CHAPTER 6

DISCUSSION

Chapter 1 reviewed the relationships between obesity and the related metabolic comorbidities, highlighting the contributions of both abdominal and peripheral adiposity to metabolic syndrome disturbances and to disease risk, particularly to type 2 DM and cardiovascular diseases. The abdominal obesity relevance to ectopic fat storage or lipotoxicity, mainly in the skeletal muscle and liver were also examined. Furthermore, the metabolic abnormalities resulting from fat accumulation in these non-adipose tissues were additionally addressed. The three articles collected in this thesis were designed to study some of these associations, not only to extend the current knowledge regarding the independent abdominal and peripheral adiposity relevance to metabolic syndrome and disease risk, but also to shed light upon some of the several questions that were still waiting to be answered. Briefly, we sought to extend the current evidence that a smaller HC, for a given WC, is associated to an increased risk for metabolic syndrome abnormalities. In addition, we also examined the abdominal and thigh AT contributions to CVD risk factors and to liver ectopic fat storage, as well as the relationships between metabolic syndrome disturbances and liver fat. The methodology used in the present investigation was described in *Chapter 2*.

Waist and hip circumference vs. health risk

Worldwide obesity prevalence has been increasing tremendously, contributing to the observed increase in CVD, type 2 DM, and metabolic syndrome incidence[1, 2]. This increase will probably raise in the next decade, being estimated that type 2 DM and CVD-related mortality will represent almost three-fourth of all deaths[3]. It has been recognized that body fat distribution seems to be more important to health risk than the overall body fat amount[4]. While visceral adiposity has been associated to an unfavourable metabolic syndrome profile and a higher CVD and type 2 DM risk, it is still unclear if peripheral adiposity, reflecting different metabolic roles, may predict in a different way an increase in disease risk and in

metabolic abnormalities. Several assessment techniques have been used to measure body fat distribution, varying from more sophisticated methods such as DXA, CT and magnetic resonance imaging, reliable but costly, to more simple anthropometric markers such as BMI, WC and WHR, commonly used in a epidemiological context. Despite the advantages to easily screen body fat degree when used in a large population set, BMI is not able either to discriminate fat from fat-free mass or provide information regarding body fat distribution.

To overcome some of these handicaps, WC has often been used as a more reliable marker to reflect abdominal obesity. In fact, sagittal diameter or WC alone seem to be better predictors of VAT rather than WHR[5, 6]. In addition, it has also been shown that WC can equally predict VAT in young and older subjects[7]. However, since, for a given WC, VAT increases with age, it is wise to consider that absolute WC values could represent different abdominal obesity levels in younger and older subjects[8]. Therefore, based in the available evidence suggesting a close relationship between WC and abdominal obesity (mainly visceral adiposity), it is not surprising that the WC cut-points have been included in ATP III and IDF metabolic syndrome definitions[9].

However, several studies have been reporting that WHR can be a stronger predictor of type 2 DM[10] and CVD[11, 12] than WC, mainly because WHR can reflect not only a larger WC but also narrow hips, which may represent either a lower fat and or muscle mass[13]. In fact, it has been reported that, for a given WC, a larger HC was related with a lower risk of metabolic syndrome disturbances [14-16], type 2 DM [10, 15, 17-19], and CVD morbidity and mortality[17, 20-22]. However, this relative HC protective contribution to health risk, observed after statistical adjustment for WC, disappeared when WC was not taken into account. In this context, we sought in *Chapter 3* to investigate not only the independent associations of WC and HC with major metabolic syndrome components, but to further extend the current knowledge to more specific pro-inflammatory and atherothrombotic disturbances.

Additionally, we have also examined the contribution of different abdominal and thigh adipose and muscle tissue compartments to the relationships observed between these two anthropometric variables and metabolic syndrome features.

In this sense, after adjustment for age, BMI, and HC, multiple regression analyses revealed positive associations between WC and both glucose metabolism and IR markers, hypertriglyceridemia, hypercholesterolemia, and PAI-1, an impaired fibrinolysis marker associated with an atherothrombotic state[23]. WC was also inversely related with HDL-C and IL-6 plasma concentrations, a proinflammatory state marker which is more likely to be elevated in obese insulin-resistant than in obese insulin-sensitive subjects[24]. A synergistic effect promoted by a combination of hypercholesterolemia, hyperglycemia, and hyperinsulinemia has been advanced to explain the increase in PAI-1 concentrations in obese subjects[25], which may justify the results previously described. However, hyperinsulinemia alone can also increase PAI-1 concentrations in both obese and type 2 DM subjects, revealing a link between IR and PAI-1[23]. However, it is still unclear whether insulin acts directly or via IR to enhance PAI-1 values.

Furthermore, higher PAI-1 concentrations have also been found to be closely related with dyslipidemia, abdominal adiposity and hypertension[25-27]. In fact, adipose tissue-derived angiotensin II has been presented as an important link between elevated PAI-1 values and the renin-angiotensin system, and hence with hypertension[28]. On the other hand, despite the scientific debate regarding the contributions of the different AT compartments to PAI-1 secretion, it was recently suggested that VAT could be responsible for PAI-1 increase observed in metabolic syndrome patients[29], which, in fact, is closely related with WC.

