devices. Conversion of fat mass from Delphi to Prodigy was 1.079, and from Expert to Prodigy 0.954. Long term precision (CV), as measured weekly by a whole body phantom, has consistently been 2% for regional FMs for all 3 instruments, but 5.5% for total lean mass by Hologic Delphi. As pointed out in paper V, we tested the long term precision for Lunar Prodigy in humans, and found CV of 0.4% for total FM, 1.5% for leg FM, 0.8% for trunk FM and 0.3% for total lean mass.

10.2. Assessment of fat masses (Paper II, III)

Obese postmenopausal women (BMI 35.1 kg/m² ± 3.8) \( (n = 63) \) had significantly greater total, trunk and leg FM (and total lean mass) than overweight women (BMI 27.6 kg/m² ± 1.2) \( (n = 36) \) \( (P < 0.001 \) for all) (II). Although no difference in BMI \( (P = 0.18) \) or weight \( (P = 0.55) \), premenopausal women (BMI 36.0 kg/m² ± 3.8) \( (n = 36) \) had greater leg/trunk FM ratio \( (P = 0.04) \) than postmenopausal women (BMI 35.1 kg/m² ± 3.8), while fat mass or lean mass did not differ (III). Obese men \( (n = 113) \) (BMI 33.9 kg/m² ± 4.2) had greater weight because of greater lean mass, while obese women \( (n = 99) \) (pre- and postmenopausal) had a greater BMI (35.5 kg/m² ± 3.8), total FM, leg FM and leg/trunk FM ratio than men, but similar trunk FM \( (P = 0.127) \)(III).

10.3. Insulin resistance

In paper II we found that obese postmenopausal women \( (n = 63) \) were significantly more insulin resistant than overweight women \( (n = 36) \) \( (P < 0.001) \) assessed by HOMA-IR. Postmenopausal women were also more insulin resistant than premenopausal women \( (P = 0.01) \) (III), while men \( (n = 113) \) were more insulin resistant \( (P < 0.004) \) than obese pre- and postmenopausal women \( (n = 109) \) (III). Postmenopausal women \( (n = 63) \) were more insulin resistant \( (P = 0.01) \) than premenopausal women \( (n = 46) \).
10.3.1. Influence of fat masses in cross sectional studies (II, III and IV)

The main finding in paper II, is that a leg FM may confer a relative protective effect on insulin resistance as measured by HOMA-IR in obese postmenopausal women (n= 63), not found in overweight (n= 36) postmenopausal women (P < 0.001), and despite greater insulin resistance in obese subjects. In obese subjects, leg FM was favourably correlated with indices of insulin resistance and sensitivity. The relative importance of leg and trunk FM was better expressed by finding even stronger associations between leg/trunk FM ratio and indices of insulin resistance (P< 0.001). Further analysis by simple linear regression showed increasing HOMA-IR with increasing trunk FM (R= 0.22), (but not significantly, P = 0.09) and with decreasing leg FM (R = 0.29, P = 0.002) and decreasing leg/trunk FM ratio (R = 0.51, P< 0.001). In the overweight group, trunk FM nearly reached significance (R = 0.31, P = 0.06) while leg and leg/trunk FM showed no relationship with HOMA-IR (II). This means that for any given degree of trunk FM, an increase in leg FM will attenuate the negative effect of trunk FM on indices of insulin resistance. In paper III we show that this effect was not evident in obese men, and that in premenopausal women this effect was less evident than in postmenopausal women, despite greater leg FM and greater leg/trunk FM ratio in premenopausal women. In paper IV, we showed that by matching of fat masses between pre-and postmenopausal women, no differences in parameters of insulin resistance between the two groups of women were found, despite significant differences in age and oestradiol levels, thus underscoring the importance of body composition for the variability in the indices of insulin resistance.

