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Body composition in relation to type 2 diabetes mellitus and cardiovascular disease risk

The role of different fat depots and lean tissue

The study presented in this thesis was performed at the Institute for Research in Extramural Medicine (EMGO Institute) of the VU University Medical Center, Amsterdam, the Netherlands. The EMGO Institute participates in the Netherlands School of Primary Care Research (CaRe), which was re-acknowledged in 2000 by the Royal Netherlands Academy of Arts and Science (KNAW).

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Body composition in relation to type 2 diabetes mellitus and cardiovascular disease risk

The role of different fat depots and lean tissue

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Vrije Universiteit Amsterdam, op gezag van de rector magnificus prof.dr. T. Sminia, in het openbaar te verdedigen ten overstaan van de promotiecommissie van de faculteit der Geneeskunde op vrijdag 18 juni 2004 om 13.45 uur in de aula van de universiteit, De Boelelaan 1105

door Marieke Brigitte Snijder geboren te Vlissingen promotoren: prof.dr.ir. J.C. Seidell

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Introduction

The overall aim of the present thesis is to gain insight in the role of different fat depots and lean tissue in type 2 diabetes, the metabolic syndrome and cardiovascular disease risk. In the following, the main findings of the studies described in this thesis are first summarized. Some important methodological considerations are then discussed, followed by the pathophysiological interpretation, and practical consequences. Finally, we will end with suggestions for future research and conclusions.

Main findings

Anthropometry

Body fat distribution, apart from overall obesity, is considered to be an important determinant of disturbed glucose metabolism. Currently, there is a tendency to measure only waist circumference and to ignore measurements of hip or thigh circumference when assessing the health risk of abdominal obesity. Recent guidelines have recommended to use waist circumference cut points for the identification of high risk individuals (Table 2 of Chapter 1) ¹. However, results of this thesis suggest that this may result in substantial loss of information.

In a Dutch population-based cohort study that started in 1989, the Hoorn Study, we found that, as expected, larger waist circumference was associated with higher levels of fasting and postload glucose, fasting insulin, and fasting HbA1c (Chapter 2). After adjustment for waist circumference, age, and BMI, however, larger hip circumference was associated with *lower* levels of fasting and postload glucose, insulin, and HbA1c, in both sexes. For thigh circumference similar results were found but these associations were statistically significant in women only.

In 1996 all surviving cohort members of the Hoorn Study were invited to participate in a second health examination. We observed that among persons who were free of diabetes at baseline, those with a larger hip or thigh circumference at baseline were at lower risk to develop diabetes after 6 years, taking baseline age, BMI and waist circumference into account (Chapter 3). In a larger Australian population-based study, the AusDiab Study, we confirmed the cross-sectional results of the Hoorn Study by showing that larger hip circumference was independently associated with lower glucose levels (Chapter 4). Furthermore, we extended our observations by considering additional components of the metabolic syndrome as outcome, i.e., blood pressure and blood lipids. The AusDiab Study covers a wide age range (all ages above 25 years), and the independent association of a larger hip circumference with more favourable levels of glucose, triglycerides and HDL-cholesterol, was apparent across all age groups, except in persons aged 75 years or older.

In population studies in Mauritius, Rodrigues, Nauru, and Papua New Guinea, we found that the opposite associations of waist and hip circumferences with glucose and lipid levels, and to a lesser extent with blood pressure values, were consistent across different ethnic populations, despite considerable differences in body composition and susceptibility to diabetes (Chapter 5).

So, to specifically answer the main research questions as formulated in Chapter 1, waist circumference was independently of hip circumference and BMI associated with unfavourable

glucose and lipid levels and to a lesser extent blood pressure. In contrast, hip circumference was independently of waist circumference and BMI associated with more favourable levels (research question 1). These associations were present in both sexes and were consistent across ethnicity groups and all ages except above the age of 75 years (research question 2).

Dual energy X-ray absorptiometry

In the third health examination of the Hoorn Study in 2001, when participants were aged 60 to 85 years, we found that waist circumference mainly reflected trunk fat mass as determined by DXA and was hardly influenced by the amount of lean mass in the trunk, in both sexes (Chapter 6). The variability of hip circumference was mainly determined by fat mass in the legs, but was also largely dependent of the amount of leg lean mass, particularly in men. It appeared that accumulation of fat mass in the legs as well as larger lean (muscle) mass in the legs was associated with a better glucose tolerance status after adjustment for trunk fat mass.

In the same follow-up examination of the Hoorn Study we investigated whether different regional fat depots or lean tissue were determinants of arterial stiffness, which is a marker of vascular function that is related to cardiovascular disease (Chapter 7). Larger trunk fat mass was associated with higher peripheral arterial stiffness but not with central arterial stiffness. Larger leg fat mass had opposite, although weaker, associations with peripheral arterial stiffness. Larger leg lean mass was the most important determinant of lower central arterial stiffness.

Thus, in answer to research question 3, waist circumference mainly reflected trunk fat mass, whereas hip circumference reflected both leg fat and leg lean mass. Larger trunk fat mass was main determinant of high glucose levels, whereas larger leg lean mass and larger leg fat mass were associated with lower glucose levels (research question 4).

Computed tomography

In participants of the Health ABC Study (aged 70 to 79 years) we were able to separate muscle, visceral fat, and subcutaneous fat in the abdomen, and to distinguish between muscle, subcutaneous fat, and intermuscular fat at the thigh, by means of computed tomography (CT). The results confirmed our DXA observations: waist circumference was mainly determined by fat area at the abdomen. It was specifically related to subcutaneous fat area from the abdomen, and to a lesser extent to visceral fat area (Chapter 8). Thigh circumference reflected mainly subcutaneous fat in women, whereas muscle and subcutaneous fat both contributed considerably to thigh circumference in men. Furthermore, visceral fat was the strongest independent determinant of unfavourable glucose and lipid levels but abdominal subcutaneous fat also significantly contributed to unfavourable glucose and lipid levels. Thigh subcutaneous fat was independently associated with more favourable lipid levels in both sexes, and with lower glucose levels in men.

In a sub-sample of 150 subjects of the Health ABC Study population, we found that measurement of total abdominal fat mass in a 4 cm slice at the abdomen by DXA (sub-region comparable to the CT slice used) was highly correlated with total abdominal fat measured by CT. However, DXA tended to underestimate abdominal fat in relatively older people with less

abdominal fat. We also showed that regional DXA measurements (both total trunk fat and 4 cm slice sub-region) of abdominal fat were not stronger associated to visceral fat than anthropometric measures (i.e., waist circumference and sagittal diameter).

In summary, it was shown that waist circumference mainly reflects subcutaneous abdominal fat tissue (research question 3), whereas visceral fat was the main determinant of unfavourable glucose and lipid levels (research question 5). Thigh circumference reflected mainly subcutaneous thigh fat in women, whereas both subcutaneous thigh fat and thigh muscle contributed to thigh circumference in men (research question 3). Subcutaneous thigh fat was independently of abdominal subcutaneous and visceral fat associated with more favourable glucose and lipid levels (research question 5).

Methodological considerations

Study design

All studies described in this thesis were observational studies. Therefore, study participants may have been different in several other characteristics apart from body composition, which was the main determinant of interest. These characteristics may have confounded our results if they were related to both body composition and the outcome variable of interest. However, participants of the studies described in this thesis were extensively examined and many putative confounders were determined and have been adjusted for in the statistical analyses. Still, it cannot be excluded that effects of all potential confounders were not adequately controlled for (residual confounding). Since we have investigated relatively large study populations, and have confirmed our results in different, independent populations, the possibility that our results are just chance findings seems unlikely.

The Hoorn Study, the AusDiab Study, the studies in Mauritius, Rodrigues, Nauru, Papua New Guinea, and the Health ABC Study were all population-based. Therefore, it is assumed that results found in these studies can be extrapolated to all persons in the population of similar geographical region and age. However, it should be kept in mind that only persons who are physically able to come to the research centre will enter the study population. Therefore, relatively unhealthy persons are possibly underrepresented. This may lead to underestimation of the true associations, if these persons are more extreme in body composition and metabolic factors.

Participants of the second examination of the Hoorn Study (1996), six years after the baseline examinations, were relatively healthier at baseline than were the non-responders (selection bias) or those who died (healthy survivor effect). This differential mortality in prospective studies possibly also leads to an underestimation of the true associations. This phenomenon probably also occurred at the third examination of the Hoorn Study in 2001.

All our studies were cross-sectional, except one (Chapter 3), which was prospective. A well-known limitation of cross-sectional studies is that one should be cautious to draw conclusions about cause and effect. If the outcome and determinant are measured at the same point in time, it is not possible to ascertain the order of occurrence. Longitudinal studies are needed to appropriately address this issue. Still, also data from longitudinal studies cannot exclude the

possibility that variables are not causally related and that both are determined by another (not measured) underlying cause. For example, genetic factors, low birth weight, hormone concentrations, and the sympathetic nervous system could all be involved in the development of an unfavourable body composition as well as metabolic disturbances (see also below).

Confounding and effect modification

Multiple regression analysis was used to study our research questions. This method is particularly useful when the interest is in a linear association between two variables, taking the effects of other variables (confounders) into account. In addition, effect modification can be assessed easily with the use of this method. A potential confounder is associated both with the outcome and with the determinant but is not involved in the causal pathway. The latter, however, is often difficult to determine and essentially not known. Adjustment for variables that are involved in the causal pathway leads to over-adjustment of the true associations. Therefore, in case of uncertainty, we reported the unadjusted as well as adjusted associations. For example, in our longitudinal analyses in Chapter 3 we studied baseline waist and hip circumference in relation to incident diabetes. Should we adjust for baseline glucose levels, or not? If there is a causal relationship between hip circumference and glucose levels, it is possible that persons with narrow hips had increased glucose levels already at baseline, and adjustment would then be inappropriate. If baseline glucose levels and circumferences result from a shared underlying cause, adjustment would also be inappropriate. Lifestyle factors, such as smoking, physical activity and alcohol intake could have confounded our results, as they are associated with both body composition and diabetes. On the other hand, lifestyle factors can also be associated with diabetes, through the effect they have on body composition. In our data adjustment for lifestyle did not materially change our results. It may be, however, that lifestyle factors were not optimally measured because self-administered questionnaires were used (information bias).

In all analyses, we examined potential effect modification by sex. It is common use to stratify by sex in studies on body composition because of the known differences between men and women. Sex differences in body composition do not necessarily imply that the relations between body composition and outcome are different between sexes. In most cases, however, we found statistically significant effect modification by sex, and therefore we reported results for men and women separately. Another important possible effect modifier was age. In the Hoorn Study, a relatively narrow age range was captured (50 to 75 years) and we did not find statistically significant differences between different age groups within this study sample. In the AusDiab Study, however, we were able to study the total adult age range and we showed that in women the relations of anthropometry with metabolic variables disappear above the age of 75 years, whereas in men the associations disappear above 65 years. Effect modification by age can possibly explain why we found associations to be stronger in women than in men in the Hoorn Study: subjects were aged 50 to 75 years, and the association may have already started to disappear in men.

In the most recent follow-up examination of the Hoorn Study in 2001, we studied a glucose tolerance status-stratified sample for reasons of efficiency. Due to this sampling procedure the glucose intolerant subjects were relatively over-represented, which probably also caused an over-

representation of subjects with an unhealthy fat distribution or body composition compared to the general population. Depending on the research question at issue this sampling procedure may have affected our results. For example, in Chapter 6, we investigated body composition measures as independent determinants of glucose levels. In this case, the results will not be influenced by the sampling procedure, except if associations are different within each group of glucose tolerance status (effect modification). We tested for effect modification by glucose tolerance status and this was not statistically significant. In Chapter 7, we investigated the association between body composition and arterial stiffness. It is very likely that the associations that we found are stronger in this study population compared to the general population. In this case, the over-representation of glucose intolerant subjects increased the statistical power to detect the true associations. However, it is known that worse glucose tolerance status is associated with increased arterial stiffness and also with an unfavourable body composition. Therefore, it may also be a confounding variable. Indeed, adjustment for glucose tolerance status weakened some of the associations. However, it is also very likely that glucose levels, or other factors associated with glucose tolerance status, are mediators of the association and in that case adjustment is not justified.

Multi-colinearity

A potential problem in multiple regression analysis is multi-colinearity. If independent variables are highly correlated with each other, it is very difficult to separate the effects of these variables statistically. This is certainly true for components of body composition. There is as yet no widely accepted method for analysis of these data. It could be argued that the opposite associations of waist and hip circumferences, and of trunk and leg fat, with metabolic variables are (to some extent) statistical artefacts. There is a rule of thumb that says that it is unwise to include two independent variables into the multiple regression model if the correlation between these variables is larger than 0.9. Several statistical packages have a diagnostic check (e.g., the tolerance test) to check for multi-colinearity. Nevertheless, criteria to exclude multi-colinearity in these tests are also rules of thumb and it is possible for models to pass these tests, whereas results from the regression analysis are clearly unreliable, as shown by very large estimated coefficients and extraordinary large estimated standard errors.

In our data, correlations between components of body composition were never larger than 0.9, except in Chapter 7. In addition, a diagnostic test (tolerance testing) did not show disturbances due to multi-colinearity in our models, except in Chapter 7. In Chapter 7, lean mass in the trunk was highly correlated with lean mass in the legs, partially because men and women were analysed together. Therefore, we excluded trunk lean mass from the analyses in this chapter.

In addition to tolerance testing, which did not show disturbances in our models, the estimated coefficients and standard errors were not extremely and unreliably large. The final results that convinced us that opposite associations of waist and hip circumference were not caused by multi-colinearity, were figures showing mean glucose levels according to combined strata of waist and hip circumference (e.g., Figure 1 in Chapter 2 and Figure 1 in Chapter 4). In this way, no statistical modelling is performed that would potentially produce unreliable results due to multi-colinearity.

Pathophysiological interpretation

We have shown that anthropometric measures should be interpreted with caution in a physiological sense. Waist and hip circumferences do not only reflect (the distribution of) fat mass, but are also influenced by the amount of regional lean mass. This should be realized when associations of anthropometric measures with glucose metabolism and other components of the metabolic syndrome are interpreted. Larger lean mass was generally beneficial for glucose and lipid metabolism, and vascular function. Furthermore, it was concluded that not all fat depots are detrimental and that some depots may even be beneficial. We now explore some pathophysiological mechanisms possibly underlying the observed associations.

Beneficial effects of muscle

Larger lean mass in the legs measured by DXA, which mainly reflects larger muscle mass, was related to better insulin sensitivity (Chapter 6). Although insulin resistance affects all tissues, skeletal muscle is the main target of insulin and the major site of insulin resistance. Fat depots also have a reduced glucose uptake in obese persons, however, because of enlargement of the fat mass, insulin resistance of adipose tissue does not seem to contribute substantially to the reduced insulin stimulated whole-body glucose uptake in obesity ².

During aging, the number and size of muscle fibers decrease and a change in muscle fiber type occurs (relative larger loss of type 2 fibers) ³, which is probably involved in increasing the risk of metabolic disturbances leading to increased health risk in the elderly. Skeletal muscle fibers have a great adaptive potential resulting from the ability to change their molecular, functional, and metabolic properties in response to altered demands, such as physical activity. Depending on the intensity and duration of physical activity, muscle fibers undergo fiber type transitions (e.g. from type 2B to type 2A) ⁴. Fiber type could play a role in determining insulin action because type 2B fibers are less insulin sensitive compared to type 1 and type 2A fibers. Decreased insulin action is related to an increase in the proportion of type 2B fiber type and also to decreased density of capillary supply to the skeletal muscle ⁵. There may be biochemical changes related to oxidative capacity in muscle cells or impaired diffusion of insulin when capillaries are more widely spaced.

Exercise may slow down the aging-related fiber type transition and may induce a favourable fiber type transition in physically inactive persons. Our data, however, suggest that the relation of larger muscle mass with better metabolic outcome is independent of age and physical activity. It is very difficult, however, to accurately measure physical activity. In our studies it was assessed by questionnaires. It may be that muscle mass is actually a better estimate of physical activity.

In contrast to the results when DXA was used (Chapter 6), we did not find associations of larger muscle areas by CT with more favourable metabolic variables in the Health ABC Study (Chapter 8). It is possible that a single CT slice at the thigh is not representative of the total muscle mass in the legs.

Detrimental effects of abdominal fat

More abdominal fat, in particular visceral fat, was the most important determinant of unfavourable glucose and lipid levels. As explained in the General Introduction (Chapter 1), this could be due to the fact that visceral fat has a high FFA turnover rate compared to other fat depots. Therefore, visceral fat is more likely to release free fatty acids into the circulation causing increased free fatty acid levels, which may lead to ectopic fat storage in muscle, liver and pancreas ⁶⁻⁹. This in turn leads to insulin resistance in these organs, and impairment of insulin secretion due to beta-cell deterioration. In addition, due to the release of these FFA from visceral fat into the portal vein that directly leads to the liver, they also cause reduced hepatic insulin clearance and increased gluconeogenesis in the liver. Removal of visceral fat reversed hepatic insulin resistance in rats ¹⁰. And a pilot study in humans suggests that omentectomy (removal of part of visceral fat) might improve the metabolic profile ¹¹.

Results from the literature are not consistent on whether abdominal subcutaneous fat mass also independently contributes to the increased risk of an unfavourable metabolic profile 12 . In the Health ABC Study (Chapter 8), the associations of abdominal subcutaneous fat were weaker compared to visceral fat, but substantially and independently contributed to higher glucose and lipid levels. This is in agreement with previous, smaller studies 13,14 . In another study in six obese women, surgical removal of abdominal subcutaneous fat (55 to 65 % of subcutaneous abdominal fat corresponding to 4.3 \pm 1.1 litres) by liposuction, led to improvement of insulin sensitivity and glucose levels after 3 to 4 weeks 15 . There were no changes in lipid levels, however, and long-term consequences are yet unknown and should be investigated.

Abdominal subcutaneous fat can be further divided into deep and superficial subcutaneous adipose tissue. It was demonstrated that the amount of deep subcutaneous adipose tissue had a much stronger relationship with insulin resistance than superficial subcutaneous fat, which may be due to differences in lipolysis ¹⁶. This may contribute to the inconsistent results regarding total abdominal subcutaneous fat in relation to insulin resistance.

Beneficial effects of femoral-gluteal fat

We observed larger leg fat mass, particularly subcutaneous fat, to be associated with *better* glucose and lipid levels after adjustment for trunk fat mass and lean mass. This finding can be explained by a mechanism that has been recently proposed ¹⁷.