On the contrary, we have observed that, for a given WC, a large HC was associated with a lower fasting insulin, Hb A1c and PAI-1 concentrations, as well as with a lower TC/HDL-C ratio. Our results reinforce the previous observations reporting a relative

protective role of a larger HC, when WC is taken into account, to IR and dyslipidemia[15, 21, 30], and thus to type 2 DM risk[10, 13, 16, 31]. Furthermore, our results extend this notion to atherothrombotic disturbances and other CVD risk factors. In this context, other studies have also been reporting that higher peripheral fat mass, measured by DXA, was independently associated with a lower CVD risk[15, 17, 32]. Furthermore, the protective contribution of larger hips to morbidity has also been observed in the risk of premature mortality, after adjustment for BMI[17, 20].

Consistently with other reports[33], in our study, a larger WC was associated with higher VAT and Ab SAT areas, representing a morbidogenic body composition phenotype, while a larger HC was independent and inversely related with both abdominal AT compartments. In addition, larger hips were associated with both gluteofemoral AT compartments and thigh muscle mass. Therefore, in this context of the opposite contributions of WC and HC to metabolic risk, as a unique feature of this paper, it was the first time that it was investigated the independent contribution of abdominal and thigh AT compartments, and muscle tissue distribution, measured by CT, to the relative HC protection to the metabolic disturbances previously described. For a given WC, higher TTAT and TTSAT mass were both associated with lower Hb A1c concentrations and a lower LDL-C/HDL-C ratio. Additionally, a higher TTSAT mass was also inversely related with both HDL-C and fasting insulin concentrations. In contrast, TTSFAT did not reveal associations with any of the metabolic markers studied. On the other hand, for a given WC, a higher TTMT mass was related with lower PAI-1 and fibrinogen concentrations.

The results observed in the present work suggest that, in overweight or obese women, the protective HC role in dyslipidemia and IR, observed when WC is taken into account, can be mediated by subcutaneous femoral-gluteal AT. Moreover, it has been observed that, for a given amount of abdominal fat, low subcutaneous fat in the legs is associated with an

atherogenic lipid profile[34]. Underlying hormonal factors, such as estrogens concentrations, may regulate preferential thigh AT accumulation[35]. In addition, compared to VAT adipocytes, femoral-gluteal adipocytes are more sensitive for anti-lipolytic stimuli and less sensitive to catecholamine-stimulated lipolysis[36]. Furthermore, these thigh AT depots present a higher lipoprotein lipase (LPL) activity, which promote a higher rate of the free fatty acids uptake from circulation. This "buffer" capacity may provide not only an anti-atherogenic and anti-diabetogenic effect, but can also prevent liver, pancreas and skeletal muscle lipotoxicity[37]. However, there are several aspects that might underlie and confound the observed associations, being relevant to highlight the glucocorticoid and growth hormone disturbances, as well as behavioral factors, such as diet and physical activity[38].

Despite evidence has been highlighting the contribution of muscle tissue to a better metabolic profile[39], and lower insulin metabolism[40] and fatty acid oxidation capacity disturbances[18, 22], our results suggest that thigh muscle tissue seem also to be relevant to the observed protection against atherosclerotic and prothrombotic disturbances. In fact, these are novel observations that need further research to clarify not only the mechanisms responsible for these associations but also to extend the knowledge to other specific metabolic syndrome features.

Abdominal and thigh adiposity vs. health risk

WC has been largely used, especially in an epidemiological context, as marker of abdominal obesity, and has been found to be a stronger VAT predictor than WHR[5, 6]. However, some studies have reported that WC is not always a better CVD[11, 12] and type 2 DM[10] predictor than WHR. On the other hand, it has been showed that in two persons with the same WC, VAT and Ab SAT areas could present a wide variation range, representing a different disease risk assumption. In addition, for a given WC, VAT mass seems to increase

with age[8]. Therefore, absolute WC values could represent different visceral adiposity levels in both younger and older subjects. To overcome these difficulties, more sophisticated body composition assessment methods such as CT or MRI have been applied to discriminate Ab SAT from VAT. Studies using CT have been showing a consistent association between VAT area and metabolic or disease risk[34, 41, 42].

However, it is not clear if Ab SAT is similarly associated with an unfavourable metabolic syndrome profile independently of VAT[43]. Although some studies have suggested that higher Ab SAT areas were associated with IR markers and lipid metabolism disturbances, these associations were weaker than those verified for VAT[31, 34, 44]. Concomitantly, little is known about thigh AT compartments relevance to metabolic syndrome outcomes and disease risk. Therefore, the author and his colleagues investigated the independent relationships between both abdominal and thigh AT compartments with major metabolic syndrome components, as well as with several other metabolic syndrome features associated with a proinflammatory and an atherothrombotic state (*Chapter 4*).