10.3.2. Influence of weight loss (V and VI)

In paper V, 35 women (12 pre- and 23 postmenopausal) with a BMI of 34.5 ± 3.2 kg/m², having attained a weight loss at least 4 kg after a one – year weight loss program, were studied. The main finding was a WL of 9.6% and a proportionally equal reduction of trunk
and leg FM (14.9 and 14.7%). A significant improvement of all indices of insulin resistance was found. Among these HOMA-IR was reduced by 34.7%. In men (VI) (n = 9) with initial BMI 34.9 ± 3.3kg/m², also with a WL of at least 4kg, mean WL was 10.8 %. However trunk FM was reduced proportionally more (30.1 %) than leg FM (21.3 %), such that leg/trunk FM ratio increased by 13.2%. All indices of insulin resistance were improved with a 63.2% reduction in HOMA-IR. In women, reductions of leg and trunk FMs were correlated with the improvement in HOMA-IR (P < 0.01 and P < 0.001, respectively) demonstrating equal importance of reduction in trunk and leg FM on insulin resistance. In multivariate analysis improvement in HOMA-IR was explained by total FM (R² = 0.20, P = 0.004) only. For the improvement in QUICKI, reduction in leg FM came out as a favourable predictor (R² = 0.33, P < 0.001). In men, a different pattern was revealed: significant correlations between the reduction in trunk FM (P < 0.001) and leg/trunk FM ratio (P < 0.05), but not with leg FM (P = 0.11), and improvement in insulin resistance were found. Multivariate analysis revealed that improvement in fasting insulin was explained by an increase in leg/ trunk FM ratio (P = 0.013) while improvement in ln HOMA-IR was explained by reduction in trunk FM (P = 0.06), but not by reduction in leg FM. As an additional part of study V and VI, the potential predictive effect of baseline values of initial level of insulin resistance on reduction of FMs, showed that in women the initial HOMA-IR predicted the reduction in trunk FM, while in men none of the baseline indices of insulin resistance predicted loss of FMs.

10.3.3. Influence of age and premenopausal status (III, IV)

In paper III multiple regression analysis showed that postmenopausal status was an explanatory variable for HOMA-IR (R² = 0.29, P = 0.015) and MCRestOGTT (R² = 0.29; P < 0.001), while age was not. In paper III, we also demonstrate by general linear analysis considerable interaction between pre- and postmenopausal women. Postmenopausal women with low leg/trunk FM ratio had the highest HOMA-IR values, while premenopausal women
with high leg/trunk FM ratio had the lowest HOMA-IR values. In this test menopausal status came out significantly (P = 0.001) for HOMA-IR as well as MCREstOGTT (P = 0.002).

Although age in paper II was found to be important in the combined group of overweight (n=36) and obese women (n = 63) (R²= 0.31, P = 0.005), age was not correlated to any parameter of insulin resistance in obese women or men, nor in regression analysis (III and IV).

10.3.4. Influence of hormonal factors (IV)

After matching the two groups of pre- and postmenopausal women combined for fat masses, no differences in levels of cortisol, total testosterone, SHBG, free testosterone or IGF-1 were found. Total testosterone and free testosterone index were negatively correlated with MCREstOGTT (P = 0.04 and 0.004, respectively). SHBG was positively correlated with MCREstOGTT (P = 0.006) and negatively with insulin 2h value during OGTT (P = 0.003). Free thyroxine showed a positive correlation to fasting insulin (P = 0.03), 2 h insulin (P = 0.003) and C-Peptide 2h value (P < 0.001). In multiple regression analysis HOMA-IR correlated positively (unfavourably) to free thyroxine (R² = 0.14, P = 0.023), while IGF-1 (R² = 0.28; P = 0.005) and testosterone (R² = 0.36; P = 0.048) exhibited significant effects on insulin resistance, being negative (unfavourable) explanatory variables for MCREstOGGT.

10.3.5. Influence of medications (II, III)

We found no impact of medications between categories of users of oestrogens, antihypertensives, thyroxin or statins on parameters of insulin resistance (II). However, women without any medication (n = 41) had more favourable values for HOMA-IR (P = 0.028) as well as the other parameters of insulin resistance (fasting insulin (P = 0.038), insulin 2 h value during OGTT (p < 0.001), fasting C.-peptide (P = 0.004), 2 h value for C-Peptide during OGTT (P = 0.005), than those taking medications (III). In men greater HOMA-IR was found between those taking antihypertensives (P = 0.0042) than those taking other
medications or no medication (III). No difference in HOMA-IR between smokers and no smokers was found, neither in men nor women (III).