It is suggested that the femoral-gluteal fat depot plays a protective role, by acting as a 'sink' for circulating FFA. Adipocytes in the femoral region are relatively insensitive to lipolytic stimuli and have a high sensitivity for anti-lipolytic stimuli. The enzyme lipoprotein lipase (LPL) plays an important role in the uptake of free fatty acids from the circulation, whereas hormone sensitive lipase (HSL) stimulates lipolysis. Particularly in women, the femoral fat depot has a relatively high LPL activity and relatively low rate of basal and stimulated lipolysis ^{18,19}. Therefore, the femoral-gluteal region is more likely to effectively take up FFA from the circulation and is less likely to release them readily. By this FFA uptake, ectopic fat storage in the liver, skeletal muscle and pancreas, which causes insulin resistance and beta cell dysfunction, is prevented ²⁰. In other words, if one is more capable to store lipids in the femoral-gluteal adipose tissue (which results in larger hips), circulatory lipid levels will be lower and therefore cause less damage to organs,

resulting in a lower risk of a disturbed glucose and lipid metabolism (Figure 1). Our findings are consistent with recent published data on the protection by peripheral fat mass by DXA in women from metabolic cardiovascular risk factors ²¹, and from aortic calcification which is an early marker of arteriosclerosis ²².

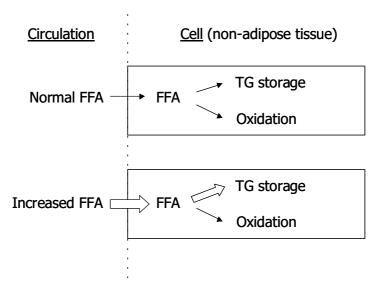


Figure 1. Schematic representation of intracellular disposition of fatty acids in non-adipose tissue. Increased FFA in the circulation (possibly due to reduced FFA uptake and storage by femoral-gluteal adipose tissue) lead to increased lipid accumulation in muscle, liver and pancreas cells (ectopic fat storage), which is related to insulin resistance and beta-cell dysfunction ^{17,20}

In line with this new potential mechanism, transplantation of subcutaneous adipose tissue in lipoatrophic animals reversed the elevated glucose levels ²³. Subcutaneous lipectomy caused metabolic disturbances in hamsters ²⁴. In humans the critical role of subcutaneous adipose tissue is shown by the observation that adipose tissue deficiency (lipodystrophy or lipoatrophy) is also accompanied by ectopic fat storage, and insulin resistance and diabetes mellitus ²⁰. Since these persons have a very low total body fat content, this used to be an unexplained paradox. There are no studies that investigated the differential effects of liposuction in the femoral-gluteal region compared to liposuction in the abdominal region.

It has been observed that the medical drugs thiazolidinediones increased the insulin sensitivity of insulin resistant patients, whereas at the same time these persons accumulated a considerable amount of total body fat. These drugs promote preadipocyte differentiation into mature adipocytes, in particular in the gluteal regions ²⁵. These new mature adipocytes have a better capacity to store lipids, which results in less ectopic fat deposition, and consequently in less insulin resistance and beta-cell dysfunction.

Adipose tissue as a metabolic organ

Apart from storage and release of free fatty acids, adipose tissue secretes many signalling proteins and cytokines with broad biological activity and critical functions. These proteins act in a paracrine or endocrine fashion. Some of these adipokines may be involved in the development of insulin

resistance in obesity ²⁶, possibly mediating the relation between adipose tissue and glucose and lipid levels. Leptin, for example, plays a critical role in the control of energy balance, by suppressing appetite via the hypothalamus. But leptin is probably also directly involved in the insulin sensitivity and secretion ²⁶. Since plasma levels are positively correlated with BMI, obesity seems to reflect a leptin-resistant state. Leptin seems to have an insulin-sensitising effect on the whole body in vivo. Therefore, resistance for this effect could induce insulin resistance. One explanation might be that high leptin levels interfere with insulin signalling ²⁶.

Adiponectin, another protein secreted by fat cells, stimulates fatty acid oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing insulin sensitivity ²⁶⁻²⁸. In addition, adiponectin inhibits the inflammatory process and possibly arteriosclerosis. Paradoxically, the blood concentration of adiponectin is decreased in obese and patients of type 2 diabetes, while it is secreted by fat cells. This could point to a paracrine or autocrine regulation.

Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) are cytokines that are also produced by adipocytes. IL-6 increases hepatic glucose production, reduces LPL activity, stimulates lipolysis and increases hepatic triglyceride production ^{26,29}. The role of TNF-alpha in the induction of insulin resistance in humans is not as clearly shown as it is in rodents ²⁶. C-reactive protein (CRP) is an acute-phase reactant synthesised in the liver, largely regulated by cytokines, in particular IL-6. Plasma concentration of CRP shows a marked increase (>10 mg/L) in response to a variety of acute stimuli including infection, burns, surgery, major trauma and other inflammatory conditions ³⁰. Slightly elevated, but conventionally normal, CRP levels (<10 mg/L) may reflect a chronic, low-grade systemic inflammation. These slightly increased levels have also been found to be associated with (abdominal) obesity, diabetes, insulin resistance, and lipid levels 31-34. Prospective studies, however, show inconsistent findings, in particular between sexes. In a study described by Han et al. 35, a relation was found between baseline levels of CRP and the incidence of type 2 diabetes in women but not in men. Within the Hoorn Study, however, CRP was associated with high risk for developing type 2 diabetes in men only (see Appendix). This association was largely explained by WHR, which is in line with the hypothesis that abdominal obesity-induced increase of low-grade chronic inflammation (IL-6) possibly contributes to the development of type 2 diabetes ³⁶.

The secretory functions of adipose tissue is probably also subject to regional variations ³⁷. Therefore, it might be that the different associations of abdominal and femoral-gluteal subcutaneous fat depots with glucose and lipid levels are mediated by adipokines that are differently secreted by abdominal compared to femoral-gluteal fat depots. There are known differences in endocrine secretion of leptin, adiponectin and IL-6 between abdominal subcutaneous fat and visceral fat ³⁸⁻⁴⁰, whereas regional differences in secretion of plasminogen activator inhibitor-1 (PAI-1) and TNF-alpha are controversial ³⁷. Less is known, however, about differences in secretion of these adipokines between abdominal and femoral-gluteal subcutaneous fat. In addition, there are probably many more yet undiscovered proteins, differently secreted by different fat depots, that might influence metabolic function. Clearly, more research in this area is needed.

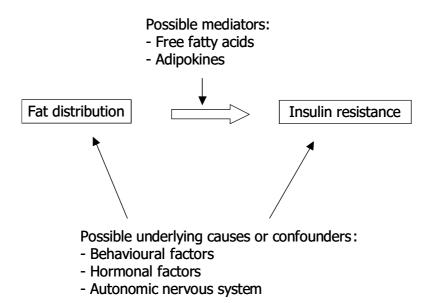


Figure 2. Simplified schematic presentation of the relation between fat distribution and insulin resistance with possible underlying mechanisms, mediators and confounders

Possible underlying causes or confounders

Disturbances in glucocorticoid metabolism have been shown to be associated with alterations in fat distribution and alterations in glucose metabolism ⁴¹. Björntorp suggested that these hormonal aberrations due to an increased sensitivity of the hypothalamic-pituitary-adrenal axis, may provide a cause for both visceral fat accumulation and for metabolic disturbances ⁴². This mechanism also provides a possible link between factors such as smoking and mental stress, and health risks ⁴³.

The observed changes in body composition in women after menopause, or during lactation, led to the hypothesis of regulation of fat distribution by sex hormones. Hyperandrogenicity in women and hypoandrogenicity in men has been associated with an unfavourable fat distribution, with increased insulin insensitivity, and the development of type 2 diabetes ⁴¹. Estrogens, for example, stimulate the accumulation of femoral-gluteal fat and are related to an improved cardiovascular risk ⁴⁴.

Finally, it is suggested that low growth hormone levels are associated with an unfavourable fat distribution and cardiovascular risk. Growth hormone deficiency is associated with increased cardiovascular morbidity and treatment favourably changes fat distribution and lipid levels ^{45,46}. Insulin sensitivity, however, is decreased with growth hormone replacement therapy ⁴⁶.

Taken together, hormones potentially underlie or confound the relation between fat distribution and metabolic risk that we found (Figure 2). In our studies, however, hormones were not determined so their influence could not be studied.

Apart from endocrine factors, body fat is related to autonomic nervous system function. It was recently shown that fat depots are innervated by the parasympathetic nervous system and stimulation promotes lipid accumulation (anabolic function) ⁴⁷. This complements the earlier finding of a sympathetic innervation that stimulates lipolysis in adipose tissue (catabolic function). Obesity appears to be associated with a reduced sympathetic nervous system reactivity to change in the

physiological environment ⁴⁸. It was demonstrated that decreased activation of the parasympathetic nervous system, which occurs in obesity and type 2 diabetes, resulted in reduced insulin-dependent uptake of glucose and free fatty acids in fat tissue and increased hormone sensitive lipase (HSL) activity resulting in increased lipolysis. Each of the fat depots (subcutaneous and intra-abdominal) is supplied by nerves originating from different parts of the central nervous system. This could possibly explain differences in the lipolytic rate between fat depots in different anatomic locations. Kreier et al. propose an unbalanced and disturbed autonomic nervous system function as a major cause of changed body fat storage and the metabolic syndrome. As a result of environmental changes (abundant food availability, shift of food intake to the end of the day, decreased physical activity) the susceptible brain loses its feeling for internal and external rhythm and the biological clock mechanisms become metabolically flattened and arrhythmic ⁴⁹. Therefore, a disturbed autonomic nervous system function is also a potential confounder or underlying cause of the relationship between fat distribution and metabolic risk, which was not considered in the present thesis.

Practical consequences

Anthropometry as a predictor of health risk

Our results suggest, irrespective of the underlying mechanisms, that both waist *and* hip circumference are important independent determinants of glucose metabolism and cardiovascular risk, in addition to overall obesity (BMI). Should all these measures therefore be considered in clinical practice to better predict the risk of diabetes and cardiovascular disease?

The use of these measures to identify persons with an increased health risk in clinical practice was not the aim of the present thesis, and was therefore not specifically investigated. We did not estimate the difference in risk assessment when hip or thigh circumferences are additionally included in a risk model already containing waist circumference, which requires specific diagnostic analyses.

In the baseline Hoorn Study examination (age 50 to 75 years), among persons that were not previously diagnosed with diabetes, 15.1 % was obese and 49.8 % was overweight based on BMI cut points ¹. Still, of the persons with normal BMI 55.5% had an overweight WHR and 28.8% had an overweight waist circumference (for cut points see Table 2 in Chapter 1), particularly in the oldest persons if stratified by age. In obese subjects, only 2.1% had a normal WHR and 0.3% had a normal waist circumference. We know, however, that even within the group of obese persons, those with a relatively higher WHR have an increased risk for type 2 diabetes and cardiovascular disease compared to those with lower WHR ^{50,51} (see also Chapter 1). These data suggests that the generally utilized cut points might be invalid and it may be necessary to develop BMI- and age-specific cut points.

Waist and hip circumferences should preferably not be combined in a ratio (such as WHR) because both measures show opposite associations with metabolic risk factors and combining them in a ratio could imply loss of information. Research should be performed to investigate the additional predictive value of each measure to estimate health risk. We expect that at very old age (\geq 75 years) waist and hip circumferences might be less useful due to age-related changes in body

composition, because at this age these measures showed weaker associations with cardiovascular risk factors, independent of BMI (Chapter 4). Further research in the clinical practice area is needed, which should also consider the extra time and effort that is needed to perform both measurements.

Hip circumference might lack sufficient explanatory power for routine use in clinical settings, but it is very useful for research proposes because it can give important information of physiological mechanisms. Also for research, it should preferably not be combined with waist circumference in a ratio. Apart from interpretative problems, the use of ratios can introduce spurious correlations between the ratio and other variables, and normality assumptions of statistics may be violated ⁵². Preferably continuous variables should be used in the analyses, because current cut points may lead to significant misclassification regarding health risks in specific groups.

Modification of regional fat depots and muscle by intervention

Lifestyle interventions and recommendations to improve health usually aim at weight loss ¹. However, it is possibly also relevant to consider modification of fat distribution and body composition.

Most studies on weight loss, either by diet or by exercise, report a proportional preferential mobilization of abdominal fat (particularly of visceral fat) compared to subcutaneous gluteal fat ⁵³⁻⁵⁷. In addition, physical activity is associated with an increased thigh muscle mass ⁵⁸. Apart from its influence on skeletal muscle mass, physical activity induces favourable fiber type transitions in the skeletal muscle tissue itself ⁴. Exercise also decreases fat deposition in thigh muscle ⁵⁸. Therefore, even without weight loss, increased physical activity can lead to better fat distribution and improved muscle mass and morphology.

Waist and hip circumferences and the tissues contributing to their variation may also be influenced by behavioural characteristics such as smoking and alcohol consumption. Persons who smoke have a higher WHR than non-smokers, due to larger waists and smaller hips ⁵⁹. It has been shown that the relation with waist circumference is mainly due to visceral fat rather than abdominal subcutaneous fat ⁶⁰. High WHR in heavy drinkers is caused by larger waists, but it is also related to reduced gluteal muscle mass ^{59,60}. Therefore, smoking cessation and drinking less heavily may be potential lifestyle strategies to improve fat distribution and their metabolic consequences. In a prospective mail survey including women only, in case of weight gain during a 10-year period, the likelihood of abdominal weight gain exceeded that of peripheral weight gain for cigarette smokers, frequent liquor users, and high meat eaters, whereas peripheral weight gain was more likely for high vegetable eaters and women who exercised more than 4 hrs a week ⁶¹.

In the Netherlands, two types of drugs are available for weight reduction (sibutramine and orlistat). Orlistat is a lipase inhibitor, which inhibits the lipid uptake from the gut. There are no studies that investigated the effect of orlistat on fat distribution, but it is likely that effects are similar to weight loss by diet. After weight loss by sibutramine, the proportional changes in visceral fat were larger than those in abdominal subcutaneous fat ⁶². Sibutramine inhibits the re-uptake of noradrenalin and serotonine in the hypothalamus, and thereby it induces the satiety and increases thermogenesis. In addition, after sibutramine use the waist circumference and WHR were reduced and were related to improvements of glucose and insulin levels ⁶³.

Suggestions for future research and conclusion

In addition to the well-known detrimental effects of visceral fat, we have shown that abdominal and femoral-gluteal subcutaneous fat depots are differently related to the metabolic profile. Taking abdominal fat into account, the fat depot in the femoral-gluteal region has no detrimental effect and may even be beneficial. The exact underlying mechanisms, however, remain unclear and more research in this area is needed.

First, our findings using DXA and CT measurements should be confirmed in other populations. In particular, studies including DXA and/or CT measurements should be performed in younger cohorts. If possible, the role of several lifestyle factors, such as physical activity, diet, and smoking, should be carefully investigated using more precise and objective measurements. It is important to know whether these lifestyle factors act as a confounder, effect modifier, or underlying cause.

In addition, future research should focus on metabolic differences between different fat depots, particularly metabolic differences between abdominal and femoral-gluteal subcutaneous fat depots. Differences in secretion of certain adipokines may explain our findings. It is relevant to know whether these metabolic characteristics change with aging. Also the mechanism causing the relation of larger muscle mass with vascular function deserves more attention.

To test the hypothesis of a reduced health risk with increased femoral-gluteal fat accumulation, it would be relevant to investigate the metabolic consequences of liposuction specifically from this region. Because liposuction becomes a popular cosmetic technique, it is important to know the potentially detrimental effects of this surgery.

It remains to be established whether the increased health risk associated with decreased hip circumference should be considered in clinical practice to predict health risk of the individual. In addition, it should be investigated whether the currently used cut points to assess increased health risk should be age- and BMI-specific. A future perspective could be to develop tables for clinical practice that indicate health risk for various levels of waist circumference for given age and BMI, which may more accurately than the BMI cut points presently used. Supplementary measurement of the hip circumference may improve the prediction of health risk.

In conclusion, this thesis provides a better understanding of the associations between body composition and fat distribution in relation to metabolic and cardiovascular risk. Apart from overall obesity, body fat distribution and muscle mass are important determinants of insulin resistance, the metabolic syndrome, diabetes and cardiovascular risk. In addition to the well-known detrimental effects of visceral fat, it was shown that abdominal and femoral-gluteal subcutaneous fat depots are differently related to the metabolic profile. The abdominal subcutaneous fat depot also has a detrimental association, in addition to visceral fat, whereas the fat depot in the femoral-gluteal region has no detrimental effect and may even be beneficial. Larger skeletal muscle mass is related to a more favourable metabolic profile.

Anthropometric measures, such as waist and hip circumferences, sagittal diameter, or BMI, cannot accurately distinguish between these body composition characteristics (different fat depots

and lean tissue). This should be kept in mind when interpreting results of studies on body composition in relation to health risk.

This thesis provides important insight in the relation between body composition, type 2 diabetes, and cardiovascular disease risk. This insight and future additional knowledge of underlying mechanisms may contribute to the development of prevention strategies targeted on nutrition, lifestyle and drugs to stop the increase in prevalence of type 2 diabetes and cardiovascular disease.

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Type 2 diabetes and the metabolic syndrome

Due to the increased availability of food and the greater energy density of food in combination with a more sedentary lifestyle there is a worldwide increase in the prevalence of obesity (severe overweight) ¹. Type 2 diabetes mellitus is the most apparent health consequence of obesity ². In 2001 the prevalence of self-reported type 2 diabetes in the United States was 7.9% ³. In the Netherlands the prevalence of type 2 diabetes was estimated at 3.6% in women and 4.4% in men in the year 2000, based on four epidemiological population studies ⁴. It is estimated (based on projections of age and urbanization) that during the first quarter of the 21st century the number of people with type 2 diabetes in the world will rise enormously from 150 million in 2000 to 300 million in 2025 ⁵.

Type 2 diabetes is associated with microvascular complications, such as renal disease, retinopathy and neuropathy ⁶, and with a 2- to 4- fold higher risk of cardiovascular complications ^{7,8}. Therefore, type 2 diabetes has a large and increasing impact on the health care system and on health related quality of life. Type 2 diabetes is most prevalent among middle-aged and elderly people, however, due to the alarming increase of obesity among people of all ages, type 2 diabetes is increasingly being diagnosed in children as well ⁹.