The primary findings were that VAT reflected an unfavourable metabolic syndrome profile, being associated with fasting glycemia, TG, LDL-C and PAI-1 concentrations, as well as with higher TC/HDL-C and LDL-C/HDL-C ratios, after controlling for age, BMI and VO_{2max}. A higher VAT area was additionally related with lower HDL-C concentrations. The observed VAT mean area was higher than 110 cm^2, a suggested cut-point to observe severe dyslipidemia and glucose metabolism disturbances[45], which might justify the previous associations reported. VAT remained significantly associated with the same metabolic features after adjustment for both Ab SAT and deep Ab SAT, revealing, in the last case, an additional association with CRP.

Although both VAT and Ab SAT have been demonstrating cross-sectional associations with insulin sensitivity[31, 44] and IR[46], VAT seems to assume a greater clinical

importance. Despite the underlying mechanisms are not totally clear, it has been advanced that visceral-derived FFA drained into portal circulation might be responsible for primary hepatic IR[47, 48]. However, in abdominal obese women, the increased lipolysis rate in subcutaneous adipocytes, existing in higher amounts, increase FFA release into systemic circulation, contributing to an increased peripheral IR[49]. In addition, adipose tissue is an important endocrine organ[50], being responsible for expression of several hormones, growth factors and cytokines. In fact, both VAT and Ab SAT can secrete leptin, TNF-α, IL-6, PAI-1 and several other adipokines that might worsen IR and increase disease risk. In our study, we found a close relation between VAT and PAI-1 concentrations. It is known that PAI-1, through inhibition of tissue plasminogen activator, is the main regulator of endogenous fibrinolytic system, favouring the development of thromboembolic disturbances[51]. These fibrinolytic disorders might underlie the association between obesity and both CVD[52] and IR[52, 53]. Moreover, it has been advanced that insulin and the transforming growth factor-beta (TGF-β), an adipokine that increases preadipocytes cell proliferation, appear to be the main responsibles for PAI-1 synthesis in AT[54].

It is also noteworthy that, after control for deep Ab SAT, a higher VAT area was associated with higher CRP and lower adiponectin concentrations. Mainly regulated by circulating IL-6, CRP is an endothelial dysfunction marker which can independently predict IR[55, 56] and coronary heart disease[57]. Similarly to our results, another recent study has also reported that abdominal AT was related with a low chronic inflammatory state, linking abdominal obesity with CVD[56].

On the other hand, it is now well established that there is a close and consistent inverse association between adiponectin and both IR[58] and inflammation[56]. Additionally, adiponectin is also inversely related with other CVD risk factors such as LDL-C, TG and blood pressure[59], and it is also considered to be an independent predictor of CVD[60, 61]. Due

to an insulin sensitizing effect in skeletal muscle, adiponectin may increase muscle glucose uptake and fatty acid oxidation through muscle adenosine monophosphate-activated protein kinase activation[62], preventing peripheral IR. Adiponectin concentrations are reduced in obese and diabetes patients, which is consistent with our results. In this sense, these findings reinforce the notion of an independent VAT relevance to an unfavourable metabolic syndrome profile and to an inflammatory and atherothrombotic state in overweight and obese women.

Although both visceral and subcutaneous abdominal AT have been related with an unfavourable metabolic profile independently of ectopic liver fat storage, CRF levels[63], and even in absence of obesity[64], we found that Ab SAT was uniquely related with TNF-α concentrations, after adjustment for age, BMI and VO_{2max}. When controlling for VAT, Ab SAT revealed additional associations with several other inflammatory and thromboembolic disturbances but none with IR or dyslipidemia markers. Despite the fact that omental adipocytes can produce threefold more IL-6 than subcutaneous adipocytes[65], we found that IL-6 was related with Ab SAT but not with VAT. Similarly, leptin was a significant correlate of Ab SAT but it was not associated with VAT. In light of these findings, Ab SAT may predict an inflammatory and atherogenic state but it was not associated with IR and lipid metabolism disturbances in our study.

Due to its metabolic differences, we examined in further analysis the associations of both superficial and deep Ab SAT compartments with metabolic syndrome features. After controlling for VAT, while superficial Ab SAT was identically related to the same inflammatory and atherogenic risk factors as those observed for Ab SAT, only fibrinogen and uric acid were significant correlates of deep Ab SAT. Contrarily to our results, which revealed a more consistent superficial Ab SAT relevance to disease risk, other studies have

reported that deep Ab SAT may be a better predictor of an unfavourable metabolic profile independently of VAT[44, 66]. Therefore, further studies are needed to clarify these findings.

Less evidence is linking thigh adiposity with metabolic disturbances. However, it has been reported that obesity may lead to ectopic fat storage in skeletal muscle, located between muscle fibbers or intramyocytes, which may contribute to muscle IR and metabolic inflexibility in obese patients with or without type 2 DM[67, 68]. We found that, after control for age, BMI and VO_{2max}, TTAT was inversely related with Hb A1c and PAI-1 concentrations, as well as with LDL-C/HDL-C ratio. On the contrary, when controlling for TTMT, several inflammatory and thromboembolic risk factors were significant correlates of TTAT. Similar associations were observed for TTSAT, not only when adjusting for age, BMI, and VO_{2max} but also when controlling for TTMT.