10.4. Insulin sensitivity.

Measurements of QUICKI largely followed the information given by HOMA-IR, which is logical, as QUICKI is defined as inverse index relative to HOMA, and as such provided no additional conclusive information (II, IV, V and VI) (omitted in paper III). MCRest OGTT provided no useful additional information (II, III) compared to HOMA-IR. However, in pre and postmenopausal women matched for FMs, IGF-1 and testosterone predicted MCRest OGTT in multiple forward regression analysis (IV).

10.5. Insulin secretion (Beta cell function)

Measurement of HOMA secr provided results that varied in concert with HOMA-IR (II, III) indicating normal beta cell function.

10.6. Serum lipids

10.6.1. Influence of regional fat masses in cross sectional studies (II, III and IV)

Overweight women had a more favourable lipid profile than obese women (P < 0.001 for HDL cholesterol and P < 0.01 for total cholesterol/HDL cholesterol ratio) (II). Obese women (n = 109) (III) had a more favourable lipid profile than obese men (n = 113) (P < 0.001 for HDL-cholesterol and total cholesterol/HDL-cholesterol ratio) despite similar trunk FM.

In obese postmenopausal women (II) (n = 63) leg FM was favourably correlated to total cholesterol/HDL cholesterol ratio (P = 0.012) while leg/trunk FM ratio was favourably correlated to triglycerides (P = 0.012) and total cholesterol/HDL cholesterol ratio (P = 0.048). Trunk FM was unfavourably correlated to triglycerides, while none of the fat masses were correlated to serum lipids in the overweight group. In the whole group of obese pre- and postmenopausal women (III) (n = 109) similar results were obtained: leg/trunk FM ratio was
favourably correlated with cholesterol/HDL cholesterol and triglycerides (P < 0.001) as well as total cholesterol and HDL-cholesterol (P < 0.05). By matching pre- and postmenopausal women for FMs (IV), thus making the results independent of FM distribution, HDL-cholesterol was higher (P = 0.025) and cholesterol/HDL cholesterol ratio lower (P = 0.026) in postmenopausal women than in premenopausal women. In men (III) (n = 113), however, no relationships between FMs and indices of lipid metabolism were found.

10.6.2. Influence of weight loss (V,VI)

In women only serum triglycerides were significantly reduced (P = 0.002) (V) while in men serum lipids were not significantly changed during the one-year weight loss program (VI).

11. Discussion


11.1.1. DXA.

A crucial question is the validity of assessment of trunk FM as representative for abdominal visceral fat. In this regard, a conventional assessment of trunk FM by DXA has been shown to have a comparably predictive value as assessment of an abdominal subregion (region of interest or ROI) above the iliac crest compared with CT assessment of visceral fat at the L4-L5 level in non-obese elderly (Snijder 2002) (46) and obese men and women (Kamel 2000) (54). Glickman, 2004 (167) found DXA L1-L4 ROI compared with CT proved to be both reliable and accurate method to determine abdominal adiposity in subjects with a broad range of body fat (8.0-58 %). In agreement with these studies of abdominal subregions by DXA, Bredella et al. 2010 (168) found that DXA trunk FM and leg FM were highly correlated with CT visceral fat at L5 (r = 0.95, P < 0.0001) in strictly obese premenopausal women, although DXA underestimated trunk FM and leg FM with increasing weight (Bland-Altman analysis).
Because trunk FM is provided as a standard variable by the DXA software, and also as an easier and more practicable method than the measurement of the abdominal subregion, we chose to use trunk FM as an estimate of abdominal fat.