Diabetes mellitus is characterised by chronically elevated blood glucose concentrations. There are two major forms: type 1 and type 2 diabetes. Type 1 diabetes usually becomes apparent in childhood. Because of autoimmune destruction of the pancreatic beta-cells, there is an absolute deficiency of its product insulin. Insulin plays a key role in the uptake of glucose into the cells. This thesis focuses on type 2 diabetes, characterized by a relative lack of insulin, due to resistance of target cells to insulin action and an insufficient compensatory insulin secretion. Type 2 diabetes mostly develops gradually, and a disturbed glucose metabolism can be present without clinical symptoms. Therefore approximately 30% - 50% of cases are currently undiagnosed ^{10,11}. The clinical diagnosis of type 2 diabetes (Table 1) is based on elevated fasting blood glucose levels or elevated glucose levels 2 hours after a standardised 75-g oral glucose load ¹². Persons with an impaired glucose metabolism, but no diabetic glucose levels (often referred to as 'pre-diabetes') have a high risk of developing type 2 diabetes as well as an elevated risk of cardiovascular disease ^{8,13,14}

Table 1. WHO-criteria for the diagnosis of type 2 diabetes mellitus ¹²

	Fasting glucose ^a		2-h postload glucose ^a		
Glucose tolerance status	(mmol/l)		(mmol/l)		
Normal glucose tolerance (NGT)	< 6.1	and	< 7.8		
Impaired fasting glucose (IFG) ^b	\geq 6.1 and < 7.0	and	< 7.8		
Impaired glucose tolerance (IGT) ^b	< 7.0	and	\geq 7.8 and < 11.1		
Diabetes mellitus (DM)	≥ 7.0	or	≥ 11.1		

^a Glucose measured in venous plasma

^b IFG and IGT categories are often combined as impaired glucose metabolism (IGM) category

In addition to high glucose levels, type 2 diabetes is usually characterised by an adverse cardiovascular risk profile including high levels of triglycerides, low levels of high-density lipoprotein cholesterol, hypertension and hyperinsulinaemia. Together with obesity, the clustering of these risk factors is now referred to as 'the metabolic syndrome', which has currently several slightly different definitions ^{12,15,16}.

The importance of fat distribution

Overweight is commonly defined as having a body mass index (BMI) between 25 and 30 kg/m². BMI is calculated as weight divided as height squared. Obesity, or severe overweight, is defined as BMI \geq 30 kg/m² ¹⁷ (Table 2). BMI can be considered as a height-adjusted weight measure, but it takes neither body composition nor the distribution of fat mass over the body into account.

Table 2. Definitions of (abdominal) overweight and obesity according to body mass index (BMI), waist circumference and waist-to-hip circumference ratio (WHR) ^{17,18}

	BMI (kg/m²)	Waist circumference (cm)		W	HR
		Men	Women	Men	Women
Underweight	< 18.5	-	-	-	-
Normal weight	18.5 – 24.9	< 94.0	< 80.0	< 0.90	< 0.80
Overweight	25.0 – 29.9	94.0 - 101.9	80.0 - 87.9	0.90 - 0.99	0.80 - 0.84
Obesity	≥ 30.0	≥ 102.0	≥ 88.0	≥ 1.00	≥ 0.85

Since the 1950's it is recognised that apart from overall obesity the distribution of body fat might influence health risk ¹⁹. In particular an android (upper body) fat distribution is associated with metabolic disturbances and increased risk of cardiovascular disease and type 2 diabetes. An android fat distribution, or abdominal obesity, is usually assessed by the waist-to-hip circumference ratio (WHR), or the less frequently used waist-to-thigh circumference ratio (WTR). A WHR of more than 1.0 in men and 0.85 in women is considered to indicate abdominal obesity according to the World Health Organisation ¹⁷ (Table 2). Persons with a BMI in the normal-weight range can still be at increased risk of type 2 diabetes if the WHR is increased, and particularly the combination of a high BMI and a high WHR results in the highest risk of type 2 diabetes ^{20,21} (Figure 1). The WHR (or WTR) is assumed to reflect abdominal fat accumulation. Since the waist circumference alone is a better measure of total abdominal fat, recent guidelines focus on waist circumference to estimate health risk (Table 2) as suggested by Lean et al ¹⁸.

Current pathophysiological hypothesis

Although the concept that obesity, in particular abdominal obesity, is an important causal factor leading to type 2 diabetes is generally accepted, the exact pathophysiological mechanisms are not completely known. For a long time, adipose tissue was considered to be passive, with as main function to store energy. Nowadays, we know that the processes of fat storage and mobilisation are regulated in a highly coordinated manner.

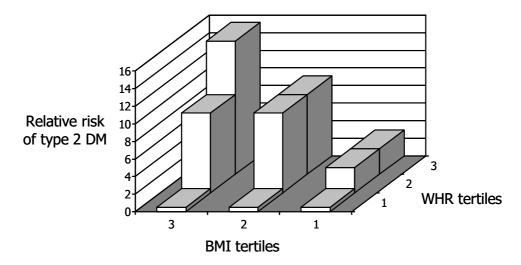


Figure 1. Risk of developing type 2 diabetes mellitus (DM) during 13.5 years of follow-up within tertiles of BMI and WHR. Adapted from Ohlson et al ²⁰.

Dietary fat and carbohydrates can both be used as energy source in the human body, however, excess fuel is predominantly stored as fat. The excess fat is stored in the form of triglycerides. Triglycerides are taken up from the circulation into the fat cell after hydrolyzation into fatty acids stimulated by lipoprotein lipase. In the fat cell fatty acids can be re-esterified to triglycerides. During fasting or exercise, when energy expenditure exceeds energy intake, lipolysis mobilizes these triglycerides to free fatty acids (also referred to as non-esterified fatty acids) and glycerol into the circulation. These free fatty acids in plasma are than used by the muscle, liver and other organs for oxidation.

Fatty acids play an important role in the development of diabetes ²²⁻²⁴. When free fatty acid levels in the circulation are high (as in obesity), glucose uptake and oxidation by muscle and other organs is inhibited through several mechanisms. The pancreas will compensate the diminished glucose uptake by increasing insulin secretion, but in many of the insulin resistant persons, the beta-cell eventually fails. In addition, free fatty acids will accumulate in organs, particularly in the muscle and in the liver, but also in the pancreas (so-called 'lipotoxicity' or 'ectopic fat storage'). Accumulation of fat in non-adipose tissue may further promote insulin resistance and impair beta-cell function, which are the two key features in the development of type 2 diabetes ^{23,25}. Excess free fatty acids also provide substrates for hepatic triglyceride production and thus may contribute to dyslipidemia as usually seen in type 2 diabetes.

Abdominal fat can be distinguished into subcutaneous abdominal fat and intra-abdominal fat. Intra-abdominal fat (also referred to as 'visceral fat') is more sensitive to lipolytic stimuli, and less sensitive to anti-lipolytic stimuli (such as insulin), compared to subcutaneous fat, and therefore visceral fat is more likely to release free fatty acids into the circulation. It is therefore suggested that visceral fat is a better marker for high whole body free fatty acid exposure than other fat depots ²⁶. In addition, it has been postulated that visceral fat is detrimental because it directly releases free fatty acids into the portal vein, causing reduced hepatic insulin clearance,

increased gluconeogenesis and increased dyslipidemia ^{27,28}. Although the visceral fat depot has been found to be most strongly related to the development of metabolic disturbances, the contribution of abdominal subcutaneous fat is still controversial ²⁹.

Objectives of this thesis

The use of sophisticated imaging techniques (such as magnetic resonance imaging (MRI) and computed tomography (CT)) to distinguish different fat depots at the waist level, or to determine lipid accumulation in muscle or liver, are expensive and time-consuming. Therefore, epidemiological studies generally use simple anthropometric measures (i.e. BMI, WHR, WTR, waist circumference) to estimate obesity and abdominal obesity. However, these measures may have several disadvantages resulting in invalid physiological interpretation.

A higher BMI is assumed to represent more body fat. BMI, however, does not distinguish between fat mass and lean (non-fat) mass. For example, well-trained body builders have a very low percentage body fat, but their BMI may be in the overweight range because of their large lean (muscle) mass. In addition, BMI may not correspond to the same level of body fatness in different ethnic populations. For instance, high health risk in Asians starts at lower levels of BMI because they have a higher body fat percentage compared to Caucasians with similar BMI ^{30,31}. In addition, the distribution of fat over the body may differ between ethnic populations, which is not measured by the BMI. The relation between BMI and body fatness also changes with age. During aging there is a loss of lean mass, which will mask a relative increase of body fat mass, even with stable BMI. In addition, during aging fat redistributes from subcutaneous fat to visceral fat, which is not captured by the BMI.

From the anthropometric measures, the waist circumference is the strongest correlate of visceral fat. The waist circumference, however, is not always a stronger predictor of type 2 diabetes ¹³ and other cardiovascular risk ²¹ than the WHR. Because a higher WHR can be the result of a larger waist as well as a smaller hip, this suggests that a smaller hip (representing either lower muscle and/or fat mass at the hips) may be involved in determining the increased risk associated with an increased WHR.

In the present thesis we investigate different aspects of body composition in relation to type 2 diabetes and cardiovascular risk. Specifically, we formulated the following main research questions:

- 1. What are the separate associations of waist and hip circumferences, independently of each other and of overall obesity (BMI), with glucose metabolism and other components of the metabolic syndrome?
- 2. Do these associations differ between men and women, between different age groups, and between different ethnic populations?
- 3. Which tissues (lean or fat tissue) are reflected by the waist circumference and by the hip circumference?

- 4. Are these tissues independently associated with glucose levels and other components of the metabolic syndrome?
- 5. What are the independent relations of visceral fat, abdominal subcutaneous fat, subcutaneous thigh fat, intermuscular thigh fat with glucose levels and other components of the metabolic syndrome?

Outline of the thesis

The chapters have been ordered according to increasing precision in the measurements of body composition. The first chapters (Chapter 2 to Chapter 5) are based on anthropometric measures of body composition. In these chapters, the independent associations of the waist circumference and the hip circumference with glucose levels, diabetes and other components of the metabolic syndrome were studied. In **Chapter 2** cross-sectional associations of waist and hip circumferences with measures of glucose metabolism are described in the Hoorn Study (age participants 50 to 75 years), while in **Chapter 3** these associations were studied longitudinally, with 6 years of follow-up. To confirm these findings in other observational studies, we repeated the analyses in a large Australian cohort, the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). This cohort also covers a wider age range (25 years and older) than the Hoorn Study, and additional components of the metabolic syndrome, i.e. blood pressure and lipid levels, were considered next to glucose levels (**Chapter 4**). Because body composition differs greatly between ethnic groups, we were interested in whether the observed associations between waist and hip circumference and the metabolic profile were also present in other ethnic populations. Results of several ethnic groups living on islands in the Pacific and Indian Oceans are described in **Chapter 5**.

Dual energy x-ray absorptiometry (DXA) is used to measure body composition in the next two chapters. Use of this method enables us to distinguish between fat mass and lean mass in the total body, but also at specific regions of the body. In **Chapter 6** we investigate first which body tissue (fat or lean tissue) is reflected by anthropometric measures, and secondly whether these tissues are related to the glucose metabolism. In **Chapter 7** we explore body composition in relation to arterial stiffness, a marker of vascular function.

In the last two chapters, measurement of body composition is refined to computed tomography (CT). Use of this method enables distinction between visceral fat and subcutaneous fat in the abdomen, and distinction between subcutaneous and intermuscular fat in the legs. In **Chapter 8** we related all these separate fat depots to components of the metabolic syndrome. Because visceral fat is probably the most important culprit for risk of the metabolic syndrome and cardiovascular disease, but often too expensive and time-consuming to measure in clinical practice and epidemiological studies, it is important to be able to assess this fat depot by more practical measures. This is explored in the study described in **Chapter 9**.

Finally, in the general discussion in **Chapter 10**, methodological and pathophysiological issues are discussed, and practical implications and suggestions for future research are addressed.

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Abstract

Objective

A higher waist-to-hip ratio, which can be due to a larger waist circumference, a smaller hip circumference, or both, is associated with higher glucose levels and incident diabetes. A lower hip circumference could reflect either lower fat mass or lower muscle mass. Muscle mass might be better reflected by thigh circumference. The aim of this study was to investigate the contributions of thigh and hip circumferences, independent of waist circumference, to measures of glucose metabolism.

Research Methods and Procedures

For this cross-sectional study we used baseline data from the Hoorn Study, a population-based cohort study of glucose tolerance among 2484 men and women aged 50 to 75. Glucose tolerance was assessed by 75-g OGTT; HbA1c and fasting insulin were also measured. Anthropometric measurements included BMI and waist, hip and thigh circumferences.

Results

Stratified analyses and multiple linear regression showed that after adjustment for age, BMI and waist circumference, thigh circumference was negatively associated with markers of glucose metabolism in women, but not in men. Standardized betas in women were -0.164 for fasting, -0.206 for postload glucose, -0.190 for HbA1c (all p<0.001) and -0.065 for In-insulin levels (p=0.061). Hip circumference was negatively associated with markers of glucose metabolism in both sexes (standardized betas ranging from -0.093 to -0.296, p<0.05) except for insulin in men. Waist was positively associated with glucose metabolism.

Discussion

Thigh circumference in women and hip circumference in both sexes are negatively associated with markers of glucose metabolism independently of the waist circumference, BMI and age. Both fat and muscle tissues may contribute to these associations.

Introduction

It is firmly established that obesity is associated with a higher prevalence of type 2 diabetes mellitus. The accumulation of visceral fat is particularly assumed to play an important role in the aetiology of the disease, notably by the overexposure of the liver to free fatty acids, which results in insulin resistance and hyperinsulinaemia 1,2 .

Waist circumference and waist-to-hip ratio (WHR) are widely used as indicators of abdominal obesity in population studies. The majority of current studies agree that waist circumference is probably a better indicator of visceral fat than is WHR. Indeed, several studies found waist circumference to be a better marker of visceral fat and to correlate more strongly with cardiovascular risk factors than WHR ^{3,4}. However, the WHR is a robust risk factor in many population studies, and other studies found WHR or waist-to-thigh ratio (WTR) a better predictor for type 2 diabetes than waist circumference alone ⁵. Moreover, a larger hip circumference has been associated with a lower risk of type 2 diabetes, independently of waist circumference ⁶. The underlying mechanism for this phenomenon is not yet clear, but may be related to the amount of muscle mass, the amount of fat mass, or both.

Thigh circumference might be a better indicator for leg muscle mass than hip circumference, because it might be less influenced by bone (pelvic width) and gluteal fat. In this study we consider the independent contributions of thigh or hip circumferences to several measures of glucose metabolism after adjustment for waist circumference. As lifestyle factors are known to influence fat distribution and glucose metabolism ^{7,8}, we studied potential confounding by smoking, alcohol intake and physical activity. We used data from the Hoorn Study, a cohort study of glucose tolerance in 2,484 subjects.

Research Methods and Procedures

Subjects

The Hoorn Study is a population-based cohort study of glucose tolerance among 2484 Caucasian men and women aged 50 to 75, which started in 1989 and has been described in detail previously ⁹. For the present study baseline measurements were used. Subjects who were already known to have diabetes were excluded from analyses (n=90), and 14 subjects had missing data for glucose measures and/or anthropometry; therefore analyses were performed in 2380 subjects (1099 men and 1281 women). Informed consent was obtained from all participants, and ethical approval for the study was obtained from the local ethics committee.

Measurements

Fasting glucose and postload glucose levels after a 75-g oral glucose tolerance test (OGTT) were measured as previously described ⁹. Subjects were classified according the 1999 WHO criteria ¹⁰. Glycated haemoglobin (HbA1c), used as a long-term indicator of glucose levels, and fasting specific insulin level were measured as previously described ¹¹. Fasting insulin levels can be used as an estimate of insulin insensitivity ¹².

Weight and height were measured in barefoot subjects wearing light clothes only, and body mass index (BMI) was calculated as weight divided by height squared. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest, and the hip circumference at the widest level over the trochanters. Thigh circumference was measured directly below the gluteal fold at the left leg. The mean value of two measurements was used in the analyses. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference and waist-to-thigh ratio (WTR) as waist circumference divided by thigh circumference.

Information on lifestyle factors was obtained by questionnaire. Smoking was expressed in cigarette-years for smokers or former smokers. Alcohol intake was categorized in 4 groups: non-drinkers, up to 10 g/day, 10-30 g/day and >30 g/day. Physical activity was expressed as hours per day. The activities included sports, bicycling, gardening, walking, doing odd jobs, and housekeeping.

Statistical methods

All statistical analyses were performed separately for men and women because of the known differences in fat distribution between sexes. Baseline characteristics are reported according to glucose tolerance status. Differences between men and women were examined by Student's t-test. Because the distribution of insulin levels was skewed geometric means are presented.

To study the contribution of thigh circumference to glucose metabolism independently of waist circumference, sex-specific tertiles of waist circumference and of thigh circumference were created. We divided the population of each sex into 9 groups by creating a 3x3 table according these tertiles, and calculated the unadjusted means of age, BMI, and glucose metabolism variables (fasting and postload glucose, HbA1c, and fasting insulin) in each group. Additionally we calculated means of glucose variables adjusted for age and overall obesity (BMI). These analyses were repeated using hip circumference instead of thigh circumference. To test for trend, the categorical variable of thigh or hip tertiles was entered in a linear regression model as a continuous variable with the glucose metabolism variable as dependent variable. Insulin levels were In-transformed, because of their skewed distribution.

We also studied the independent contribution of thigh and waist circumferences to glucose metabolism in a multiple linear regression model using thigh and waist circumferences as continuous variables, with adjustment for age and BMI. To make regression coefficients more comparable, we report standardized betas. A standardized beta of 0.1 indicates that if the independent variable changes one SD, the dependent variable changes 0.1 SD. In an additional model, further adjustments were made for lifestyle factors (smoking, alcohol intake and physical activity). We repeated these analyses using hip circumference instead of thigh circumference. Multi-colinearity in these regression models was studied by examining the tolerance, which is a statistic used to determine how much the independent variables are linearly related to one another. It is calculated as 1 minus R^2 for an independent variable when it is predicted by the other independent variables already included in the analysis. The stability of the regression model was considered to be disturbed by multi-colinearity if tolerance < 0.1. All analyses were performed using SPSS (version 10.1 for Windows; SPSS, Inc., Chicago, IL).