The inverse associations verified between both TTAT and TTSAT with PAI-1 are novel observations that have not been previously reported in overweight and obese women, suggesting a relative protective role of gluteal-femoral AT against atherothrombotic and IR risk factors when age, BMI and VO_{2max} are taken into account. In this sense, it has been suggested that gluteal-femoral AT my act as a buffer for circulating FFA[69], preventing muscle and liver ectopic fat storage and, consequently, lipotoxicity[37]. Clearly, these results suggest that further investigation is needed to clarify the associations observed.

After statistical control for age, BMI and VO_{2max}, TTSFAT was related with fasting glycemia, thrombotic markers, and with microalbuminuria concentrations. TTSFAT remain significantly associated with the same variables and revealed additional associations with lipid metabolism, atherogenic and inflammatory risk factors, after adjustment for TTMT. Additionally, TTSFAT was, in both cases, inversely correlated with leptin. These findings suggest that thigh lipid pools are also related with adipocytokines expression and could act as important local modulators of skeletal muscle metabolism. In this sense, more research is

needed to understand the differences in cytokine expression and secretion by abdominal and thigh AT compartments and its influence in metabolic function.

Body fat distribution and liver fat

As reviewed in *Chapter 3* and *Chapter 4*, it is generally accepted that higher WC, reflecting abdominal obesity is associated with several metabolic syndrome clinical outcomes. The increased circulating FFA commonly observed in abdominal obese patients predispose to glucose metabolism disturbances in the liver, muscle, pancreas and several other organs[70]. The pancreatic beta-cells which have increased insulin secretion to compensate glucose uptake and oxidation impairments will eventually fail, leading to type 2 DM[37]. Concomitantly, the increased circulating FFA are accumulated in several organs, promoting the so-called lipotoxicity or ectopic fat storage in the liver, pancreas, heart and skeletal muscle[71]. In fact, even a modest excess of fat storage in some of these lean tissues may induce lipid cardiomyophaty and type 2 DM[71].

On the other hand, liver fat storage has been associated with IR and dyslipidemia in obese[72] and type 2 DM patients[73]. Moreover, it has been suggested that hepatic steatosis, clinically characterized by hepatocyte fat infiltration and defined as the first stage of NAFLD, emerge from a combination of increased FFA influx to liver, increased liver FFA synthesis[74], and decreased FFA oxidation and VLDL synthesis. In fact, hepatic steatosis is associated with IR, dyslipidemia and major metabolic syndrome outcomes not only in obese patients but also in lean subjects without glucose intolerance[75]. Hepatic steatosis has been also associated with inflammatory markers of non-specific hepatitis[76]. In this sense, liver fat storage, clinically expressed by a reliable index designated as liver-to-spleen ratio[77], seems to be related with visceral adiposity[78-80], IR[75, 79, 81, 82] and several other metabolic syndrome disturbances[78, 79, 82].

Although evidence has been demonstrating an independent contribution of VAT, and liver fat storage to an unfavourable metabolic syndrome profile and IR in obese or type 2 DM patients, it is still not totally clear if liver fat is additionally associated with other specific inflammatory and atherothrombotic risk factors in overweight and obese women. Furthermore, despite the recognized contribution of abdominal obesity to ectopic liver fat storage, less is known about the relationships of both specific abdominal and thigh AT compartments to liver fat. In this particular context, to our knowledge, only one study developed in type 2 DM patients has reported that thigh subfascial AT was correlated with both liver fat and IR[82]. Therefore, the authors have examined in *Chapter 5* the separate contributions of abdominal and thigh adipose and muscle tissue compartments to liver fat. Further analyses were developed to investigate the independent associations of major metabolic syndrome components and pro-inflammatory and atherothrombotic risk factors with liver fat.

Our primary findings were that a higher thigh SFAT area was associated with either a higher LSR or a lower liver attenuation, representing a lower liver fat storage, independently of age and BMI. Furthermore, it was observed that, for a given WC, increased thigh SFAT areas were also independently related with a higher LSR. To our knowledge, these associations between thigh SFAT and both LSR and liver attenuation are novel observations that may suggest an indirect preventive role of this thigh AT depot against ectopic liver fat storage in overweight or obese women. As reviewed in *Chapter 3* and *Chapter 4*, it has been suggested that femoral-gluteal AT may function as a "sink" for circulating FFA[83]. In addition, the fact that these thigh adipocytes are less sensitive to catecholamine-stimulated lipolysis and present a relatively high lipoprotein lipase activity, important in FFA uptake from the circulation[84], may prevent liver lipotoxicity and counteract the inevitable

physiologic cascade responsible for IR and several other disturbances currently observed in abdominal obese subjects.