Although considered as the “gold standard”, some limitations of CT are to be mentioned: Compared with CT, exposure to the DXA radiation is substantially less, only 0.01-0.015 mGy, representing only approximately 10% of the radiation dose given by one ordinary X-ray of the lungs. Because of the high radiation exposure by CT, the standard procedure is to take only one slice. However, in obese subjects, CT has shown a great variability in VAT at five different abdominal levels (Chowdhury et al. 1993) (169). The use of the L4-L5 region as standard may give different results, especially from the scan at the umbilicus, where the highest percentage of body fat is located and best allows differentiation of SAT from VAT. Further, the high costs and requirement for a high degree of technical skill limit its clinical applicability (Van der Kooy et al. 1993) (52). In contrast, the examination by DXA is rapid and takes only 10 minutes for a whole body scan, thus providing immediate results of the whole body as well as regional body composition (trunk, legs and arms).

**Limitations in validity of DXA:** As pointed out in paper I, significant differences between the 3 instruments with regard to measurement of body composition were recorded, most importantly an overestimation of fat mass by Expert with increasing fat mass (Bland-Altman analysis). This may be related to the phenomenon of beam-hardening, i.e change in the beam spectrum by preferential attenuation of the low-energy photons (change in the attenuation constant) with increasing absorber thickness. This may explain too high values of FM measured by Expert, also found by J Hilsted in extreme obesity (Gotfredsen and Hilsted 1997) (170). La Forgia 2009 et al. (171) suggest that this error may be due to differences in tissue thickness, indicating that the DXA device used may not be able to accurately account for beam hardening in obese cohorts.
Inadequacy of the software algorithms for discrimination between soft tissue composition in areas containing bone, may result in an erroneous measurement of fat in the trunk and to a greater extent in fatter subjects. Error in the detection of FM, may result from the method used by DXA to determine soft tissue composition directly above and below bone. (DXA uses the average soft tissue composition surrounding bone to estimate the soft tissue composition above and below bone). This fact may possibly influence the results because of the quantity of bone in the trunk region. However, inaccurate estimates of fat mass by DXA did not appear to contribute to the slight difference between DXA and a 4-compartment model in a study by Prior 1997 (172) of 172 young adults who varied in gender, race, athletic status, body size, musculoskeletal development, and body fatness.

In obese subjects, fat distribution, i.e. “gynoid” vs. “android” fat distribution may make delineation difficult, whether on the pelvic/abdominal or leg side of the line through the femoral neck (figure 2) as pointed out by Park et al. (2002) (173). Despite these limitations Lee et al. (2008) (174) found that any single measurement of abdominal fat, whether by DXA (including measurements of trunk FM and leg FM and their ratio) or CT-assessed VAT was of comparable utility in predicting metabolic risk factors in obese women. In this regard, the use of DXA therefore seems to be the most appropriate method in a clinical setting.

11.1.2. Insulin resistance, beta cell function and sensitivity.

In section 7, we have discussed the validity of surrogate measures of insulin resistance (HOMA-IR), insulin sensitivity i.e. QUICKI, MCRest OGTT and beta cell function (HOMAsecr) with special reference to the obese state. We conclude that the use of these indices are valid compared to the hyperinsulinemic-euglycemic clamp method. HOMA IR and HOMA secr have been used to assess IR and beta- cell function in over 150 epidemiological studies examining subjects of various ethnic origins and with varying degrees of glucose tolerance (Wallace al. 2004) (70). Some aspects should however be recognized:
1. HOMA IR, or HOMAsecr is a measure of basal resistance and beta cell function, in contrast to stimulated states as measured by clamp studies. However, good correlations are obtained between clamp studies and surrogate measures in obese subjects (see section 7). Wallace et al. (2004) (70) pointed out that these indices are not usually normally distributed as also were found in our studies. They recommended the use of logarithmically transformed HOMA (Wallace et al. 2004) (70). We used log HOMA only in paper VI because of the low number of subjects (n = 9). However, QUICKI uses a log transform of the insulin glucose product and should be recognized as simply being log HOMA-IR (Wallace et al. 2004) (70). As QUICKI varied in concert with HOMA in all respects, QUICKI may be looked upon as an assurance against divergent results.