Table 1. Population characteristics according to sex and glucose tolerance status, expressed as mean (SD) or geometric mean

		~	Men			M	Women	
	Total	NGT	IGT and/or IFG	DM	Total	NGT	IGT and/or IFG	DM
Z	1099	807	209	81	1281	977	213	83
Age (years)	61.2 (7.3)*	60.7 (7.2)	62.3 (7.2)	$63.7 (7.1)^{\S}$	61.8 (7.4)	60.9 (7.2)	64.7 (7.2)	$65.4 (7.0)^{\S}$
Fasting glucose (mmol/I)	$5.68 (1.08)^*$	5.34 (0.40)	6.13(0.45)	7.97 (2.62) §	5.51(1.09)	5.20 (0.42)	5.96 (0.52)	$7.98 (2.80)^{\S}$
Postload glucose (mmol/I)	5.92 (2.93)*	4.95 (1.29)	7.07 (2.00)	$12.65 (5.63)^{\S}$	6.19(2.89)	5.23 (1.17)	7.82 (1.72)	$13.47 (5.81)^{\S}$
HbA1c (%)	5.43 (0.69)	5.33 (0.47)	5.46 (0.52)	$6.41 (1.56)^{\S}$	5.40 (0.68)	5.28 (0.47)	5.57 (0.46)	$6.46 (1.64)^{\S}$
Fasting insulin (pmol/l)	79.22	74.21	93.16	100.04^{\S}	77.28	73.14	87.34	108.40^{\S}
BMI (kg/m^2)	26.2 (2.9)*	25.7 (2.6)	27.1 (3.2)	$28.3 (3.2)^{\S}$	26.7 (3.9)	26.3 (3.7)	28.0 (4.0)	$28.5 (4.6)^{\S}$
Waist circumference (cm)	95.25 (9.14)* 93.74 (8.19)	93.74 (8.19)	98.35 (10.64)	$102.23 (8.64)^{\S}$	86.82 (10.52)	85.33 (9.90)	90.98 (10.70)	$94.04 (11.54)^{\S}$
Thigh circumference (cm)	56.55 (4.79)*	56.19 (4.63)	57.25 (4.74)	$58.27 (5.91)^{\S}$	59.36 (5.80)	59.50 (5.88)	59.08 (5.45)	58.55 (5.66)
Hip circumference (cm)	100.26 (5.39)* 99.79 (5.03)	99.79 (5.03)	101.11 (6.35)	$102.91 (5.18)^{\S}$	102.73 (7.49)	102.42 7.34)	103.61 (7.77)	$104.27 (8.27)^{\S}$
WHR	0.95 (0.06)* 0.94 (0.06)	0.94 (0.06)	0.97 (0.07)	$^{8}(20.0)$ 66.0	0.84 (0.07)	0.83 (0.07)	0.88 (0.07)	$0.90 (0.08)^{\S}$
WTR	1.69 (0.15)* 1.67 (0.15)	1.67 (0.15)	1.72 (0.16)	$1.76 (0.16)^{\S}$	1.47 (0.17)	1.44 (0.16)	1.54 (0.15)	$1.61 (0.19)^{\S}$

*p < 0.05 (comparing men and women)

§ p trend < 0.01 (comparing glucose tolerance categories)

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; DM, diabetes mellitus; HbA1c, glycated haemoglobin; WHR, waist-to-hip ratio; WTR, waist-to-thigh ratio

Glucose tolerance status could not be assessed in 10 subjects (2 men, 8 women) because of missing postload glucose level

Results

Baseline characteristics are presented in Table 1. As expected, age, BMI, waist circumference, WHR and WTR increased with worsening glucose tolerance status. Also, the hip and thigh circumference were significantly positively associated; however, the associations were less strongly and the association with thigh circumference in women was not significant (p=0.106) and tended to reverse. The correlation between waist and hip circumference was higher (0.73 for men and 0.71 for women) than the correlation between waist and thigh circumference (0.48 for men and 0.43 for women).

Table 2. Unadjusted means of age, BMI, and glucose metabolism markers in subgroups of low, medium, and high (1 through 3) thigh and waist circumferences

			Men			Women	
		Te	rtiles of th	nigh	Τe	ertiles of t	high
Tertiles of		1	2	3	1	2	3
waist		≤ 54.4 cm		\geq 58.3 cm	\leq 56.5 cm		\geq 61.5 cm
1	n	194	115	61	216	142	70
	Age (years)	61.7	58.7	56.5**	61.9	58.4	57.3**
\leq 91.0 cm	BMI (kg/m²)	22.7	24.8	25.2**	22.4	24.0	25.5**
(men)	Fasting glucose ^a	5.38	5.45	5.42*	5.23	5.19	5.17
\leq 81.7 cm	Postload glucose ^a	5.20	5.12	4.68**	5.66	5.47	4.99*
(women)	HbA1c (%)	5.41	5.30	5.25**	5.34	5.20	5.13**
	Fasting insulin ^b	62.66	64.81	73.75	60.33	67.09	68.87**
2	n	104	135	119	144	152	135
	Age (years)	63.4	61.3	58.2**	63.3	62.5	60.4**
	BMI (kg/m²)	24.8	25.8	27.2**	24.8	26.7	27.9**
	Fasting glucose ^a	5.55	5.62	5.59	5.49	5.34	5.45
	Postload glucose ^a	5.77	5.90	5.33	6.36	5.87	5.66**
	HbA1c (%)	5.47	5.30	5.28**	5.43	5.36	5.31*
	Fasting insulin ^b	78.23	74.41	80.83	77.60	72.26	74.72
3	n	61	125	185	65	129	228
	Age (years)	69.0	63.0	60.8**	65.5	63.6	62.8**
\geq 99.0 cm	BMI (kg/m²)	26.7	27.8	30.0**	27.5	29.0	32.0**
(men)	Fasting glucose ^a	5.95	6.00	6.10	6.38	6.00	5.69**
\geq 90.6 cm	Postload glucose ^a	7.02	6.73	7.18	8.65	7.49	6.49**
(women)	HbA1c (%)	5.65	5.58	5.59	5.88	5.67	5.46**
-	Fasting insulin ^b	94.15	93.38	103.2*	106.61	96.87	94.16*

^{**} p < 0.05 and * p < 0.1 (testing for trend with increasing thigh circumference)

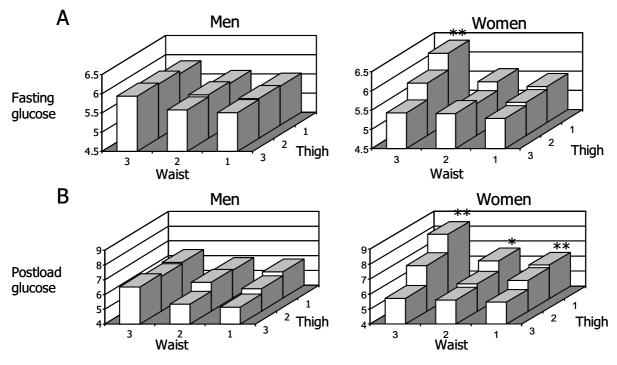
Stratified analyses

Table 2 shows that a higher waist circumference was associated with older age, higher BMI, and higher glucose and insulin levels. In men with a small waist, there was a statistically significant negative association of thigh circumference with HbA1c and postload glucose levels, despite the

a mmol/l

b pmol/l, geometric mean

positive association between thigh and BMI. Insulin was positively associated with thigh circumference. In women the negative associations of thigh circumference with glucose levels and HbA1c were more pronounced. Fasting insulin was positively associated with thigh circumference in women with a low waist circumference, but in women with a high waist circumference the association inverted. The same analyses were performed using tertiles of hip circumference instead of thigh circumference to create the 9 groups for each sex. Similar results were found (data not shown), but the number of subjects in the subgroups with low waist and high hip (and vice versa) was relatively small (range n=27 to n=36).



* p < 0.1 and ** p < 0.05 (testing for trend within tertiles of waist circumference)

Figure 1. BMI- and age-adjusted means of fasting glucose levels (A) and postload glucose levels (B) in subgroups of low, medium and high (1 through 3) waist and thigh circumferences, for men and women separately

Figure 1 (A and B) illustrates the means of fasting glucose and postload glucose levels, respectively, adjusted for age and BMI. In women, but not in men, there was an inverse association of thigh circumference with glucose levels. When testing for trend within tertiles of waist circumference, there was a significant negative association between thigh circumference and fasting glucose levels in women in the uppermost tertile of waist circumference (p < 0.001). The negative association of thigh circumference with postload glucose levels was shown in women in all waist tertiles ($p=0.047,\ 0.080$ and <0.001 for low, medium and high waist circumference, respectively). In men, no significant association of thigh circumference with glucose measures was shown. The figures also suggest interaction (effect modification) between waist and thigh circumference in women. This indicates that in women with a large waist circumference in particular, a greater thigh circumference is associated with lower glucose levels. A similar pattern

was observed for adjusted HbA1c and fasting insulin levels (data not shown). When we used tertiles of hip circumference instead of thigh circumference, similar results were obtained in women. In men, the inverse association of hip circumference with fasting and postload glucose levels (but not with HbA1c or fasting insulin) was also shown, although this association was significant only for postload glucose with medium waist circumference (p trend=0.028) (data not shown).

Multiple linear regression

Table 3 shows that both in men and in women, the waist circumference was strongly positively associated with all measures of the glucose metabolism (fasting and postload glucose, HbA1c, and insulin) after adjustment for age, BMI and thigh circumference (model 1). In the same model, thigh circumference was negatively associated with markers of the glucose metabolism in women only.

Table 3. Independent contributions (standardised betas) of waist and thigh circumferences (model 1) and waist and hip circumferences (model 2) to glucose metabolism markers, in linear regression models adjusted for age and BMI

Model 1 ^a		Waist circumference	р	Thigh circumference	р
Fasting glucose	Men	0.179	0.001	-0.019	0.651
	Women	0.280	0.000	-0.164	0.000
Postload glucose	Men	0.154	0.003	-0.057	0.163
	Women	0.249	0.000	-0.206	0.000
HbA1c	Men	0.132	0.013	-0.053	0.208
	Women	0.277	0.000	-0.190	0.000
Ln-Insulin	Men	0.294	0.000	0.018	0.637
	Women	0.280	0.000	-0.065	0.061
Model 2 ^b		Waist circumference	р	Hip circumference	р
Fasting glucose	Men	0.221	0.000	-0.103	0.029
	Women	0.351	0.000	-0.222	0.000
Postload glucose	Men	0.243	0.000	-0.212	0.000
	Women	0.339	0.000	-0.296	0.000
HbA1c	Men	0.175	0.002	-0.097	0.043
	Women	0.348	0.000	-0.183	0.000
1 T 11		0.000	0.000	0.022	0.000
Ln-Insulin	Men	0.283	0.000	0.023	0.602

^a Model 1 includes waist circumference, thigh circumference, BMI and age as independent variables

We tested the potential interaction suggested by Figure 1 between thigh and waist circumference by adding an interaction term of thigh and waist to the regression model. In men, we did not find significant interaction for glucose and insulin levels. In women, we observed significant interaction of waist and thigh in the models with fasting and postload glucose levels and fasting insulin levels (p = 0.005, 0.008 and 0.030, respectively) but not for HbA1c. In an additional regression model, we also adjusted for the lifestyle factors smoking, physical activity, and alcohol

^b Model 2 includes waist circumference, hip circumference, BMI and age as independent variables

intake. These factors did not markedly change our results (data not shown). When applying the same multiple regression analyses using hip circumference instead of thigh circumference (Table 3, model 2), we found that both waist and hip contributed significantly to the glucose variables in both sexes, except for fasting insulin in men. No interaction between hip and waist was observed. Additional adjustment for lifestyle factors did not substantially change these results (data not shown). In none of the regression models was the stability of estimated parameters influenced by multi-colinearity.

Discussion

This study demonstrates that after adjustment for age and overall obesity (BMI), thigh circumference was strongly and negatively associated with markers of glucose metabolism in women, but not in men. Hip circumference was negatively associated with markers of glucose metabolism in both sexes. These associations were independent of waist circumference, which was positively associated with these markers. Only in women, we observed a significant interaction between thigh and waist circumference, indicating that especially women with a combination of large waist and small thigh circumferences seem to have worse glucose levels than women with smaller waists or larger thighs. Adjustment for lifestyle factors did not change the associations.

These results are in accordance with previously published data concerning the specific contribution of hip circumference to disease risk ^{6,13,14}. Seidell et al. recently reported that the hip circumference was independently and negatively associated with several cardiovascular risk factors, including fasting insulin and fasting glucose, in the Quebec Family Study ¹⁵. A limitation of the latter study was the inclusion of younger subjects (age range 18-84 years), which might have weakened the associations, because disturbances in glucose metabolism usually appear at an older age. In addition, only fasting glucose and insulin were measured. Our study extends these findings by also including postload glucose and HbA1c measurements. Furthermore, to our knowledge this is the first study to include both thigh and hip circumference to compare their independent influence on glucose metabolism.

The increased risk of unfavourable glucose levels in subjects with higher WHR or WTR is generally thought to be attributable to increased visceral fat mass ^{1,2}. Indeed we confirm a strong positive association of waist circumference with markers of glucose metabolism. A higher WHR or WTR, however, may also result from a lower hip or thigh circumference. Indeed, a higher WHR was found to be associated with a decreased muscle mass in the legs and gluteal region ¹⁶. Therefore, it has been speculated that the contribution of larger hip circumference to lower glucose levels may be due to higher muscle mass ⁶. Skeletal muscle is the main target of insulin, as well as one of the sites of insulin resistance. Low muscle mass has been associated with insulin resistance. Chowdhury et al. showed that higher glucose levels in Indian compared to Swedish men of the same age and BMI, was not due to differences in visceral fat, but to their lower leg muscle mass ¹⁷. Also, a high WHR has been associated with a higher proportion of type IIb muscle fibers and lower capillary density at the thigh, which may be associated with decreased glucose transport and reduced insulin sensitivity ^{18,19}.

Larger thigh and hip circumferences could also reflect increased femoral and gluteal subcutaneous fat respectively. Particularly in women these depots have relatively high lipoprotein lipase (LPL) activity and relatively low rate of basal and stimulated lipolysis ²⁰. These depots may protect the liver and muscle to high exposure to free fatty acids, through uptake and storage. The regional differences in adipocyte metabolism are more pronounced in women than in men. This could possibly explain why we did find a negative association between thigh circumference and glucose levels in women, but not in men. In contrast, hip circumference was associated with glucose metabolism in both sexes. Interpretation of the hip circumference, however, may be different between men and women. It is plausible that variation in hip circumference in women is explained mostly by variation in gluteal fat mass and pelvic width, while in men muscle mass might be the main determinant.

Underlying hormonal factors may influence waist, thigh and hip circumferences as well as insulin resistance. Disturbances in glucocorticoid metabolism, growth hormone (GH) metabolism, and sex hormone balance have all been shown to be associated with alterations in fat distribution as well as deterioration in insulin resistance and glucose metabolism ²¹. Cushing's syndrome, which is characterized by high cortisol levels, leads to increased visceral fat and decreased muscle mass in the legs, and hyperglycaemia ²². Striking similarities exist between the metabolic syndrome and untreated GH deficiency ²³. Low-dose GH treatment combined with dietary restriction resulted in a decrease of fat, and in an increase of muscle mass with a consequent improvement of the insulin resistance ²⁴. Finally, sex steroids have also been shown to influence both fat distribution and insulin sensitivity ^{21,25}. For instance, high androgen levels in women and low testosterone levels in men are associated with measures of fat distribution and insulin resistance ²⁶⁻²⁹. Estrogens stimulate accumulation of subcutaneous fat at the gluteal and femoral depots in women. The mechanisms by which endocrine disturbances lead to abdominal fat distribution and decreased insulin sensitivity are not completely clear. It has been suggested that the effect of these hormones on LPL activity in adipose tissue may be involved ^{13,22,25}.

Adipose tissue is an endocrine organ secreting many peptides. These peptides include leptin, resistin, adiponectin, angiotensinogen, interleukin-6, tumor necrosis factor-alpha, adipsin and plasminogen activator inhibitor-1 30 . Regional differences in secretion of these peptides could also be an alternative or additional explanation for the relationships between circumferences and glucose levels that we found.

Another underlying factor that possibly contributes to a disturbed glucose metabolism as well as an abdominal fat distribution could be intra-uterine growth retardation (low birth weight). A low birth weight is associated with increased adult WHR and insulin resistance ³¹. Studies examining the foetal origins hypothesis suggest that small birth size may be a marker of foetal adaptations that program future vulnerability to adult disease ³². For type 2 diabetes this may result from an altered development and insulin secreting capacity of the endocrine pancreas, or by altered insulin sensitivity of target tissues ³³.

A limitation of the present study was its cross-sectional nature. Thus, the possibility that a smaller hip circumference is not a risk factor for high glucose levels, but rather the consequence of the same underlying factor, cannot be excluded. Recently, however, Lissner showed that smaller

hip circumferences predicted the incidence of self-reported diabetes in women in a prospective study ³⁴.

In conclusion, thigh circumference in women and hip circumference in both sexes are negatively associated with markers of glucose metabolism, independently of waist circumference, BMI and age. Further investigation, particularly prospective research, is needed to elucidate the underlying mechanisms that lead to the negative association of thigh and hip circumferences with glucose levels as observed in this study.

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Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: the Hoorn Study

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Abstract

Background

The higher risk for type 2 diabetes in persons with high waist-to-hip ratio (WHR) or waist-to-thigh ratio (WTR) has mostly been attributed to increased visceral fat accumulation. However, smaller hip or thigh circumferences may also explain the predictive value of WHR or WTR for type 2 diabetes.

Objective

This study considered prospectively the association of hip and thigh circumferences, independent of waist circumference, with the incidence of type 2 diabetes.

Design

The Hoorn Study is a population-based cohort study of diabetes. A total of 1357 men and women aged 50-75 and non-diabetic at baseline participated in the 6 years follow-up examination. Glucose tolerance was assessed by use of a 75-g OGTT. Baseline anthropometric measurements included BMI and waist, hip, and thigh circumferences.

Results

Logistic regression analyses showed that a 1-SD larger hip circumference gave an odds ratio (OR) for developing diabetes of 0.55 (95% CI 0.36-0.85) in men and 0.63 (0.42-0.94) in women, after adjustment for age, BMI, and waist circumference. The adjusted ORs for a 1-SD larger thigh circumference were 0.79 (0.53-1.19) in men and 0.64 (0.46-0.93) in women. In contrast with the hip and thigh circumference, waist circumference was positively associated with the incidence of type 2 diabetes in these models (ORs ranging from 1.60 to 2.66).

Conclusion

Large hip and thigh circumferences are associated with a lower risk of type 2 diabetes, independently of BMI, age and waist circumference, whereas a larger waist circumference is associated with a higher risk of type 2 diabetes.

Introduction

Although the worldwide dramatic increase in the incidence of obesity, and consequently the incidence of type 2 diabetes mellitus, has been recognized, the exact etiologic link between these two still remains unclear. It was observed in the Hoorn Study that the waist-to-hip ratio (WHR) and not BMI is an important independent predictor of incident diabetes in 50-75-year-old individuals ¹. This result indicates that fat distribution may be a better predictor for progression to type 2 diabetes than is BMI, which is also suggested by studies that examined the WHR or the waist-to-thigh ratio (WTR) ²⁻⁶. In particular, the accumulation of visceral fat is assumed to play an important role in the aetiology of diabetes by overexposing the liver to free fatty acids, resulting in insulin resistance and hyperinsulinaemia ⁷⁻⁹.

Evidence, however, that the strong predictive value of the WHR or WTR for type 2 diabetes is not solely due to abdominal fat accumulation (as indicated by waist circumference) is growing. Cross-sectional studies showed that a larger hip circumference is associated with a lower prevalence of self-reported type 2 diabetes and lower fasting glucose concentrations, independently of BMI and waist circumference ^{10,11}. We obtained similar results in the Hoorn Study for both hip and thigh circumferences ¹².