However, it is noteworthy that previous studies have been relating thigh SFAT not only with IR[68, 82], but also liver fat[82]. In a recent study developed with non-insulin dependent DM patients, it was reported that liver fat was not only negatively associated with visceral adiposity[82], but also with thigh subfascial AT independently of the effects of VAT and BMI. More than interpreting these observations as evidence supporting the notion that thigh SFAT can contribute to fatty liver pathogenesis, the authors have suggested that both TSFAT and fatty liver are special adiposity depots related with IR pathogenicity in type 2 DM. In this sense, these results obtained in type 2 DM patients contrast with our observations in overweight and obese women, suggesting that this body composition area warrants more research.

On the other hand, we have observed that higher concentrations of fasting insulin, TG, liver transaminases, PAI-1 and uric acid concentrations were independent and inversely related with LSR. In addition, higher TC/HDL-C and LDL-C/HDL-C ratios were also predictors of a lower LSR. These findings highlight the contribution of hyperinsulinemia, hypertriglyceridemia and hypercholesterolemia to the pathophysiological metabolic cascade that mediates liver disturbances in overweight and obese women. Furthermore, it has been advanced that hyperinsulinemia alone may inhibit hepatocyte FFA oxidation, which might additionally contribute to liver lipotoxicity. Other studies have also reported associations between both IR[78] and lipid metabolism [78, 85] markers and liver fat. In addition, the hepatic steatosis might be accompanied by a low chronic inflammatory state[86]. In this sense, it was found in the current work that both atherothrombotic and inflammatory risk factors were inversely associated LSR, emphasizing the associations of a low chronic inflammatory and atherothrombotic state with hepatic fat storage. It is known that increased liver transaminases

concentrations can predict liver injury degree[87], being also related with obesity severity. In our study, both ALT and AST were independent predictors of liver fat storage.

When adjusted for VAT, excepting uric acid and PAI-1, same metabolic risk factors remained significantly associated with LSR, being these associations slightly stronger than those observed when controlling for age and BMI. As discussed in *Chapter 4*, both VAT and Ab SAT are independent predictors of metabolic and disease risk[63]. However, although some reports have been suggesting that liver fat storage is normally preceded by VAT accumulation[78], it was already observed that hepatic steatosis remains independently associated with major metabolic syndrome components independently of total and visceral adiposity[88, 89]. In this sense, our results reinforce the independent associations of an unfavourable inflammatory and atherothrombotic metabolic profile with liver fat, independently of VAT.

Obesity, and more specifically, abdominal obesity contribution to liver fat storage and consequent metabolic disturbances was already addressed in several studies. In a study with 144 patients with NASH, BMI was the unique independent predictor of hepatic steatosis degree[90]. Other studies have also reported that both in obese patients[87] and in living liver donors[91], the hepatic steatosis severity was also associated to BMI. Similarly, we found that BMI, was inversely associated with LSR, independently of age. However, BMI is not always an independent predictor of liver fat storage. In a study with 221 patients with chronic hepatitis C, VAT rather than BMI, was a predictor of hepatic steatosis[92]. In fact, central obesity markers, such as WC[80, 81], WHR[80, 93], VAT[78], VAT/TAAT ratio[78], and Ab SAT[80]seem to be close and consistently related with liver fat. Similarly, after adjusting for age and BMI, we found that a higher VAT area and an increased SD were independently associated with lower a LSR, emphasizing that liver lipotoxicity is associated not only with an unfavourable metabolic syndrome profile but can also reflect abdominal obesity. This

liver lipotoxicity may be responsible for other metabolic disturbances, including increased liver FFA synthesis, adipocyte proliferation failure and insufficient FFA oxidation[71, 79, 86]. In this context, our results are consistent with some recent observations[78], suggesting that liver fat is associated not only with an unfavourable metabolic syndrome profile but can also reflect abdominal obesity.

Despite hyperleptinemia often present in visceral obese patients, may aggravate IR, promoting intrahepatocyte fat storage[94], and therefore, fatty liver, leptin was not a associated with LSR or liver attenuation. Inflammatory cytokines such as TNF-α and IL-6, commonly overexpressed in obese patients or overweight subjects with type 2 DM have been related with ectopic liver fat storage and NASH pathogenesis[86]. However, both inflammatory and atherothrombotic risk factors were not predictors of lower LSR in our study.

There are some limitations in this work that warrants mention. In fact, it is noteworthy to highlight that liver fat attenuation can not reflect absolute liver fat, since attenuation of each voxel is a function of its lipid, lean tissue and water composition. Conversely, variations of each component may change the attenuation value, adding data interpretation difficulties. In addition, despite the rigorous protocol to obtain fasting blood samples, being controlled the stage of menstrual cycle to avoid lipid blood profile variations induced by phase changes, we did not control diet composition prior blood sampling.

REFERENCES

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature.* Dec 13 2001;414(6865):782-787.

2. Grundy SM. Metabolic Syndrome: Connecting and Reconciling Cardiovascular and Diabetes World. *J Am Coll Cardiol.* 2006;47:1093-1100.

3. WHO. *Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation.* Geneva, Switzerland: World Health Organization; 2002.