2. The CV for HOMA-IR varies considerably depending on the number of fasting samples obtained and the type of insulin assay used (Muniyappa et al.) (76). In this respect, it is important to note that over wide ranges of insulin sensitivity/resistance, log HOMA transforms the skewed distribution of fasting insulin values to yield a much stronger linear correlation with glucose clamp estimates of insulin sensitivity (Katz et al. 2000) (73) (Muniyappa et al. 2007) (76). Because HOMA IR and QUICKI was in agreement in our studies, and as such complemented one another, we conclude that the combined use of these indices, in retrospect provided an appropriate estimate of insulin resistance and sensitivity in our obese subjects.

3. MCRest OGTT have been shown to most useful in obese states (See section 7.3.2), and as measure of insulin sensitivity in agreement with QUICKI in our obese subjects.
11.2. General discussion

11.2.1. Insulin resistance and dyslipidaemia in cross sectional studies

Comparison between overweight and obese postmenopausal women.

In paper II we show that a high leg/trunk FM ratio has a more favourable effect than lower ratios on indices of insulin resistance and sensitivity. Most importantly, this favourable effect was not found in overweight subjects. This beneficial effect is more evident not only with increasing leg/trunk FM ratio, but also with increasing leg FM per se. Numerous studies using anthropometry (Heitmann et al.) (175) 2004, (Snijder et al.2003) (176), (Snijder et al.2004) (177), (Seidell et al. 2001) (178), or by DXA (Williams et al. 2004) (27), (Tankö et al. 2003) (28), (Van Pelt et al. 2002) (29), (Snijder et al. 2004) (179) have suggested that leg FM attenuates the unfavourable effect of abdominal fat on insulin resistance in cohorts of mixed normal, overweight and obese subjects. Examination by CT of thigh SAT (Goodpaster et al. 2005) (180), (Goodpaster et al. 1997) (26) are in accordance with these results. We suggest that the favourable results in these studies are attributable to the obese participants. The importance of the favourable effect of leg FM/ trunk FM ratio has recently been confirmed by Tousignant et al. 2008 (181) using DXA and CT, on indices of insulin resistance (AUC during OGTT and hyperinsulinemic clamp) in 124 overweight and obese postmenopausal women concluding that this ratio added substantially to the prediction of insulin sensitivity over VAT or CFM (central fat mass) alone. However, no distinction between overweight and obese women was made in that study, and to our knowledge no other studies using leg/trunk FM ratio are available in this regard. Likewise, no studies comparing obese and overweight subjects are available in regard to serum lipids. Although no difference in serum lipids were recorded between pre- and postmenopausal women (Paper III), we found by the matching of fat masses between the two groups a higher HDL cholesterol in post-than in premenopausal women, somewhat unexpected, as insulin resistance was not different (Paper IV).
conclusion the leg/trunk fat mass ratio seems to be an important parameter for assessment of individual risk for diabetes and cardiovascular disease in obese subjects as opposed to overweight subjects.

**Comparison between obese women versus obese men.**

In paper III, we found that obese men were more insulin resistant than women despite similar BMI and trunk FM. However, men had lower leg FM and leg/trunk FM ratio relative to women. These findings suggest that lower absolute and relative leg FM may be responsible for the lack of beneficial effect of leg fat on indices of insulin resistance seen in women. These data also suggest that there may be a minimum threshold for leg FM that is necessary for a favourable effect on insulin resistance to occur. Interestingly, Goodpaster et al. (2005) (180) found that higher thigh SAT was less associated with the metabolic syndrome in obese than in normal and overweight women and men. An inverse association between more thigh fat mass and the metabolic syndrome was found in obese women and men. Thus, it is reasonable to assume that it is the absolute or relative levels of leg FM that determines if fat mass is protective or not, explaining differences observed in our obese women and men. Indeed, low levels of subcutaneous leg FM have been associated with increased risk of unfavourable effects, irrespective of abdominal FM (Snijder et al. 2005) (182). The effect of lipodystrophy is another example (Seip et al.) (183). Our findings of a relatively favourable effect of leg/trunk FM ratio on serum lipids in obese women are supported by recent studies. Wiklund et al. 2008) (184) in 175 men and 417 women with a broad range of BMI (15-41 kg/m²), mean ~ 25 kg/m²), using DXA. They found significant positive relationships between triglycerides and abdominal as well as gynoid fat mass (corresponding to leg FM) and abdominal to gynoid fat ratio in both women and men. As pointed out in paper III, the amount of leg FM may be determinant for a protective effect of this fat mass to occur. Being much lower in men than in women, both in the study of
Wiklund (184) and in our obese men, it is reasonable to suggest that the amount of gynoid fat mass (or leg FM in our study) was not sufficient to exert measurable protective effects as opposed to obese women. Thus, we suggest that different minimal amounts of leg FM may exist for men and women.