In one prospective study of Chinese men and women hip circumference was positively associated with the incidence of type 2 diabetes ¹³. In that study, however, neither waist circumference nor BMI was taken into account. To our knowledge, only one prospective study of the specific association of hip circumference with the incidence of diabetes has been carried out in Caucasian persons. That study found a larger hip circumference to be associated with lower incidence of several cardiovascular endpoints and diabetes, independently of waist circumference ¹⁴. The latter study, however, was limited to women and in both prospective studies the presence of diabetes was not examined on the basis of an oral glucose tolerance test (OGTT).

In the Hoorn Study, a population-based cohort study of glucose tolerance, both men and women were included and a 75-g OGTT was performed. The aim of the present study was to investigate the associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes and with changes in fasting and postload glucose concentrations during approximately 6 years of follow-up.

Subjects and Methods

Subjects

The Hoorn Study is a population-based cohort study of glucose tolerance among 2484 Caucasian men and women aged 50 to 75 that started in 1989 and has been described elsewhere ¹⁵. In 1996-1998 a follow-up examination was carried out. Of the 2484 subjects, 150 subjects had died and 108 subjects had moved out of Hoorn. Another 140 subjects were not invited because of logistic reasons. Of the remaining 2086 subjects who were invited for follow-up examination, 1513 subjects (72.5%) participated. After exclusion of the subjects with type 2 diabetes at baseline (49 men and 44 women) and exclusion of subjects with missing anthropometric data, prospective analyses were performed in 1357 subjects (619 men and 738 women). Informed consent was

obtained from all participants and ethical approval for the study was obtained from the local ethics committee.

Measurements

Fasting glucose concentration and 2 hour postload glucose concentration after a 75-g OGTT were measured in plasma (mmol/L) with the glucose dehydrogenase method (Merck, Darmstadt, Germany) at baseline ¹⁵ and hexokinase method (Boehringer Mannheim, Germany) at follow-up, except in subjects who were already known to have diabetes. Fasting and postload glucose concentrations were used to classify subjects according to the 1999 World Health Organisation criteria ¹⁶.

Weight and height were measured, while subjects were barefoot and wearing light clothes only, and body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest, and hip circumference was measured at the widest level over the greater trochanters. Thigh circumference was measured on the left leg directly below the gluteal fold. The mean value of two measurements was used in the analyses. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference, and waist-to-thigh ratio (WTR) was calculated as waist circumference divided by thigh circumference.

Information on lifestyle factors was obtained by questionnaire. Smoking was expressed in cigarette-years (number of cigarettes smoked per day times the number of years smoked) for smokers or former smokers. Alcohol intake was categorized in 4 groups: non-drinkers, up to 10 g/day, 10-30 g/day and >30 g/day. Habitual physical activity was expressed as hours per day. The activities included sports, bicycling, gardening, walking, odd jobs, and housekeeping.

Statistical methods

Differences in baseline characteristics between men and women were tested by Student's t-test for normally distributed variables and by Mann-Whitney's test for variables with a skewed distribution. Differences in proportions were tested by the chi-square test.

Logistic regression analyses were performed to study the association of baseline anthropometric measures (BMI, WHR, WTR, and waist, hip and thigh circumferences) with the incidence of type 2 diabetes. Associations are expressed as odds ratios (ORs) with their 95% confidence interval (CI) per (sex-specific) 1-SD increase in the anthropometric variable involved. An OR can be interpreted as a relative risk. All models were adjusted for age and then additionally for baseline glucose concentrations. The influence of possible confounding by lifestyle factors was studied by adding these factors to the regression model. Possible interaction (effect modification) by sex, age, and anthropometric characteristics was studied by adding product terms to the model. Because the follow-up duration was not the same for each individual, we repeated the analyses with additional adjustment for follow-up duration.

To use cut off values for fasting and postload glucose concentrations and combine them for the definition of type 2 diabetes ¹⁶ implicates a loss of quantitative information. To examine whether the results of the logistic regression analyses for hip, thigh and waist circumferences were influenced by the use of these criteria, linear regression analyses were performed with continuous

fasting and continuous postload glucose concentrations at follow-up as outcome variables. These regression models were adjusted for age and BMI and then additionally for baseline fasting or postload glucose concentrations. Standardized betas are reported to make regression coefficients directly comparable between the different anthropometric measures. A standardized beta of 0.1 indicates that if the independent variable changes 1 SD, the dependent variable changes 0.1 SD. The stability of the models was considered to be disturbed by multi-colinearity if tolerance was <0.1. The tolerance is a statistic used to determine how much the independent variables are linearly related to one another. It is calculated as 1 minus R squared for an independent variable when it is predicted by the other independent variables already included in the analyses. All analyses were performed using SPSS for Windows (version 10.1.0, SPSS Ins, Chicago, IL).

Results

Baseline characteristics by sex are shown in Table 1. Men and women had the same age, but differed significantly in all anthropometric measures: women had higher BMI, and thigh and hip circumferences, but lower waist circumference, WHR and WTR. Men had higher fasting glucose concentrations, whereas women had higher postload glucose concentrations, although the differences in glucose concentrations were relatively small. Follow-up duration did not differ between men and women (6.4 ± 0.5 years in both sexes), and ranged from 4.4 to 8.1 years in men and from 4.5 to 7.9 in women. The women were more physically active, smoked less and had a lower alcohol intake than did the men (data not shown).

Table 1. Baseline characteristics for men and women separately

	Men	Women
N	619	738
Age (years)	60.2 (6.9)	60.4 (6.9)
BMI (kg/m²)	25.9 (2.7)*	26.5 (3.5)
WHR	0.94 (0.06)*	0.83 (0.07)
WTR	1.68 (0.15)*	1.46 (0.16)
Waist circumference (cm)	94.2 (8.4)*	85.8 (9.9)
Hip circumference (cm)	100.2 (5.1)*	102.9 (6.9)
Thigh circumference (cm)	56.6 (4.6)*	59.6 (5.2)
Fasting glucose (mmol/l)	5.46 (0.49)*	5.31 (0.53)
Postload glucose (mmol/l)	5.22 (1.65)*	5.54 (1.59)
		<u>`</u> _

^{*} men significantly different from women (p Student's t-test < 0.05)

WHR, waist-to-hip ratio; WTR, waist-to-thigh ratio

During follow-up, 64 men (10.3%) and 68 women (9.2%) developed type 2 diabetes. Only 6 men and 12 women were diagnosed by the general practitioner before the follow-up examination took place; the remaining patients were diagnosed at this examination. Because sex was a significant effect modifier in the relation between anthropometry and incident diabetes, we performed all analyses for men and women separately. The results in Table 2 show that after adjustment for age, WHR was a strong predictor for type 2 diabetes in both sexes (model 2),

Table 2. Relative risk (odds ratio, OR) for the development of type 2 diabetes per 1-SD increase in risk factor: logistic regression analyses

		Men	(n=619)	Wome	en (n=738)
	Risk factors in model	OR	95% CI	OR	95% CI
Model 1	ВМІ	1.03	0.79 - 1.34	1.53	1.21 - 1.95**
Model 2	WHR	1.55	1.17 - 2.06**	2.15	1.63 - 2.83**
Model 3	BMI	0.79	0.57 - 1.08	1.20	0.91 - 1.58
	WHR	1.75	1.27 - 2.41**	1.98	1.47 - 2.67**
Model 4	Waist circumference	1.23	0.95 - 2.64	1.98	1.54 - 2.55**
Model 5	Hip circumference	0.87	0.66 - 1.14	1.27	1.00 - 1.62*
Model 6	Waist circumference	1.94	1.31 - 2.88**	2.75	1.90 - 3.97**
	Hip circumference	0.54	0.36 - 0.79**	0.65	0.45 - 0.92*

^{**} p < 0.01, * p < 0.05

WHR, waist-to-hip ratio; All models were adjusted for age

whereas BMI was a significant predictor in women only (model 1). After mutual adjustment (model 3), only WHR seemed to be important in predicting diabetes. Waist circumference (model 4) was less strongly associated with incident diabetes after adjustment for age than the WHR, suggesting a predictive role for hip circumference. To examine whether the association of WHR was largely due to the waist circumference or to the hip circumference, we added these variables separately into one regression model (model 6). Both circumferences appeared to be significantly associated in opposite directions with the risk of diabetes in both sexes, also after adjustment for BMI (Figure 1). BMI was not significantly associated with the incidence of type 2 diabetes in this model. No interactions were observed between anthropometric variables or between age and anthropometric variables. Similar analyses were performed for the WTR and thigh circumference (Table 3). The results were similar, except that in men, thigh circumference was not significantly associated with a lower risk of diabetes (model 6). Adjustment for BMI did not change this result (Figure 2). Adjustment for lifestyle factors (smoking, alcohol intake, and physical activity) also did not change the results (data not shown).

Table 3. Relative risk (odds ratio, OR) for the development of type 2 diabetes per 1-SD increase in risk factor: logistic regression analyses

		Men	(n=619)	Wome	en (n=738)
	Risk factors in model	OR	95% CI	OR	95% CI
Model 1	BMI	1.03	0.79 – 1.34	1.53	1.21 – 1.95**
Model 2	WTR	1.41	1.05 - 1.89*	2.08	1.59 - 2.72**
Model 3	BMI	0.97	0.73 - 1.27	1.31	1.02 - 1.69*
	WTR	1.42	1.05 - 1.92*	1.92	1.45 – 2.54**
Model 4	Waist circumference	1.23	0.95 - 2.64	1.98	1.54 - 2.55**
Model 5	Thigh circumference	0.90	0.68 - 1.19	1.00	0.79 - 1.28
Model 6	Waist circumference	1.44	1.05 - 1.96*	2.37	1.78 - 3.17**
	Thigh circumference	0.73	0.52 - 1.04	0.68	0.50 - 0.92*

^{**} p < 0.01, * p < 0.05

WTR, waist-to-thigh ratio; All models were adjusted for age

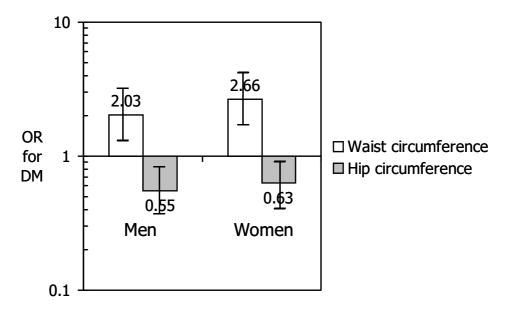


Figure 1. Odds ratios (ORs) for developing type 2 diabetes (DM) per 1-SD larger waist and hip circumferences. Waist circumference was adjusted for hip circumference, BMI, and age; hip circumference was adjusted for waist circumference, BMI and age

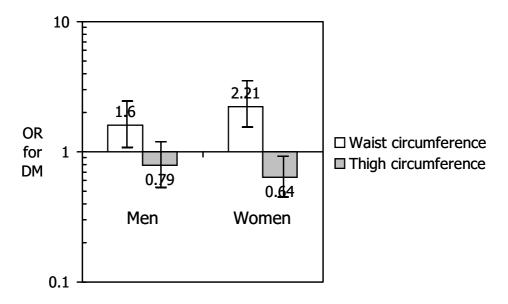


Figure 2. Odds ratios (ORs) for developing type 2 diabetes (DM) per 1-SD larger waist and thigh circumferences. Waist circumference was adjusted for thigh circumference, BMI, and age; thigh circumference was adjusted for waist circumference, BMI and age

The ORs for a 1-SD larger hip circumference after adjustment for waist circumference and additional adjustment for all lifestyle factors were 0.52~(0.33-0.80) for men and 0.65~(0.43-0.98) for women. A 1-SD larger thigh circumference after adjustment for waist circumference and all lifestyle factors resulted in ORs of 0.80~(0.53-1.20) for men and 0.64~(0.44-0.93) for women. Adjustment for follow-up duration did not change any of the observed associations (data not shown). After adjustment for baseline fasting and postload glucose concentrations, only the waist circumference in women remained significantly associated with the incidence of type 2 diabetes (data not shown).

Tables 4 and 5 present the results of the multiple linear regression models for associations of baseline body circumferences with follow-up fasting and postload glucose concentrations, respectively. We adjusted for age and BMI (models 1 and 2) and then additionally for baseline fasting or postload glucose concentrations (model 3 and 4). In accordance with the results of the logistic regression analyses, glucose concentrations (fasting as well as postload) were positively associated with baseline waist circumference and negatively associated with baseline hip or thigh circumferences, although the results were not statistically significant for thigh circumference in men (models 1 and 2). After adjustment for baseline glucose concentrations (models 3 and 4), most associations became non-significant. In men waist circumference was still significantly and positively associated with fasting glucose, whereas in women only thigh circumference was significantly associated with postload glucose concentrations in men, whereas in women waist circumference was positively associated and thigh circumference was negatively associated after adjustment for baseline postload glucose concentration.

The correlation between waist and hip circumferences was 0.71 in men and 0.69 in women, and the correlation between waist and thigh circumferences was 0.46 in men and 0.42 in women. The regression models were not disturbed by multi-colinearity.

Table 4. Associations (standardized betas) of baseline body circumferences with continuous fasting glucose concentrations at follow-up: multiple linear regression analyses

		Men (r	n=619)	Women	(n=738)
	Independent variables	Beta	р	Beta	р
Model 1 ^a	Waist circumference	0.284	0.000	0.216	0.000
	Hip circumference	-0.178	0.005	-0.154	0.008
Model 2 ^a	Waist circumference	0.219	0.001	0.157	0.011
	Thigh circumference	-0.036	0.528	-0.134	0.005
Model 3 ^b	Waist circumference	0.159	0.008	0.094	0.082
	Hip circumference	-0.102	0.064	-0.076	0.134
Model 4 ^b	Waist circumference	0.120	0.036	0.060	0.267
	Thigh circumference	-0.024	0.633	-0.084	0.043

^a Adjusted for age and BMI

^b Adjusted for age, BMI, and baseline fasting glucose

Table 5. Associations (standardized betas) of baseline body circumferences with continuous postload glucose concentrations at follow-up: multiple linear regression analyses

		Men (r	n=619)	Women	(n=738)
	Independent variables	Beta	р	Beta	р
Model 1 ^a	Waist circumference	0.203	0.003	0.253	0.000
	Hip circumference	-0.180	0.005	-0.203	0.000
Model 2 ^a	Waist circumference	0.137	0.036	0.182	0.003
	Thigh circumference	-0.037	0.517	-0.148	0.002
Model 3 ^b	Waist circumference	0.083	0.162	0.119	0.021
	Hip circumference	-0.074	0.180	-0.057	0.240
Model 4 ^b	Waist circumference	0.056	0.322	0.093	0.072
	Thigh circumference	-0.007	0.889	-0.066	0.097

^a Adjusted for age and BMI

Discussion

In the present study, we showed that the body circumference ratios (WHR and WTR) are better predictors of future type 2 diabetes than is overall obesity measured by BMI. Both waist and hip circumference have important, but opposite, associations with the risk of diabetes after adjustment for age and BMI. A larger waist circumference is associated with a higher risk of diabetes, whereas a larger hip circumference is associated with a lower risk of diabetes. The same results apply for the thigh circumference, although the protective effect only reached statistical significance in women. The measurements of continuous glucose concentrations showed that fasting and postload glucose concentrations were positively associated with baseline waist circumference and negatively associated with baseline hip and thigh circumferences.

Of the 2086 persons who were invited for the follow-up examination, 72.5 % participated. These participants were healthier at baseline than were the non-participants ¹. Therefore, it is possible that we have underestimated the true incidence of diabetes and consequently underestimated the associations with waist, hip, and thigh circumferences.

When incident diabetes or continuous glucose concentrations after follow-up are used as the study outcome, there is often a discussion of whether to adjust for baseline glucose concentrations. Adjustment for baseline glucose answers the question of whether knowledge about the thigh or hip circumference contributes to the prediction of incident diabetes once baseline glucose is taken into account. In our study, thigh or hip circumference did not independently contribute to incident diabetes or continuous glucose concentrations after adjustment for baseline glucose. However, if baseline glucose concentrations and baseline thigh or hip circumferences result from the same etiological process, adjustment for glucose concentrations would be inappropriate. Alternatively, if we assume that there is a causal association of thigh or hip circumference with impaired glucose metabolism, it is possible that persons with narrow hips or small thighs had increased glucose concentrations already at baseline. If we then adjust for these baseline glucose concentrations, the effects of thigh or hip circumferences disappear. The observed cross-sectional association between body circumferences and glucose levels ¹² reinforce

^b Adjusted for age, BMI, and baseline postload glucose

these suggestions. Waist circumference is more likely to remain a significant predictor of type 2 diabetes after adjustment for baseline glucose levels.

Our observation that central obesity, independent of overall obesity, is an important determinant of type 2 diabetes is not new ³⁻⁶. The abdominal visceral fat is considered to be possibly etiologically important through increased fatty acid release into the portal system ⁷⁻⁹. Our results extend these findings by showing an independent association of waist and hip circumferences to the development of diabetes. Our results agree with the results of the scarce cross-sectional studies ^{10,11}. One previous prospective study, showing an independent protective effect of the hip circumference on the development of diabetes, was limited to women ¹⁴. Furthermore, the presence of diabetes was examined on the basis of an OGTT. In the present prospective study, an OGTT was performed in both men and women. In addition, we included measurements of thigh as well as hip circumference.

The negative association of the hip circumference with glucose metabolism was proposed to be caused by a greater muscle mass at the gluteal region ¹⁰. Muscle mass is one of the sites of insulin resistance as well as the main target of insulin. WHR has been related to both larger visceral fat and smaller leg muscle areas in men ¹⁷. Also, the higher prevalence of glucose tolerance in Indian men than in Swedish men was related to lower muscle mass ¹⁸. Thigh circumference might better reflect muscle mass than hip circumference, because it is less likely to be influenced by frame size (pelvic width). Our results, however, show that hip circumference has a stronger negative association with glucose concentrations than does thigh circumference, especially in men.

Larger thigh and hip circumferences could also reflect increased femoral and gluteal subcutaneous fat mass. Particularly in women, these depots have relatively high lipoprotein lipase (LPL) activity and relatively low rates of basal and stimulated lipolysis ¹⁹. These depots may protect the liver and muscle from high exposure to free fatty acids through uptake and storage. Recently, Van Pelt et al ²⁰ showed that larger leg fat mass measured by dual-energy X-ray absorptiometry was associated with better insulin sensitivity and a better lipid profile after adjustment for trunk fat.

Regional differences in adipocyte metabolism (LPL activity and lipolysis) are more pronounced in women than in men ¹⁹. This may explain why the observed protective role of larger thighs was stronger in women than in men. Furthermore, the interpretation of hip circumference may differ between men and women. Gluteal fat mass and pelvic width may be the main determinants of hip circumference in women, whereas pelvic width and muscle mass may be the main determinants in men.