4. Vague J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, athrosclerosis, gout, and uric calculous disease. *Am J Clin Nutr.* 1956;4:20-34.

5. Pouliot MC, Despres JP, Lemieux S, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol.* 1994;73:460-468.

6. Rankinen T, Kim SY, Perusse L, Despres JP, Bouchard C. The prediction of abdominal visceral fat level from body composition and anthropometry: ROC analysis. *Int J Obes Relat Metab Disord.* Aug 1999;23(8):801-809.

7. Seidell JC, Oosterlee A, Deurenberg P, Hautvast JG, Ruijs JH. Abdominal fat depots measured with computed tomography: effects of degree of obesity, sex, and age. *Eur J Clin Nutr.* Sep 1988;42(9):805-815.

8. Han TS, McNeill G, Seidell JC, Lean ME. Predicting intra-abdominal fatness from anthropometric measures: the influence of stature. *Int J Obes Relat Metab Disord.* Jul 1997;21(7):587-593.

9. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation.* Dec 17 2002;106(25):3143-3421.

10. Snijder MB, Dekker JM, Visser M, et al. Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: the Hoorn Study. *Am J Clin Nutr.* 2003;77(5):1192-1197.

11. Rexrode KM, Carey VJ, Hennekens CH, et al. Abdominal adiposity and coronary artery disease in women. *JAMA.* 1999;281(24):2284-2285.

12. Silventoinen K, Jousilahti P, Vartiainen E, Tuomilehto J. Appropriateness of anthropometric obesity indicators in assessment of coronary heart disease risk among Finnish men and women. *Scand J Public Health.* 2003;31(4):283-290.

13. Snijder MB, Dekker JM, Visser M, et al. Trunk fat and leg fat have independent and opposite associations with fasting and postload glucose levels: the Hoorn study. *Diabetes Care.* Feb 2004;27(2):372-377.

14. Snijder MB, Dekker JM, Visser M, et al. Larger thigh and hip circumferences are associated with better glucose tolerance: the Hoorn study. *Obes Res.* Jan 2003;11(1):104-111.

15. Snijder MB, Zimmet PZ, Visser M, Dekker JM, Seidell JC, Shaw JE. Independent and opposite associations of waist and hip circumferences with diabetes, hypertension and dyslipidemia: the AusDiab Study. *Int J Obes Relat Metab Disord.* Mar 2004;28(3):402-409.

16. Snijder MB, Zimmet PZ, Visser M, Dekker JM, Seidell JC, Shaw JE. Independent association of hip circumference with metabolic profile in different ethnic groups. *Obes Res.* 2004;12(9):1370-1374.

17. Lissner L, Bjorkelund C, Heitmann BL, Seidell JC, Bengtsson C. Larger hip circumference independently predicts health and longevity in a Swedish female cohort. *Obes Res.* Oct 2001;9(10):644-646.
18. Seidell JC, Han TS, Feskens EJ, Lean ME. Narrow hips and broad waist circumferences independently contribute to increased risk of non-insulin-dependent diabetes mellitus. *J Intern med.* Nov 1997;242(5):401-406.
19. Seidell JC, Han TS, Feskens EJ, Lean ME. Narrow hips and broad waist circumferences independently contribute to increased risk of non-insulin-dependent diabetes mellitus. *J Intern med.* 1997;242(5):401-406.
20. Heitmann BL, Frederiksen P, Lissner L. Hip circumference and cardiovascular morbidity and mortality in men and women. *Obes Res.* Mar 2004;12(3):482-487.
21. Tanko LB, Bagger YZ, Alexandersen P, Larsen PJ, Christiansen C. Peripheral adiposity exhibits an independent dominant antiatherogenic effect in elderly women. *Circulation.* Apr 1 2003;107(12):1626-1631.
22. Seidell JC, Perusse L, Despres JP, Bouchard C. Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. *Am J Clin Nutr.* Sep 2001;74(3):315-321.
23. Bastard JP, Pieroni L, Hainque B. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. *Diabetes Metab Res Rev.* May-Jun 2000;16(3):192-201.
24. McLaughlin T, Abbasi F, Lamendola C, et al. Differentiation between obesity and insulin resistance in the association with C-reactive protein. *Circulation.* Dec 3 2002;106(23):2908-2912.
25. Calles-Escandon J, Mirza SA, Sobel BE, Schneider DJ. Induction of hyperinsulinemia combined with hyperglycemia and hypertriglyceridemia increases plasminogen activator inhibitor 1 in blood in normal subjects. *Diabetes.* 1998;47:290-293.
26. Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE. The cardiovascular risk factor plasminogen activator inhibitor type 1 is related to insulin resistance. *Metabolism.* Aug 1993;42(8):945-949.
27. Festa A, D'Agostino R, Jr., Mykkanen L, et al. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance. The Insulin Resistance Atherosclerosis Study (IRAS). *Arterioscler Thromb Vasc Biol.* Mar 1999;19(3):562-568.
28. Schling P, Mallow H, Trindl A, Loffler G. Evidence for a local renin angiotensin system in primary cultured human preadipocytes. *Int J Obes Relat Metab Disord.* Apr 1999;23(4):336-341.
29. Yudkin JS, Coppack SW, Bulmer K, Rawesh A, Mohamed-Ali V. Lack of evidence for secretion of plasminogen activator inhibitor-1 by human subcutaneous adipose tissue in vivo. *Thromb Res.* 1999;96:1-9.
30. Tatsukawa M, Kurokawa M, Tamari Y, Yoshimatsu H, Sakata T. Regional fat deposition in the legs is useful as a presumptive marker of antiatherogenesity in Japanese. *Proc Soc Exp Biol Med.* Feb 2000;223(2):156-162.
31. Abate N, Garg A, Peshock RM, Stray-Gunderson J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest.* 1995;96:88-98.
32. Tanko LB, Bagger YZ, Alexandersen P, Larsen PJ, Christiansen C. Central and peripheral fat mass have contrasting effect on the progression of aortic calcification in postmenopausal women. *Eur Heart J.* Aug 2003;24(16):1531-1537.