Gan et al. 2003 (185) comparing insulin resistance and serum lipids in relation to regional FMs, found that in the more overweight and obese men (BMI > ~29 kg/m²), serum lipids did not correlate to most regional FMs. This may indicate the existence of a threshold value of leg FM above which the correlations are attenuated or lost. This may explain why we did not find relationships between regional FMs and serum lipids in our cohort of strict obese men. Other studies, give support to our suggestion: Paradisi et al. (1999) (63) in 24 obese men (BMI 28.9 kg/m² ± 2.8), found by DXA that a relatively high amount of leg FM showed favourable significant correlations with triglyceride and total cholesterol (P < 0.05), however, not found in lean men. The same was also found by Pouliot et al. (1992) (25). Most recently, Porter et al. (2009) (186) in a large study of participants in the Framingham study (n = 3001 men and women) confirmed that cardiometabolic risk increases significantly with increases in VAT. The prevalence of many risk factors, including hypertension and the metabolic syndrome, also increases with increasing SAAT for all VAT and BMI tertiles. Among those in the lower two-thirds of VAT distribution, more SAAT is also associated with increased risk of most other risk factors, suggesting that SAAT is not protective in these individuals. Among those with the most VAT, however, increase in SAAT was associated with lower triglycerides, suggesting that SAAT may be associated with beneficial effects on triglyceride levels in the obese. Most importantly, they also suggested that a lack of increase in LDL cholesterol and impaired fasting glucose with increasing SAAT among the obese may be due to a threshold effect. Thus, all these data, including our own, suggest a favourable impact of subcutaneous tissue on cardiovascular risk factors in obese women and men. However this effect was not...
confirmed in our cohort of obese men (III). This difference may be due to a threshold effect represented by a minimum of leg/trunk fat mass ratio or a minimum of absolute leg fat mass necessary for being associated with cardiometabolic risk factors.

**Influence of menopausal status.**

As pointed out in paper III, postmenopausal women had a more favourable effect of leg FM and leg/trunk FM ratio on indices of insulin resistance than premenopausal women, in spite of a greater leg/trunk FM in the latter (P = 0.04). Forward multiple regression analysis and interaction analysis showed that menopausal status was of significant importance with a larger distribution of unfavourable leg/trunk FM ratios on insulin resistance among postmenopausal women. Menopausal status therefore seems important for the change in distribution of fat mass, also shown by others (Ley et al. 1992) (187), (Svendsen et al. 1995) (188), (Trémolières et al.1996) (189), (Toth et al.2000) (190), but menopausal status is not per se associated with increased insulin resistance (Toth et al. 2000) (191). Thus, the prevailing opinion favours that the increase in insulin resistance seen in postmenopausal women is related to the change in distribution of fat mass.