Adrenal and sex steroid concentrations and growth hormone (GH) concentrations may influence visceral fat accumulation as well as the development of insulin resistance ²¹⁻²⁴. For example, hyperandrogenicity in women and hypoandrogenicity in men has been associated with insulin sensitivity and the development of type 2 diabetes ^{25,26} and with fat distribution ²⁷⁻²⁹. These steroids may influence adipose tissue by changing the LPL activity ^{7,22,25,30}. In the present study, no information on hormones was available.

Although the exact mechanisms need to be further explored, the results of the present study show that body tissue distribution is an important factor in the development of type 2

diabetes in older people, even more than is BMI. Therefore, lifestyle interventions aimed at the prevention of type 2 diabetes not only should focus on weight loss, but should preferably combine approaches that decrease waist circumference and increase hip or thigh circumference. Increased physical activity, drinking less heavily, and smoking cessation have been shown to do both ³¹. Caloric restriction tends to decrease both waist and hip or thigh circumference. Increased physical activity results in a better body composition, by an increase of the muscle mass in the legs and a decrease in visceral fat accumulation. The better body composition achieved by smoking cessation, and less heavy drinking, is suggested to be caused by the influence these have on hormones, as discussed above. Further research on prevention strategies, however, is needed.

In summary, larger hip or thigh circumference is associated with a lower risk of type 2 diabetes in both men and women, independently of BMI, age, and waist circumference, whereas larger waist circumference is associated with higher risk. Further research should be aimed at determining the underlying etiologic mechanisms of this association and whether our results can be extrapolated to other ethnic groups.

Acknowledgements

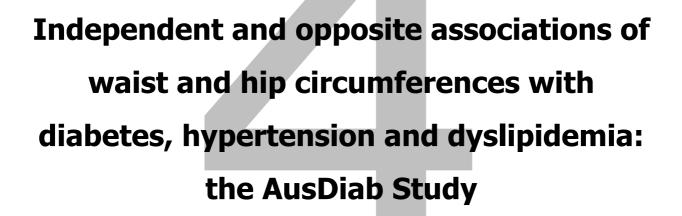
MBS performed the data analyses and wrote the drafts and the final article. JMD, MV, LMB, CDAS, JSY, RJH, GN and JCS provided advice in the presentation and/or interpretation of the results. JMD, LMB, CDAS, RJH and GN were responsible for the design and data collection of the Hoorn Study. PJK provided statistical advice. All authors declare that they had no duality or conflicts of interest.

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Abstract

Objective

Fat distribution as measured by waist-to-hip ratio has been shown to be an important independent predictor of glucose intolerance. Few studies, however, have considered the contributions of the waist and hip circumferences independently. The aim of this study was to investigate the independent associations of waist and hip circumference with diabetes in a large population-based study, and to investigate whether they also apply to other major components of the metabolic syndrome (hypertension and dyslipidemia). In addition, as previous studies were performed in older persons, we investigated whether these associations were present across adult age groups.

Methods

Weight, height, waist and hip circumferences were measured in 11247 participants of the nationally representative Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. HDL-cholesterol, triglycerides, fasting and 2-hour postload glucose were determined, and diastolic and systolic blood pressure measured. After exclusion of persons already known to have diabetes, hypertension or dyslipidemia, logistic and linear regression were used to study cross-sectional associations of anthropometric variables with newly diagnosed diabetes, hypertension and dyslipidemia, and with continuous metabolic measures, all separately for men (n=3818) and women (n=4582). Analyses were repeated in the same population stratified for age.

Results

After adjustment for age, BMI and waist, a larger hip circumference was associated with a *lower* prevalence of undiagnosed diabetes (odds ratio (OR) per one standard deviation increase in hip circumference 0.55 (95% CI 0.41-0.73) in men and 0.42 (0.27-0.65) in women) and undiagnosed dyslipidemia (OR 0.58 (0.50-0.67) in men and 0.37 (0.30-0.45) in women). Associations with undiagnosed hypertension were weaker (OR 0.80 (0.69-0.93) in men and 0.88 (0.70-1.11) in women). As expected, larger waist circumference was associated with *higher* prevalence of these conditions. Similar associations were found using continuous metabolic variables as outcomes in linear regression analyses. Height partly explained the negative associations with hip circumference. When these analyses were performed stratified for age, associations became weaker or disappeared in the oldest age groups (age \geq 75 years in particular), except for HDL-cholesterol.

Conclusion

We found independent and opposite associations of waist and hip circumference with diabetes, dyslipidemia and less strongly with hypertension in a large population-based survey. These results emphasize that waist and hip circumference are important predictors for the metabolic syndrome and should both be considered in epidemiological studies. The associations were consistent in all age groups, except in age \geq 75. Further research should be aimed at verifying hypotheses explaining the 'protective' effect of larger hips.

Introduction

Fat distribution, apart from overall obesity, is an important risk factor for type 2 diabetes and cardiovascular diseases ¹⁻⁴. Most studies use the waist-to-hip ratio (WHR) for measuring fat distribution, or simply the waist circumference because the waist circumference alone is more strongly correlated to visceral fat than the WHR ⁵. Visceral fat has been shown to be strongly correlated to components of the metabolic syndrome, and is generally assumed to be a causal factor by releasing free fatty acids into the portal vein ⁶⁻⁸. However, results are not consistent, and some studies showed abdominal subcutaneous fat to be more strongly associated ^{9,10}.

A high WHR is generally taken to indicate an excess of visceral or abdominal fat, however, it can also be due to a smaller hip circumference ^{11,12}. Therefore, recent studies have investigated the separate contributions of waist and hip circumferences to the glucose levels and type 2 diabetes ^{2,11,13-15}. A larger waist circumference was associated with higher glucose levels and risk for developing type 2 diabetes. In contrast, a larger hip circumference was consistently associated with lower glucose levels and risk of type 2 diabetes, independently of waist circumference. These results also remained after adjustment for age and body mass index (BMI), possibly indicating a protective role of a larger hip circumference. The underlying mechanisms explaining these associations are unclear.

To gain further insights into the inverse association between hip circumference and glucose tolerance status, we were interested in seeing whether these associations could be extended to other populations and to other components of the metabolic syndrome apart from blood glucose levels. In addition, we were interested in examining if these associations were present in all age groups, as body composition changes with age. Therefore, we investigated the relationship of waist and hip circumferences with diabetes, hypertension and dyslipidemia in the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study.

Methods

Study population

The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) is a large, nationally representative cross-sectional study among 11247 participants aged \geq 25 years living in Australia. A detailed description of the methodology has been published elsewhere ^{16,17}. Briefly, a nationally representative sample of the population was drawn from 42 randomly selected urban and non-urban areas (Census Collector Districts, CDs) across Australia. CDs containing < 100 individuals aged \geq 25 years, CDs classified as 100% rural, or CDs with a population comprising > 10% Aboriginal or Torres Strait Islanders were excluded. All subjects aged \geq 25 years were invited to attend the survey, which consisted of a short household interview followed by a biomedical examination at a study examination site within or close to the selected residential areas. From the 11247 participants in the study, those already known to have diabetes (263 men and 212 women) and persons receiving medical treatment for hypertension (758 men and 1010 women) or dyslipidemia (471 men and 488 women) were excluded from the present analyses, because treatment could possibly influence the associations that we study. Also women who reported to be

pregnant (n=60) and subjects with missing data on BMI, waist or hip circumference were excluded (n=267). Therefore, analyses were performed in 8400 participants (3818 men and 4582 women). All responders gave written informed consent and ethical approval for the AusDiab Study was obtained from the ethics committee of the International Diabetes Institute.

Measurements

Height was measured to the nearest 0.5 cm without shoes using a stadiometer. Weight was recorded to the nearest 0.1 kg with subjects wearing lights clothes only and no shoes, using a mechanical beam balance. Waist circumference was measured halfway between the lower rib and the iliac crest using a steel measuring tape. Hip circumference was measured at the widest point over the buttocks. Waist and hip circumference were measured in duplicate to the nearest 0.5 cm. If the difference between the 2 measurements was greater than 2 cm, a third measurement was taken and the mean of the two closest measurements was calculated. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²) and waist-to-hip ratio (WHR) was defined as waist circumference divided by hip circumference. Total body fat percentage was assessed by bio-impedance, using the Tanita body fat analyser (Model TBF-105, Tanita Corporation, Tokyo, Japan), which calculates body fat as a function of sex, height, weight and impedance.

A 75-g oral glucose tolerance test was performed to determine fasting and 2-hour postload levels in all subjects, except those on insulin or oral diabetes medication. Plasma glucose levels, serum HDL-cholesterol and triglycerides were determined enzymatically using an Olympus AU600 automated analyser (Olympus Optical Co Ltd, Tokyo, Japan), which uses the glucose oxidase method for glucose levels. Subjects who reported a history of physician-diagnosed diabetes and who 1) were taking oral glucose-lowering drugs or using insulin injections, or 2) had a fasting glucose value \geq 7.0 mmol/l or postload glucose \geq 11.1 mmol/l 18 were classified as known diabetes. Dyslipidemia was defined as HDL-cholesterol < 1.0 mmol/l or triglycerides \geq 2.0 mmol/l

Blood pressure (BP) was measured in a seated position after at least 5 minutes rest with a Dinamap semi-automatic oscillometric recorder. A cuff of suitable size was applied to the participant's upper arm (the arm not used for blood collection), which was supported by the table at heart level. Three readings were taken at one-minute intervals. In one state only, BP was measured with a standard mercury sphygmomanometer using the first and fifth Korotkoff sounds, to the nearest 2 mmHg. Based on a comparison study, adjustment was made to all diastolic BP readings recorded using the sphygmomanometer. The mean of the first two readings was calculated unless the difference was greater than 10 mmHg. In that case the two closest of three measurements was used. Participants were considered to be hypertensive if systolic BP was \geq 140 mmHg or diastolic BP \geq 90 mmHg 20 .

Statistical methods

All statistical analyses were performed separately for men and women. Differences in mean characteristics between men and women were tested for statistical significance by Student's t-test. Because of their skewed distribution, median and interquartile range are reported for triglyceride

and HDL-cholesterol levels, and differences between men and women were tested by Mann-Whitney's nonparametric test.

Sex-specific tertiles of waist circumference and of hip circumference were created, and unadjusted and BMI- and age-adjusted prevalences of undiagnosed diabetes, hypertension and dyslipidemia were calculated in nine subgroups created according these tertiles (3 X 3 table).

Logistic regression was used to study the independent associations of continuous waist and hip circumferences with undiagnosed diabetes, hypertension and dyslipidemia. Odds ratios with 95% confidence intervals are reported, expressed per one standard deviation increase in waist or hip circumference.

Because using cut points for defining the metabolic disorders implies a loss of information, we also studied relationships between anthropometric variables and continuous metabolic variables by use of linear regression. Waist and hip circumference were both included as independent variables with each of the metabolic variables (fasting glucose, postload glucose, systolic BP, diastolic BP, triglycerides or HDL-cholesterol) as the dependent variable. Triglycerides and HDL-cholesterol were In-transformed because of their skewed distribution. Regression analyses were also performed additionally adjusted for age, BMI or height. Effect modification by age was tested by adding interaction terms (waist x age and hip x age) to the regression models that already included age. In addition, regression analyses were also performed stratified for age, using 10-year intervals. Multi-colinearity was studied by the tolerance statistic. If the tolerance was < 0.1, the stability of the regression model was considered to be disturbed by multi-colinearity. To make the results of linear regression models comparable among different independent variables we report standardized beta values. All analyses were performed using SPSS Version 10.0.5 for Windows (SPSS, Chicago, IL).

Results

The characteristics of the study population are shown in Table 1. Although men had a higher BMI and WHR than women, women had a higher total body fat percentage. Except for body fat percentage and postload glucose, men had a worse metabolic profile than women.

Figure 1 presents the age- and BMI-adjusted prevalence of undiagnosed diabetes, hypertension and dyslipidemia separately for men and women, in tertiles of waist and hip circumferences. In persons with a large waist and a small hip circumference the prevalence of undiagnosed diabetes was the highest. A similar pattern was shown for the prevalence of undiagnosed hypertension and undiagnosed dyslipidemia.

Adding both waist and hip circumferences as continuous variables to one logistic regression model, a larger waist circumference was associated with a greater risk for having undiagnosed diabetes, hypertension and dyslipidemia. In contrast, a larger hip circumference was associated with a lower risk after adjustment for waist circumference. Adjustment for BMI weakened the associations with waist circumference, but not the associations with hip circumference (Table 2). Similar results were shown using fat percentage measured by impedance instead of BMI (data not shown). If prevalent cases (i.e. already using medication) were not excluded for these analyses,

Table 1. Characteristics of the study population^a

		Men	W	/omen
N	3818		4582	
Age (years)	48.7*	(13.5)	47.9	(13.1)
Weight (kg)	83.7*	(14.2)	69.3	(14.6)
Height (cm)	176.3*	(7.2)	162.8	(6.5)
BMI (kg/m2)	26.7*	(4.0)	26.1	(5.3)
Fat percentage (%) ^b	24.2*	(7.4)	39.0	(10.5)
Waist (cm)	96.3*	(11.2)	83.4	(12.7)
Hip (cm)	103.9	(7.6)	104.3	(11.1)
WHR	0.93*	(0.06)	0.80	(0.06)
Fasting glucose (mmol/l)	5.58*	(0.78)	5.24	(0.58)
Postload glucose (mmol/l)	5.99	(2.24)	6.05	(1.91)
Triglycerides (mmol/l) ^c	1.34*	(0.94 - 2.00)	1.08	(0.76 - 1.56)
HDL-cholesterol (mmol/l) ^c	1.23*	(1.06 - 1.44)	1.54	(1.31 - 1.80)
Diastolic BP (mmHg)	73.7*	(10.7)	65.5	(10.7)
Systolic BP (mmHg)	130.0*	(15.4)	122.3	(16.7)
Prevalence diabetes (%)	3.9*		2.5	-
Prevalence hypertension (%)	23.9*		13.9	
Prevalence dyslipidemia (%)	33.1*		16.2	

^{*} p < 0.05 comparing sexes by Student's t-test, or by Mann-Whitney's U in case of skewed distribution

Table 2. Relative risk (ORs with 95% CI) for having undiagnosed diabetes, hypertension or dyslipidemia associated with one SD increase in waist or hip circumference

		Men	W	omen
	Waist	Hip	Waist	Hip
Diabetes				
Model 1	3.53 (2.69-4.63)	0.51 (0.39-0.67)	4.11 (3.01-5.60)	0.46 (0.33-0.63)
Model 2	2.26 (1.53-3.35)	0.55 (0.41-0.73)	2.68 (1.85-3.88)	0.42 (0.27-0.65)
Hypertension				
Model 1	2.37 (2.06-2.73)	0.64 (0.56-0.74)	1.95 (1.67-2.28)	0.79 (0.68-0.93)
Model 2	1.26 (1.03-1.54)	0.80 (0.69-0.93)	1.08 (0.88-1.32)	0.88 (0.70-1.11)
Dyslipidemia				
Model 1	2.50 (2.19-2.86)	0.74 (0.66-0.85)	3.88 (3.32-4.55)	0.48 (0.41-0.56)
Model 2	2.02 (1.69-2.41)	0.58 (0.50-0.67)	2.98 (2.49-3.57)	0.37 (0.30-0.45)

Model 1 includes waist and hip as independent variables

Model 2 includes waist, hip, age and BMI as independent variables

^a Data are mean (standard deviation) or percentages

^b Fat percentage missing in 54 men and 77 women

^c Median (interquartile range) presented because of skewed distribution

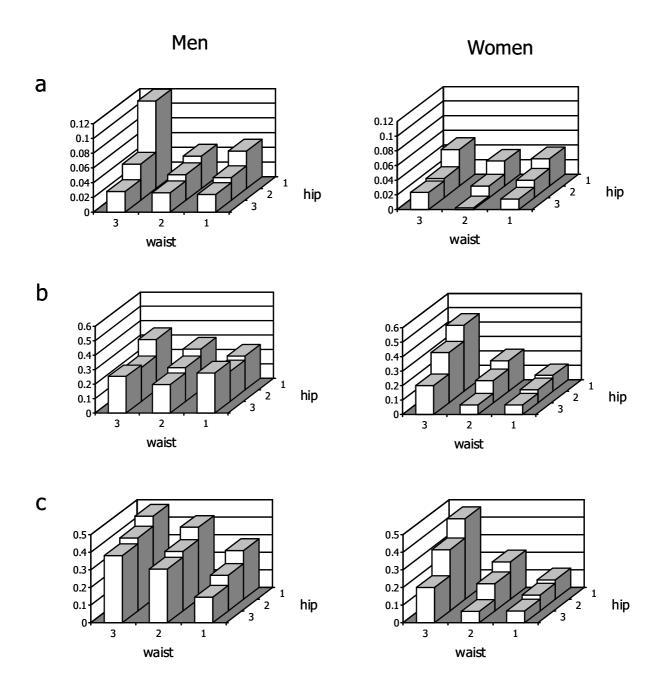


Figure 1. BMI- and age-adjusted prevalence of undiagnosed diabetes (a), hypertension (b) and dyslipidemia (c) within tertiles of low, median and high (1 thru 3) waist and hip circumferences, separately for men and women

the results were also very similar (data not shown). In univariate models, waist and hip were both significantly positively associated with a greater risk of having undiagnosed diabetes, hypertension and dyslipidemia (data not shown).