33. Pouliot MC, Despres JP, Lemieux S, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol.* Mar 1 1994;73(7):460-468.

34. Snijder MB, Visser M, Dekker JM. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC study. *Diabetologia.* 2005;48:301-308.

35. Hunter GR, Kekes-Szabo T, Snyder SW, Nicholson C, Nyikos I, Berland L. Fat distribution, physical activity, and cardiovascular risk factors. *Med Sci Spor Exer.* 1997;29(3):362-369.

36. Rebuffe-Scrive M, Eldh J, Hafstroem L-O, al. e. Metabolism of mammary, abdominal, and femoral adipocytes in women before and after menopause. *Metabolism.* 1986;35:792-797.

37. Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and faillure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann NY Acad Sci.* 2002;967:363-378.

38. Snijder MB, van Dam RM, Visser M, Seidell JC. What aspects of body fat are particularly hazardous and how do we measure them? *Int J Epidemiol.* Feb 2006;35(1):83-92.

39. Chowdhury B, Lantz H, Sjostrom L. Computed tomography-determined body composition in relation to cardiovascular risk factors in Indian and matched Swedish males. *Metabolism.* 1996;45:634-644.

40. Yki-Jarvinen H, Koivisto VA, Karonen SL. Influence of body composition on insulin clearance. *Clin Physiol.* 1985;5:45-52.

41. Goodpaster BH, Krishnaswami S, Resnick H, et al. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care.* Feb 2003;26(2):372-379.

42. Boyko EJ, Fujimoto WY, Leonetti DL, Newell-Morris L. Visceral adiposity and risk of type 2 diabetes: a prospective study among Japanese Americans. *Diabetes Care.* Apr 2000;23(4):465-471.

43. Frayn KN. Visceral fat and insulin resistance-causative or correlative? *Br J Nutr.* 2000;83(Suppl 1):S71-S77.

44. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes.* 1997;46:1579-1585.

45. Nicklas BJ, Penninx BW, Ryan AS. Visceral adipose tissue cutoffs associated with metabolic risk factors for coronary heart disease in women. *Diabetes Care.* 2003;26:1413-1420.

46. Wagenknecht LE, Langefeld CD, Scherzinger AL, et al. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes.* Oct 2003;52(10):2490-2496.

47. Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes Metab Res Rev.* Mar-Apr 2002;18 Suppl 2:S5-9.

48. Mittelman SD, Van Citters GW, Kim SP, et al. Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response. *Diabetes.* Dec 2000;49(12):2116-2125.

49. Johnson JA, Fried SK, Pi-Sunyer FX, Albu JB. Impaired insulin action in subcutaneous adipocytes from women with visceral obesity. *Am J Physiol Endocrinol Metab.* Jan 2001;280(1):E40-49.

50. Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab.* Jun 2001;280(6):E827-847.
51. Wiman B, Chmielewska J, Ranby M. Inactivation of tissue plasminogen activator in plasma. Demonstration of a complex with a new rapid inhibitor. *J Biol Chem.* Mar 25 1984;259(6):3644-3647.
52. Juhan-Vague I, Alessi MC. PAI-1, obesity, insulin resistance and risk of cardiovascular events. *Thromb Haemost.* Jul 1997;78(1):656-660.
53. Shimomura I, Funahashi T, Takahashi M, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med.* Jul 1996;2(7):800-803.
54. Samad F, Loskutoff DJ. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. *Mol Med.* Sep 1996;2(5):568-582.
55. Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation.* Jul 4 2000;102(1):42-47.
56. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol.* Apr 1999;19(4):972-978.
57. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. *Circulation.* 2004;110:380-385.
58. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* May 2001;86(5):1930-1935.
59. Kazumi T, Kawaguchi A, Sakai K, Hirano T, Yoshino G. Young men with high-normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. *Diabetes Care.* Jun 2002;25(6):971-976.
60. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol.* Jan 1 2003;23(1):85-89.
61. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA.* Apr 14 2004;291(14):1730-1737.
62. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med.* Nov 2002;8(11):1288-1295.
63. Ross R, Aru J, Freeman J, Hudson R, Janssen I. Abdominal adiposity and insulin resistance in obese men. *Am J Physiol Endocrinol Metab.* 2002;282:E657-E663.
64. Kuk JL, Nichaman MZ, Church TS, Blair SN, Ross R. Liver fat is not a marker of metabolic risk in lean premenopausal women. *Metabolism.* 2004;53(8):1066-1071.
65. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab.* Mar 1998;83(3):847-850.
66. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *Am J Physiol Endocrinol Metab.* 2000;278(5):E941-948.

67. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol.* Jul 2000;89(1):104-110.
68. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *Am J Clin Nutr.* Apr 2000;71(4):885-892.
69. Frayn KN. Adipose tissue as a buffer for daily lipid flux. *Diabetologia.* Sep 2002;45(9):1201-1210.
70. Jensen MD, Haymond MW, Rizza RA, Cryer PE, Miles JM. Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest.* 1989;83:1168-1173.
71. Unger RH. Weapons of lean body mass destruction: The role of ectopic lipids in the metabolic syndrome. *Endocrinology.* 2003;144(12):5159-5165.
72. Van Steenbergen W, Lanckmans S. Liver disturbances in obesity and diabetes mellitus. *Int J Obes.* 1995;19(Suppl 3):S27-S36.
73. Smith S, Ravussin E. Emerging paradigms for understanding fatness and diabetes risk. *Curr Diab Rep.* 2002;2:223-239.
74. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Non-alcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology.* 1994;107:1103-1109.
75. Marchesini G, Brizi M, Morselli-Labate A, et al. Association of nonalcoholic liver disease with insulin resistance. *Am J Med.* 1999;107:450-455.
76. Clain D, Lefkowitch J. Fatty liver disease in morbid obesity. *Gastroenterol Clin North Am.* 1987;16(2):239-252.
77. Pierkarski JGHI, Royal SA, Axel L, Moss AA. Difference between liver and spleen CT numbers in the normal adult: its usefulness in predicting the presence of diffuse liver disease. *Radiology.* 1980;137:727-729.
78. Banerji MA, Buckley MC, Chaiken RL. Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black man with NIDDM. *Int J Obes.* 1995;19:846-850.
79. Scheen AJ, Luyckx FH. Obesity and liver disease. *Best Pract & Res Clin Endocrinol Metab.* 2002;16(4):703-716.
80. Sabir N, Sermez Y, Kazil S, Zencir M. Correlation of abdominal fat accumulation and liver steatosis: importance of ultrasonographic and anthropometric measurements. *Eur J Ultrasound.* 2001;14:121-128.
81. Knobler H, Schattner A, Zhornicki T. Fatty liver - an adittional and treatable feature of the insulin resistance syndrome. *Q J Med.* 1999;92(73-79).
82. Kelley DE, McKolanis TM, Hegazi RA, Kuller LH, Kalhan SC. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab.* Oct 2003;285(4):E906-916.
83. Frayn KN. Insulin resistance, impaired postprandial lipid metabolism and abdominal obesity. A deadly triad. *Med Princ Pract.* 2002;11 Suppl 2:31-40.
84. Rebuffe-Scrive M, Enk L, Crona N, et al. Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy, and lactation. *J Clin Invest.* Jun 1985;75(6):1973-1976.
85. Bergstrom RW, Newell-Morris LL, Loeonetti DL, Shuman WP, Wahl PW, Fujimoto WY. Association of elevated fasting C-peptide level and increased intra-abdominal fat distribution with development of NIDDM in Japanese-American men. *Diabetes.* 1990;39:104-111.

86. Luyckx FH, Lefebvre PJ, Scheen AJ. Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss. *Diab Metab.* 2000;26(2):98-106.
87. Luyckx FH, Desaive C, Thiry A. Liver abnormalities in severely obese sbujects: effects of drastic weight loss after gastroplasty. *Int J Obes.* 1998;22:222-226.
88. Tiikainen M, Tamminen M, Hakkinen AM. Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes. *Obes Res.* 2002;10(859-867).
89. Nguyen-Duy TB, Nichaman MZ, Church TS. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab.* 2003;284:E1065-E1071.
90. Angulo P, Keach JC, Batts KP. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology.* 1999;30:1356-1362.
91. Rinella ME, Alonso E, Rao S. Body mass index as a predictor of hepatic steatosis in living liver donors. *Liv Transpl.* 2001;7:409-414.
92. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology.* Jun 2001;33(6):1358-1364.
93. Guzzaloni G, Grugni G, Minocci A. Liver steatosis in juvenile obesity: correlations with lipid profile, hepatic biochemical parameters and glycemic and insulinemic responses to an oral glucose tolerance test. *Int J Obes.* 2000;24:772-776.
94. Unger RH. Leptin physiology: a second look. *Regul Pept.* Aug 25 2000;92(1-3):87-95.