**Influence of age.**

We found no influence of age as such on indices of insulin resistance. In contrast, Pascot et al (1999) (192) suggested that even before the onset of menopause there is an age-related deterioration in the metabolic risk profile and an increase in VAT deposition in middle-aged women compared with young control subjects. Our result is in accordance with previous studies in both normal and obese subjects (Svendsen et al. 1995) (188), (Trémolières et al.1996) (189), (Toth et al. 2000) (191), (Panoutopulos et al.) (1996) (193), (Kotani et al.1994) (194), (Imbeault et al. 2003) (195). The latter concluded that visceral obesity, more than age per se, correlates with glucose-intolerance in middle-aged subjects and that aging does not influence in vitro adipose tissue glucose uptake. In paper IV we sought further
insight into potential differences other than the influence of fat masses between pre- and postmenopausal obese women such as age, menopausal stage and hormonal factors on insulin resistance by matching 38 pre- and postmenopausal women one by one for leg/trunk FM. Because this matching also led to well matched values for weight, height, BMI, total FM, trunk FM and leg FM (P = 0.52-0.98), we assumed high correlations between visceral and subcutaneous FM. Indeed, Fox et al. 2007 (196) in 3001 subjects from the Framingham Heart Study reported r = 0.71 between VAT and SAT (reflecting also overall subcutaneous tissue) in women (and r = 0.58 in men) in mixed overweight and obese subjects (mean BMI 27-28 kg/ m²). This matching led to abolishment of the differences in indices of insulin resistance between pre- and postmenopausal women reported in paper III. Because fat masses were equally distributed, we assumed no major difference in impact of other endocrine factors located within the adipocytes such as cytokines. Thus, our results indicate that the only factors between the groups, with potential influence on insulin resistance, other than fat mass distribution per se, was age and oestradiol, either as such, or mediated through an increase in abdominal fat. Although it has been hypothesized that hormonal changes may be responsible for postmenopausal changes in fat distribution (Sites et al. 2001) (146), (Sumino et al. 2003) (197), (Tschernof et al. 2004) (198), to date there is only one longitudinal study that has directly measured changes in abdominal before and after this hormonal transition. Franklin et al. 2009 (199) found that increase in VAT was accompanied with an increase in SAAT so that the ratio of visceral to subcutaneous fat remained unchanged (BMI~ 24 kg/m²) as was also shown in the Framingham study (Fox et al. 2007) (196). This is in contrast to what is the case with aging. Cross sectional studies report that aging is associated with increases in VAT independent of waist circumference in women (Enzi et al. 1986) (200), (Kotani et al. 1994) (194), (Kuk et al. 2005) (201), and also in men (Kotani et al. 1994 (194), (Kuk et al. 2005) (201), (Machann et al. 2005) (202) indicating a disproportionate increase in VAT compared to
SAAT with aging. It is reasonable to assume that in obese women both the increase in VAT and concomitant increase in SAAT, with its attenuating effect (Porter et al. 2009 (186), Goodpaster et al. 2005 (180) determine the degree of insulin resistance after menopause. Due to the suggested attenuating effect of SAT on insulin resistance after menopause, it is likely that it would be even more difficult to demonstrate a relationship between age and insulin resistance in obese women, otherwise seen in non-obese subjects. Thus, it seems that increased insulin resistance is attributable to a change in distribution of body fat with an increase in VAT consequent to the cessation of ovarian function, and possibly also to age related increase in VAT.

**Influence of hormones.**

By the matching of fat masses between pre- and postmenopausal women leading to similar degree of insulin resistance, and also cortisol, thyroxine, testosterone, SHBG and IGF-1 levels, we may suggest that the known effects of the different levels of these hormones between non-matched pre- and post menopausal women, related to age or menopausal status, are mainly related to the degree of obesity. Similarly, the difference in oestradiol levels between the two groups studied, did not influence insulin resistance, also underscoring the major importance of adiposity and its distribution per se for the level of insulin resistance.