It is shown in Table 3 that waist circumference was associated in the expected manner with all continuous metabolic variables, while hip showed opposite associations, although not statistically significant with diastolic BP in women and with HDL-cholesterol in men. Adjustment for age alone attenuated all associations with the glucose and blood pressure variables, but strengthened the associations with lipids. Adjustment for BMI alone weakened all associations with waist circumference, but strengthened all associations with hip circumference. Similar results were found if fat percentage was used instead of BMI. Adjustment for height alone affected all associations leading to weaker but still significant associations (data not shown). The associations also remained similar if only subjects with normal glucose tolerance were studied (i.e. subjects with newly detected diabetes, impaired fasting glucose and impaired glucose tolerance were excluded from the analyses), or if only subjects with normal lipids were studied (i.e. subjects with newly detected dyslipidemia were excluded). If subjects with undiagnosed hypertension were

Table 3. Associations (standardised betas) of waist and hip circumference with variables of the metabolic syndrome after adjustment for each other, and additionally adjusted for age and BMI or for age and height

	М	en	Wo	men
	Waist	Hip	Waist	Hip
Fasting glucose	0.402	-0.167	0.438	-0.142
Postload glucose	0.453	-0.249	0.463	-0.185
Diastolic BP	0.368	-0.138	0.163	0.005 ^a
Systolic BP	0.422	-0.181	0.354	-0.070
Ln triglycerides	0.556	-0.248	0.689	-0.315
Ln HDL-cholesterol	-0.346	0.019 ^a	-0.454	0.103
Additionally adjusted for age/BMI				
Fasting glucose	0.238	-0.161	0.290	-0.183
Postload glucose	0.223	-0.214	0.272	-0.273
Diastolic BP	0.131	-0.120	0.050^{a}	-0.101
Systolic BP	0.106	-0.062	0.081	-0.023 ^a
Ln triglycerides	0.388	-0.345	0.523	-0.391
Ln HDL-cholesterol	-0.294	0.153	-0.438	0.323
Additionally adjusted for age/height				
Fasting glucose	0.333	-0.093	0.351	-0.070
Postload glucose	0.316	-0.089	0.348	-0.073
Diastolic BP	0.273	-0.047 ^a	0.133	0.031 ^a
Systolic BP	0.218	0.003 ^a	0.144	0.089
Ln triglycerides	0.566	-0.229	0.599	-0.235
Ln HDL-cholesterol	-0.429	0.071	-0.532	0.146

a non significant (p>0.05)

excluded, the associations with blood pressure became weaker. In univariate models, waist and hip were both significantly positively associated with all the continuous metabolic variables (data not shown).

Results of linear regression analyses stratified for age are shown in Table 4. The number of subjects in each age group was smaller in older age groups, but was at least 166 per group. The associations of fasting and postload glucose levels with waist and hip circumferences in each age group were similar to those in the total population and reasonably consistent over all age groups, except for men aged ≥ 65 years and women ≥ 75 years. In these older age groups the associations of both waist and hip circumference became weaker, disappeared or even went in the opposite direction. Also the associations of anthropometric variables with BP disappeared in these older age groups, but the associations with BP became weak in all age groups. Associations with lipids mainly remained similar in each age group, except for the associations with triglycerides in the oldest age groups (age ≥ 75 years). None of the linear regression models was disturbed by multi-colinearity.

Discussion

In the present study, we have confirmed in a large population based sample with a wide age range that, after taking waist circumference into account, a larger hip circumference is associated with a lower prevalence of undiagnosed diabetes. We have extended these findings to other components of the metabolic syndrome, i.e. hypertension and dyslipidemia. In particular, the associations with diabetes and dyslipidemia were strong and consistent across all ages, except age ≥ 75 .

Earlier studies investigating the separate contributions of waist and hip circumferences to diabetes also have demonstrated an apparent 'protective' effect of a large hip circumference ^{11,13,15}. Similar associations were found not only for risk of future diabetes ^{2,14}, but also for incident self-reported myocardial infarction, combined cardiovascular diseases, and mortality of these diseases ¹⁴. Negative associations using thigh circumference instead of hip circumference were shown in women, but less strongly in men ^{2,15}. However, previous studies have been limited by not basing the diagnosis of diabetes on an OGGT ^{11,13,14}, or by restriction to a particular age group ^{2,11,14,15}, or gender ¹⁴.

Several mechanisms could explain the inverse association between hip circumference and metabolic disturbances. Firstly, larger hips could indicate larger skeletal frame. Adjustment for height as another measure for frame size explained part of the associations. Height can be interpreted as an indicator of muscle mass, as it is strongly correlated with lean mass ²¹. Secondly, larger hips could also directly reflect greater muscle mass. Difference in glucose metabolism between Swedish and Indian men has been demonstrated to be more strongly linked to differences in leg muscle mass and not visceral fat ¹². Although insulin resistance affects all tissues, skeletal muscle is the main target of insulin and one major site of insulin resistance. Finally, hip circumference could reflect femoral fat mass accumulation, which could have a protective effect additional to muscle mass. Fat tissue in the femoral or gluteal region has been suggested to play a protective role, because adipose tissue in these regions is less sensitive to lipolytic stimuli ²².

Table 4. Associations (standardised betas) of waist and hip circumference (adjusted for each other and for BMI) with variables of the metabolic syndrome, stratified for age

	M	en	Wo	men
	Waist	Hip	Waist	Hip
Fasting glucose				
25-34 y	0.181*	-0.158*	0.382**	-0.231**
35-44 y	0.364**	-0.111*	0.253**	-0.285**
45-54 y	0.212**	-0.145**	0.379**	-0.167**
55-64 y	0.406**	-0.251**	0.242**	-0.162**
65-74 y	-0.096	-0.153	0.365**	-0.311**
75 +	0.024	0.122	0.003	0.289
Postload glucose				
25-34 y	0.328**	-0.246**	0.101	-0.208**
35-44 y	0.234	-0.274**	0.292**	-0.341**
45-54 y	0.190**	-0.217**	0.344**	-0.350**
55-64 y	0.530**	-0.365**	0.215**	-0.371**
65-74 y	-0.063	-0.084	0.385**	-0.185
75 +	-0.018	0.065	-0.057	0.194
Diastolic BP				
25-34 y	0.334**	-0.029	-0.115	0.168*
35-44 y	0.232**	-0.097	0.114*	-0.082
45-54 y	-0.019	-0.113**	0.140**	-0.186**
55-64 y	0.076	-0.167**	0.031	-0.075
65-74 y	0.141	-0.069	-0.035	-0.298**
, 75 +	-0.032	0.044	-0.075	-0.193
Systolic BP				
25-34 y	0.032	0.075	-0.006	0.199**
35-44 y	0.303**	-0.124**	0.080	0.017
45-54 y	0.124^{\dagger}	-0.158**	0.206**	-0.187**
55-64 y	0.153	-0.218**	0.075	0.022
65-74 y	0.026	-0.042	-0.050	-0.098
75 +	0.110	-0.005	-0.039	-0.029
Ln Triglycerides				
25-34 y	0.435**	-0.304**	0.365**	-0.314**
35-44 y	0.633**	-0.460**	0.523**	-0.385**
45-54 y	0.219**	-0.312**	0.696**	-0.565**
55-64 y	0.336**	-0.284**	0.592**	-0.472**
65-74 y	0.254**	-0.260**	0.390**	-0.166**
, 75 +	0.133	-0.057	0.183	-0.017
Ln HDL-cholesterol				
25-34 y	-0.205**	0.083	-0.420**	0.411**
35-44 y	-0.414**	0.174**	-0.492**	0.250**
45-54 y	-0.235**	0.133**	-0.453**	0.327**
55-64 y	-0.309**	0.146**	-0.324**	0.470**
65-74 y	-0.165	0.150	-0.408**	0.211*
75 +	-0.382**	0.297**	-0.340**	-0.015

^{**} p < 0.05, *p < 0.10

Lipoprotein lipase (LPL) activity has been shown to be greater in femoral subcutaneous adipose fat compared to visceral fat ²³. These regions are therefore more likely to buffer the circulation of FFA after a meal, by taking them up and suppressing lipolytic release into the circulation ²⁴. A diminished entrapment capacity of adipose tissue leads to increased lipid fluxes in the circulation leading to ectopic fat storage in liver, skeletal muscle and pancreas, leading to insulin resistance and beta cell dysfunction ²⁵⁻²⁹. In humans, larger leg fat mass by dual energy x-ray absorptiometry or larger thigh fat area by computed tomography has been found to be associated with favourable levels of cardiovascular risk factors including lipids and glucose levels in a few studies now ³⁰⁻³⁵.

In line with the latter mechanism, the strongest negative associations of the hip circumference would be expected to be observed with triglycerides, which we found in the present study, followed by slightly weaker but relevant associations with glucose levels. After adjustment for BMI, blood pressure showed no strong association with the fat distribution measures. This could indicate that overall obesity plays a more important role in hypertension than does fat distribution, which is in line with recently reported data in Swedish women, where BMI was more strongly correlated with diastolic and systolic BP than was WHR. Only BMI and change in BMI predicted future hypertension ³⁶. There is also much debate regarding the role of insulin resistance in the regulation of BP and only a modest association between higher insulin sensitivity and lower risk of development of hypertension has been shown recently ³⁷. Factors as salt and alcohol intake and physical activity might be other important factors determining BP and hypertension ³⁸.

Previous studies that investigated separate contributions of waist and hip could or did not distinguish between different age groups. In the present study associations between anthropometrics and metabolic variables seemed to apply for all ages, but disappeared after the age of 75 years. The association with glucose levels disappeared at a younger age in men than in women (65 years vs. 75 years respectively). The fact that associations between anthropometric measures and disease are weaker in older age groups may be explained by the healthy survivor effect. Also other diseases may interfere with the associations of interest, and finally, anthropometric variables may reflect something different at older ages.

A limitation of the present study is that it is cross-sectional and causality cannot be assumed. Underlying factors may influence both metabolic disturbances and abdominal fat distribution. An increased activity of the sympathetic nervous system and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis have both been associated with an increased WHR and with metabolic disturbances ^{39,40}. An increased sensitivity of the HPA axis leads to high glucocorticoid and low growth hormone levels, and disturbances in sex hormones which all have been associated with insulin resistance as well as abdominal fat distribution ⁴¹. Also intrauterine growth retardation (low birth weight) has been linked with both unfavourable WHR and disease at adult age (Barker hypothesis) ^{42,43}. An altered development of the pancreas or altered insulin sensitivity of target tissues may be some of the foetal changes causing type 2 diabetes later ⁴⁴. A lower birth weight has recently been shown to be associated with lower lean mass in later life ⁴⁵.

In summary, we found independent and opposite relations of waist and hip circumferences with diabetes, hypertension, and dyslipidemia in a large population-based Australian study. The associations with diabetes and dyslipidemia were strong and consistent across all ages, except age \geq 75 years. Although the adverse effects of a higher waist circumference were stronger, the

opposite associations of a larger hip circumference were also strong and emphasize that waist and hip circumference are important predictors for the metabolic syndrome and should both be considered in epidemiological studies. Further research should explore the possible mechanisms explaining these associations.

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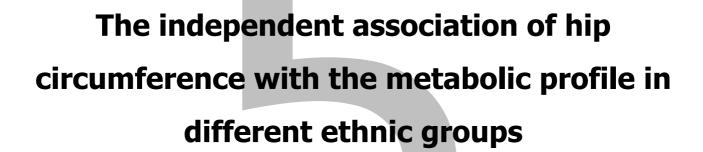
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submitted as short communication

Abstract

Objective

In Caucasians, a larger hip circumference has been shown to be associated with a better metabolic profile after adjustment for BMI and waist circumference. Our aim was to investigate this association in a variety of ethnic groups, some highly susceptible for type 2 diabetes.

Methods

We measured weight, height, waist and hip circumferences, systolic and diastolic blood pressure, fasting and 2-hour postload glucose, triglycerides and HDL-cholesterol in 1020 Melanesians, 767 Micronesians, 3697 Indians and 2710 Creoles from Pacific and Indian Ocean islands. Leptin and body fat percent were determined in Indian and Creole Mauritians only.

Results

In all ethnic groups, a larger hip circumference was associated with lower glucose and triglyceride levels in both sexes, and higher HDL-levels in women only, after adjustment for waist circumference, BMI, and age. Adjustment for height or leptin did not materially change the results.

Discussion

In conclusion, we confirmed the protective association of relative larger hips in four non-Caucasian ethnic groups. Leptin does not appear to play a mediating role in this association.

Introduction

Increasing evidence suggests that waist and hip circumferences have independent and opposite associations with glucose and lipid levels and risk of diabetes and cardiovascular disease ¹⁻⁴. A larger hip circumference has a protective association if waist circumference is taken into account. Both fat and muscle tissue from the legs appear to contribute to this phenomenon and regional differences in fat metabolism may play a role ⁵.

The separate independent associations of waist and hip circumferences with components of the metabolic syndrome have only been investigated in Caucasian populations. Body composition, however, differs by ethnic group. Therefore, we investigated the independent association of hip circumference with components of the metabolic syndrome in several ethnic groups. In addition, we studied whether leptin mediated these associations. Data were analysed from population-based studies previously conducted in the Indian Ocean islands of Mauritius and Rodrigues, and the Pacific Ocean islands of Nauru and Papua New Guinea. Some of these populations are highly susceptible to obesity and type 2 diabetes ⁶.

Methods

Population-based surveys were performed in Mauritius and Rodrigues (islands in the Indian Ocean) in 1992, and Papua New Guinea and Nauru (Pacific Islands) in 1991 and 1987, respectively. These surveys included four different ethnic groups: 1299 Micronesians (age≥20 y), 1250 Melanesians, 4429 Indians and 3266 Creoles (age≥25 y). Creoles are people with a genetic mixture of African, European, and Malagasy ancestry. All surveys were coordinated from a single centre (International Diabetes Institute, Melbourne, Australia). Details of the studies in Mauritius, Papua New Guinea and Nauru have been described previously ⁷⁻⁹, and the same methodology was used in Rodrigues. Weight, height, waist and hip circumferences were measured and BMI and waist-to-hip ratio (WHR) calculated. Fasting glucose and 2-hr glucose after a 75-g oral glucose tolerance test were measured in all subjects not taking diabetes medication. Subjects who reported a history of physician-diagnosed diabetes and who either reported taking hypoglycaemic medication, or who had diabetic glucose levels ¹⁰ were classified as having known diabetes. Fasting triglycerides and HDL-cholesterol were also determined. Blood pressure (BP) was measured after a 5-min rest. Participants reporting taking antihypertensive medication were classified as known hypertensive. Information on lifestyle (physical activity, smoking, and alcohol intake) was obtained by questionnaires administered by trained interviewers. In the Mauritius survey only, serum leptin was analysed using radioimmunoassay kits (Linco, St Louis, MO, USA) and total body fat percentage was assessed by bioelectrical impedance.

Subjects known to have diabetes and/or hypertension were excluded (260 Micronesians (20.0%), 130 Melanesians (10.4%), 662 Indians (14.9%), 491 Creoles (15.0%)). In addition, persons with missing data on any of the studied variables and pregnant women were also excluded (272 Micronesians, 100 Melanesians, 70 Indians, 65 Creoles). Linear regression was used to study the independent associations of waist and hip circumferences with the metabolic variables. Additional adjustments were made for BMI, age, height, leptin, and lifestyle factors.

Triglycerides, HDL-cholesterol and leptin were In-transformed because of their skewed distribution. Interaction by BMI was evaluated by stratified analyses. We considered the stability of the regression models to be disturbed by multi-colinearity if the tolerance was <0.1. The tolerance is a statistic used to determine how much the independent variables are linearly related to one another. It is calculated as 1 minus R squared for an independent variable when it is predicted by

Table 1. Characteristics of the study populations^a

Ethnicity	Micronesian	Melanesian	Indian	Creole
Year	1987	1991	1992	1992
Geographic location	Nauru	Papua New	Mauritius	Mauritius and
		Guinea		Rodrigues
Men				
N	364	436	1737	1317
Age, y	35.7 (10.7)	42.4 (13.7)	43.2 (11.4)	44.2 (12.0)
BMI, kg/m²	34.2 (6.8)	27.0 (4.5)	23.8 (3.7)	24.5 (3.8)
Waist girth, cm	99.3 (13.2)	87.3 (9.6)	85.8 (8.6)	81.8 (10.1)
Hip girth, cm	110.5 (11.8)	98.2 (8.4)	93.7 (6.9)	94.1 (7.1)
WHR	0.90 (0.05)	0.89 (0.05)	0.92 (0.05)	0.87 (0.06)
Fasting glucose, mmol/l	5.94 (1.63)	6.46 (2.70)	5.82 (1.48)	5.84 (1.27)
Postload glucose, mmol/l	6.60 (3.23)	8.59 (4.97)	7.07 (3.53)	6.36 (2.92)
Diastolic BP, mmHg	80.1 (12.7)	75.8 (11.1)	74.8 (11.8)	79.9 (13.2)
Systolic BP, mmHg	130.3 (17.9)	132.1 (16.9)	120.8 (17.2)	131.4 (21.2)
Triglycerides, mmol/l	1.1 (0.8-1.6)	1.0 (0.8-1.5)	1.3 (1.0-2.0)	1.0 (0.7-1.6)
HDL-cholesterol, mmol/l	0.9 (0.8-1.0)	0.9 (0.8-1.1)	1.2 (1.0-1.4)	1.4 (1.2-1.7)
Leptin, ng/ml	-	-	4.6 (3.0-7.2) ^b	3.8 (2.5-5.7) ^b
Body fat, %	-	-	20.6 (6.8) ^c	20.4 (7.0) ^c
Women				
N	403	584	1960	1393
Age, y	35.2 (11.0)	41.3 (14.7)	43.7 (12.1)	44.5 (12.5)
BMI, kg/m²	35.2 (7.7)	26.0 (5.3)	25.0 (4.8)	26.8 (5.2)
Waist girth, cm	94.4 (13.7)	82.0 (10.8)	81.4 (10.6)	80.1 (11.3)
Hip girth, cm	114.0 (13.6)	99.5 (12.3)	96.3 (9.7)	97.8 (9.7)
WHR	0.83 (0.06)	0.82 (0.05)	0.84 (0.06)	0.82 (0.07)
Fasting glucose, mmol/l	5.75 (1.62)	5.82 (2.19)	5.64 (1.49)	5.76 (1.67)
Postload glucose, mmol/l	6.75 (2.96)	8.18 (4.24)	7.36 (3.24)	7.28 (3.28)
Diastolic BP, mmHg	74.1 (11.4)	72.9 (10.2)	71.4 (11.3)	77.8 (13.1)
Systolic BP, mmHg	124.0 (19.8)	127.4 (18.9)	118.1 (17.0)	128.6 (21.9)
Triglycerides, mmol/l	0.8 (0.6-1.1)	0.8 (0.6-1.1)	1.0 (0.8-1.4)	0.9 (0.7-1.3)
HDL-cholesterol, mmol/l	1.0 (0.9-1.1)	1.1 (1.0-1.3)	1.3 (1.1-1.5)	1.4 (1.2-1.7)
Leptin, ng/ml	-	-	17.3 (11.0-25.7) ^b	17.3 (10.9-25.3) ^b
Body fat, %	-	-	34.4 (6.2) ^c	34.5 (5.9) ^c

^a Data are mean (standard deviation) or median (interquartile range)

^b n=1185 Indian men, 1432 Indian women, 410 Creole men and 537 Creole women

^c n= 913 Indian men, 1055 Indian women, 367 Creole men and 435 Creole women

the other independent variables already included in the model. SPSS for Windows (Version 10.0.5, SPSS, Chicago, IL) was used for all statistical analyses.

Results

The characteristics of the four ethnic populations are shown in Table 1.