**11.2.2. Influence of weight loss on insulin resistance and dyslipidaemia.**

While leg fat is favourably associated with insulin resistance, a crucial question is why loss of the same leg subcutaneous adipose tissue is favourably related to insulin resistance in the same manner as loss of abdominal adipose tissue. As pointed out by Janiszewski et al. (2008) (203), using MRI on overweight and obese men and women in a 3 months weight loss study, a cross sectional observation could not be extrapolated to indicate that the reduction in lower-body SAT through weight loss, was associated with deterioration of the cardiometabolic profile. Indeed, in agreement with our findings in women (V), they found that reductions in
lower-body SAT, and also SAAT, were associated with improvement in indices of insulin resistance during weight loss, however not independent of loss of VAT. These data may be extrapolated to our own in regard to the use of leg/trunk FM ratio (V, VI). An explanation has recently been provided by Janiszevski et al. (2008) (203): During times of negative energy balance, adipose tissue decreases in mass because of a reduction in adipocyte size, while the number of adipocytes remains unchanged. Small adipocytes have been found to be more insulin sensitive than larger ones (Olefsky et al. 1977) (204). They secrete less prothrombotic, but more antithrombotic cytokines (Gustafson et al. 2007) (117) and are associated with reduced ectopic fat storage. The reduction in size of subcutaneous adipocytes is associated with decreased triglycerides and VAT storage (Larson-Meyer et al. 2006) (205). Thus, the reduction of lower-body subcutaneous adipose tissue fat may actually improve the function of this tissue, thereby improving the cardiometabolic risk profile.

12. Conclusions

Paper I.

Despite differences for body composition measurements between the 3 fan-beam DXA instruments, we have demonstrated that establishment of conversion equations may provide a reasonably accurate conversion of body composition data from total and regional fat masses as well as lean body mass, one device to another, even on an individual level. Our study demonstrates that this is feasible in subjects covering a wide range of weights and differences in body composition. We also conclude that DXA is a suitable method for determining body composition in obese patients in clinical practice (paper II and III), as well as during weight reduction (paper V and VI).
**Paper II.**

By examining total and regional fat masses and indices of insulin resistance, we found that in obese postmenopausal women, trunk fat mass is strongly related to risk factors for diabetes and cardiovascular disease, and that absolute leg fat mass, or a high leg/trunk FM ratio, are associated with a favourable metabolic profile. This effect was not found in overweight women. This difference between obese and overweight women may be explained by the existence of a transition zone for leg FM or leg FM relative to trunk FM, above which a favourable effect on indices of insulin resistance may be manifest.

**Paper III.**

The insulin resistant state after menopause, probably explains why obese postmenopausal women had a more favourable effect of leg FM and leg/trunk FM ratio than premenopausal women on indices of insulin resistance, despite greater leg FM in the latter. Men did not display this favourable effect of leg FM on indices of insulin resistance, an effect that may be suggested to depend on the absolute leg FM value or relative to trunk FM values, both much lower in obese men than in women. These data suggest that there may be a threshold value for leg FM, absolute or relative to trunk FM, different for men and women, above which a favourable effect on insulin resistance may become manifest.

**Paper IV.**

Body composition indices were found to be the main determinants of insulin resistance and dyslipidaemia. This was found by matching fat masses in pre- and post menopausal women revealing no major impact of menopausal status, age or age-related changes in hormonal factors on glucose and lipid metabolism.

**Paper V and VI.**

After a one-year weight loss of approximately 10 % in women (V), we found that regional weight loss was proportionate, with unchanged leg /trunk FM ratio. We found equal
importance for leg and trunk fat mass reduction for the improvement of insulin resistance. After a corresponding weight loss in men (VI), however, the resulting improvement in insulin resistance points to a preferential loss of trunk FM with a decrease in leg/trunk FM ratio as the main responsible.


Although DXA provides information that differs from that obtained from CT or MRI for the understanding of the metabolic effects of regional abdominal FMs (VAT and SAAT), we have shown that assessment of trunk FM and leg FM as well as leg/trunk FM ratio by DXA, provide results that are in accordance with studies in using the “gold standards”, in relation to risk factors for diabetes and cardiovascular disease. A number of clinical studies using these FM parameters and associations with cardiometabolic risk in a variety of clinical settings, have recently been published (Wu et al. 2010) (206), (Saunders et al. 2009) (207), (Rahman et al. 2009) (208).
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Errata
p.24. and 37: Table 4 changed to Table 3 and Table 3 to Table 5, respectively.
p.60. last sentence and first sentence p 61 changed with correct ref. to paper V and VI.
p.64. ref 22 have been corrected from Vague J. 1947 to Vague.J. La differentiation....
p.64. ref 24: Theriault OBS have been corrected to Theriault G.