In univariate models, waist and hip circumferences were positively associated with all the metabolic variables (data not shown). After adjustment for each other, waist and hip circumference had opposite associations with each of the metabolic variables in all ethnic groups, such that a larger hip circumference was associated with a better metabolic profile (data not shown). Table 2 shows these associations additionally adjusted for BMI and age. In all ethnic groups a larger hip circumference was negatively associated with glucose levels, except in Melanesian women. A similar pattern was observed for triglycerides in all groups. Larger hip circumference was associated with better HDL-cholesterol levels in women, but not in men. Only among Creoles were any of the associations between hip circumference and BP significant.

Adjustment for height instead of BMI generally slightly weakened the associations with waist and hip circumferences, but most significant associations remained so (data not shown). There were no clear differences in the association in normal weight (BMI below 25 kg/m²), overweight (BMI between 25 and 30 kg/m²), and obese (BMI above 30 kg/m²) subjects, and the associations did not become weaker of stronger with increasing weight status. The associations did not change after adjustment for In-transformed leptin or lifestyle factors (data not shown). None of the regression models were disturbed by multi-colinearity.

Conclusion

These results show that previously reported independent associations of a larger hip circumference with more favourable glucose and lipid levels in Caucasian populations can be extended to other ethnic groups. There was no strong association of hip circumference with BP, which was also found in previous studies in Caucasians ^{1,11}.

The strength of the associations varied considerably, and they were stronger in the Pacific Islanders, especially in Melanesian participants. Some of the variability in significance levels could be due to the smaller size of the Micro-and Melanesian populations, but this does not affect the strength of the associations.

Several mechanisms have been proposed to explain the association of a larger hip circumference with a favourable metabolic profile ⁴. Firstly, hip circumference reflects muscle mass. Muscle mass is the main target of insulin and the major site of insulin resistance. Lean mass in the legs measured by dual energy X-ray absorptiometry (DXA) has indeed been negatively associated with glucose levels ⁵. Secondly, hip circumference also reflects gluteal fat mass. Recently, several studies found that leg fat (measured by DXA) was associated with a lower cardiovascular risk ^{5,12,13}. Leg fat may possibly protect by acting as a sink for circulating free fatty

Table 2. Associations (standardised betas) of waist and hip circumferences with glucose and lipid concentrations, adjusted for each other, and for age and BMI

	Men		Women		
	Waist	Hip	Waist	Hip	
Micronesian					
Fasting glucose	0.308**	-0.324**	0.342**	-0.243*	
Postload glucose	0.148	-0.201	0.381**	-0.290**	
Diastolic BP	0.480**	0.033	0.371**	0.019	
Systolic BP	0.267*	0.196	0.062	-0.093	
Ln-triglycerides	0.293**	-0.562**	0.422**	-0.131	
Ln-HDL	0.080	0.018	-0.353**	0.428**	
Melanesian					
Fasting glucose	0.540**	-0.375**	0.513**	0.048	
Postload glucose	0.513**	-0.455**	0.489**	-0.060	
Diastolic BP	0.425**	0.023	0.120	-0.059	
Systolic BP	0.025	-0.151	0.333**	-0.183	
Ln-triglycerides	0.504**	-0.346**	0.497**	-0.522**	
Ln-HDL	-0.274*	0.067	-0.209*	0.201	
Indian					
Fasting glucose	0.198**	-0.223**	0.206**	-0.187**	
Postload glucose	0.319**	-0.296**	0.229**	-0.223**	
Diastolic BP	0.280**	0.003	0.167**	-0.030	
Systolic BP	0.248**	-0.034	0.185**	-0.072	
Ln-triglycerides	0.367**	-0.227**	0.311**	-0.128**	
Ln-HDL	-0.084	-0.010	-0.135**	0.116*	
Creole					
Fasting glucose	0.208**	-0.166**	0.171**	-0.218**	
Postload glucose	0.391**	-0.272**	0.252**	-0.277**	
Diastolic BP	0.031	-0.140**	-0.138**	-0.056	
Systolic BP	-0.098*	-0.106*	-0.196**	-0.138**	
Ln-triglycerides	0.709**	-0.169**	0.605**	-0.113**	
Ln-HDL	-0.491**	-0.008	-0.511**	0.142**	

^{*} p < 0.1, ** p < 0.05

acids, thereby lowering the free fatty acid levels in the blood and protecting the liver from high exposure. This would lead to relatively less production of VLDL-cholesterol by the liver, increased insulin clearance and less hepatic steatosis or other ectopic fat storage ^{14,15}.

It has been observed that the relative contribution of gluteal fat and gluteal muscle to the variability of the hip circumference differs between men and women in Caucasians ⁵. Gluteal fat mainly contributed in women, whereas in men both fat and muscle equally contributed. There is no information on the contribution of fat or lean mass to the hip circumference of the ethnic groups included in the present study. Thus we cannot differentiate between the contributions of fat or lean tissue to the benefits of a larger hip circumference in these groups.

Another explanation might be the regional differences in secretion of adipose derived proteins or inflammation factors that influence the metabolism. Although leptin levels are mainly

associated with subcutaneous fat and not with visceral fat ¹⁶, in the present study we show that leptin is probably not an intermediate in the associations that we found. This does not exclude the possibility that other proteins that we did not investigate are involved. Finally, it is possible that the hip circumference is just a marker for subcutaneous fat over the entire body. It possibly refines the waist circumference in predicting the visceral fat depot, and therefore becomes negatively associated.

In summary, a larger hip circumference is associated with a favourable metabolic profile in several ethnic groups. Further research should be undertaken to gain more insight into the underlying mechanism explaining this association. More detailed measures of body composition will be needed in prospective studies that relate changes in body composition to changes in metabolic profile. Regardless of the underlying mechanism, it might be that assessment of increased risk for diabetes and cardiovascular disease should not be based on BMI and waist only, and should also include hip circumference.

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Abstract

Objective

Waist and hip circumferences have been shown to have independent and opposite associations with glucose levels. Waist circumference is positively associated with glucose levels, whereas hip circumference is negatively associated. It is unclear which tissues are involved in the pathophysiological mechanism causing these associations. The main goal was to determine which tissue in the trunk and legs, fat or lean tissue, is associated with measures of glucose metabolism.

Research Design and Methods

In 623 participants of the third examination of the Hoorn Study, whole body dual-energy x-ray absorptiometry was performed to determine fat and lean soft-tissue mass in the trunk and legs. Fasting and 2-hour postload glucose levels after 75-g OGTT were determined. After exclusion of known diabetic patients, cross-sectional analyses were performed in 275 men (140 with normal glucose metabolism, 92 with impaired glucose metabolism, and 43 with diabetes) and 281 women (148 with normal glucose metabolism, 90 with impaired glucose metabolism, and 43 with diabetes), aged 60 to 87 years.

Results

Greater trunk fat mass was associated with higher glucose levels after adjustment for age, trunk lean mass, leg lean mass, and leg fat mass. Standardized betas (95% CI) in men were 0.44 (0.25 – 0.64) for fasting and 0.41 (0.22 – 0.60) for postload glucose. For women, these were 0.49 (0.35 – 0.63) and 0.47 (0.33 – 0.61), respectively. In contrast, in the same regression models, a larger leg fat mass was associated with lower glucose levels. Standardized betas in men were -0.24 (-0.43 – -0.05) and -0.12 (-0.31 – 0.07) and in women -0.24 (-0.37 – -0.10) and -0.27 (-0.40 – 0.13) for fasting and postload glucose, respectively. In these models, larger leg lean mass was also associated with lower glucose levels but was only statistically significant in men.

Conclusions

If trunk fat is taken into account, accumulation of fat in the legs seems to be protective against a disturbed glucose metabolism, particularly in women. Further research is needed to unravel underlying pathophysiological mechanisms.

Introduction

Obesity, particularly abdominal obesity, is associated with increased risk for insulin resistance and type 2 diabetes. The pathophysiological mechanisms involved in the development of these conditions are not completely understood. The idea that an increased release of free fatty acids from visceral fat into the portal vein plays a major role in the development of insulin resistance in abdominal obesity is now widely accepted ¹⁻³. This, however, does not preclude the possibility that other tissues are involved.

In epidemiological studies, abdominal obesity or visceral fat is often estimated by the measurement of waist circumference alone or by measurement of the waist-to-hip circumference ratio (WHR) or waist-to-thigh circumference ratio (WTR). Several studies, however, have shown that the association between WHR or WTR and glucose metabolism or type 2 diabetes was not only due to a larger waist circumference but also due to a smaller hip or thigh circumference ⁴⁻⁸. This could indicate a protective role for a larger hip circumference to high glucose levels. To determine whether this protective effect of a larger hip or thigh circumference is due to a higher lean mass or to a higher fat mass in the gluteal-femoral region, accurate assessment of body composition is necessary.

To investigate how fat and lean tissue are represented by the waist and hip circumferences, we first studied the associations of waist and hip circumferences with fat and lean tissue mass in the trunk and in the legs as measured by dual-energy x-ray absorptiometry (DXA). The main goal of the present study was to investigate the association of fat and lean tissue mass in the trunk and in the legs with glucose metabolism. The study was performed within the third follow-up examination of the Hoorn Study, a population-based cohort study of glucose tolerance.

Research Design and Methods

Study sample

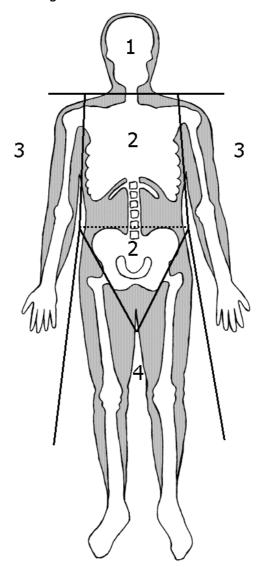
The Hoorn Study is a population-based cohort study of glucose metabolism and its complications that started in 1989 and has been described earlier ⁹. It consisted of 2484 men and women aged 50-75 years at baseline. In 2000-2001, a third examination was carried out among surviving participants who gave their permission to be re-contacted. We invited all participants who had diabetes, as determined by a 75-g oral glucose tolerance test (OGTT) or by diabetes treatment (n=176) at the second examination of the entire cohort in 1996-1998 ¹⁰. We also invited random samples of participants who had normal glucose tolerance (n=705) or impaired glucose tolerance (n=193) in 1996-1998. Of 1074 individuals invited, 648 persons (60.3 %) participated. The main reasons for not participating in the 2000-2001 follow-up examination were lack of interest (30%) or co-morbidity (23%). Other reasons were advanced age (7%), unwillingness to travel (6%), participation considered too time-consuming (6%), and miscellaneous reasons (15%); 13% gave no reason for refusal to participate in the follow-up examination.

For the present study, cross-sectional data of the 2000-2001 follow-up examination were analysed. Of the 648 participants, 25 individuals were excluded because of missing DXA data. Another 8 persons had incomplete glucose tolerance data. Participants already known to have

diabetes (n=59) were also excluded from the statistical analyses because treatment could potentially influence the relations under consideration. Therefore, our final study sample consisted of 556 persons (275 men and 281 women). The study protocol was approved by the Ethical Review Committee of the VU University Medical Center, and all participants gave their written informed consent.

Body composition

A whole body dual-energy x-ray absorptiometry (DXA) scan was performed using the fan beam technology (QDR-2000, software version 7.20D, Hologic, Brussel, Belgium). The software provides estimates of lean tissue mass, fat mass and bone mineral mass for the total body and for standard body regions. With the use of specific anatomic landmarks, regions of the head, trunk, arms and legs were distinguished as shown in Figure 1. In the analyses, we used fat and lean soft-tissue mass from the trunk and from the legs.



Legend

- 1 = head
- 2 = trunk
- 3 = arms
- 4 = legs

Figure 1. Standard regions of a dual-energy x-ray absorptiometry scan

Anthropometry and lifestyle

Weight and height were measured in barefoot persons wearing light clothes only. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest, and the hip circumference at the widest level over the greater trochanters. Waist-to-hip circumference ratio (WHR) was calculated as waist circumference divided by hip circumference. Self-reported information on physical activity (min/week), alcohol intake (g/day) and smoking (yes/no) was obtained by questionnaires.

Glucose metabolism

All participants underwent a single 75-g oral glucose tolerance test (OGTT). Fasting glucose and 2-hour postload glucose after OGTT were measured in plasma with the hexokinase-method (Roche Diagnostics GmbH, Mannheim, Germany). Glucose tolerance status was defined according to the World Health Organisation (WHO) criteria ¹¹. Subjects were classified having a normal glucose metabolism, an impaired glucose metabolism (i.e. impaired fasting glucose or impaired glucose tolerance), or diabetes mellitus. Fasting insulin was measured in plasma by a two site immunoradiometric test (Medgenix, Diagnostics, Fleurus, Belgium) and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated ¹².

Statistical analyses

All analyses were performed separately for men and women because of the statistically significant effect modification by sex of the relations under consideration. To test for linear trend in study sample characteristics over categories of glucose metabolism, a linear regression model was used, with glucose tolerance category as a linear explanatory variable. Values are expressed as means \pm SD for normally distributed variables and as medians (interquartile range) in case of skewed distribution.

To study the association of the regional fat and lean mass measured by DXA (independent variables) with the waist and hip circumferences by anthropometry (dependent variables), multiple regression analyses were performed. The associations of the regional fat and lean tissue mass with the more commonly used WHR and BMI were also investigated. Effect modification by sex, glucose tolerance status, and age was evaluated by adding product terms to the model.

The contribution of regional fat and lean tissue mass to fasting glucose, postload glucose or (Intransformed) HOMA-IR was tested using multiple regression analyses with adjustment for age. Residual plots were examined to check for violation of the normality assumptions. Effect modification by sex and glucose metabolism status was evaluated by adding product terms to the model. To facilitate direct comparisons, results of the regression analyses are reported as standardized betas. A standardized beta of 0.1 indicates that when the independent variable increases by 1 SD, the dependent variable increases by 0.1 SD. We considered the stability of the regression models to be disturbed by multi-colinearity if the tolerance was <0.1. The tolerance is a statistic used to determine how much the independent variables are linearly related to one another. It is calculated as 1 minus R squared for an independent variable when it is predicted by

Table 1. Characteristics of the study population by sex and glucose tolerance status^a

		Men				Women		
•	NGM	IGM	MQ	p _q d	NGM	IGM	DM	p _p
Z	140	92	43		148	06	43	
Age (years)	69.1 ± 6.0	69.3 ± 6.5	70.5 ± 6.4	0.26	68.3 ± 6.1	70.8 ± 6.2	73.2 ± 7.2	0.00
Fasting glucose (mmol/l)	5.46 ± 0.36	6.08 ± 0.48	7.38 ± 1.06	0.00	5.39 ± 0.37	6.08 ± 0.48	7.09 ± 1.12	0.00
Postload glucose (mmol/l)	5.45 ± 1.16	7.90 ± 0.86	10.84 ± 2.55	0.00	5.78 ± 1.12	8.13 ± 1.49	11.94 ± 2.48	0.00
Insulin (pmol/I)	47 (36 – 63)	61 (46 - 78)	79 (47 – 110)	0.00	45 (35 – 56)	75 (55 – 94)	82 (61 – 102)	0.00
HOMA-IR	1.60	2.30	3.57	0.00	1.51	2.72	3.17	0.00
	(1.19 - 2.19)	(1.65 - 2.98)	(2.07 - 5.11)		(1.16 - 1.89)	(2.12 - 3.51)	(2.35 - 5.34)	
Anthropometry								
BMI (m/kg²)	26.2 ± 3.3	27.1 ± 3.1	27.9 ± 4.1	0.00	26.2 ± 3.4	28.9 ± 4.6	29.3 ± 4.7	0.00
Waist circumference (cm)	96.3 ± 9.5	99.7 ± 9.4	102.1 ± 11.1	0.00	86.0 ± 9.8	95.1 ± 11.0	96.6 ± 12.5	0.00
Hip circumference (cm)	100.0 ± 6.2	101.0 ± 6.8	102.0 ± 6.7	0.08	102.3 ± 7.3	106.8 ± 10.0	107.9 ± 11.3	0.00
WHR	0.96 ± 0.06	90.0 ± 66.0	1.00 ± 0.07	0.00	0.84 ± 0.07	0.89 ± 0.07	0.90 ± 0.08	0.00
Body composition								
Total body fat percentage (%)	26.1 ± 6.5	29.4 ± 5.7	29.9 ± 6.1	0.00	39.9 ± 6.7	43.3 ± 5.9	43.8 ± 6.6	0.00
Total body fat (g)	21161 ± 7346	24780 ± 8305	25081 ± 7830	0.00	27880 ± 8117	33511 ± 9858	33725 ± 9919	0.00
Trunk fat mass (g)	11109 ± 4799	13647 ± 5580	14103 ± 5249	0.00	12694 ± 4701	16480 ± 5217	17322 ± 6099	0.00
Trunk lean mass (g)	27888 ± 3132	27669 ± 2880	27969 ± 3270	0.95	19881 ± 2147	20708 ± 2409	20477 ± 2429	0.03
Leg fat mass (g)	6199 ± 2020	6902 ± 2242	6762 ± 1991	0.03	10421 ± 3079	11396 ± 4173	10827 ± 3283	0.19
Leg lean mass (g)	17640 ± 2506	17423 ± 2203	17034 ± 2711	0.16	12258 ± 1694	12858 ± 1987	12620 ± 2122	0.07
				•			11.	

NGM, normal glucose metabolism; IGM, impaired glucose metabolism; DM, newly detected type 2 diabetes mellitus (WHO criteria 11)

^a Data are presented as mean ± standard deviation, for insulin and HOMA-IR the median (interquartile-range) is reported

 $^{^{}b}$ p = p for trend

the other independent variables already included in the model. All statistical analyses were performed using SPSS for Windows (version 10.1; SPSS, Chicago, IL).

Results

The characteristics of the study sample by sex and glucose metabolism status is shown in Table 1. All anthropometric measures in both sexes, and age in women, were significantly and positively associated with a worse glucose metabolism when tested for linear trend, except for hip circumference in men. Of the DXA measurements, the fat mass in the trunk as well as in the legs in men, and the fat and lean mass in the trunk in women, were significantly and positively associated with a worse glucose metabolism. After adjustment for age, the trunk fat mass and the leg fat mass remained positively associated with a worse glucose metabolism status in men. In women, besides trunk fat and lean mass, leg lean mass was also positively associated with a worse glucose metabolism status after adjustment for age (data not shown).

Anthropometry

The associations of fat and lean mass in the trunk and legs with anthropometric measures are shown in Table 2. Waist circumference was strongly positively associated with trunk fat, whereas hip circumference was positively associated with both leg fat and leg lean mass. WHR was most strongly and positively associated with trunk fat mass and was negatively associated with both

Table 2. Independent associations of trunk fat and lean mass with the waist circumference, of leg fat and lean mass with the hip circumference, and of fat and lean mass in the trunk and legs with the WHR and with the BMI

			Men	1	Women
Dependent variable	Independent	В	95% CI	В	95% CI
	variables				
Waist circumference	Trunk fat	0.81**	0.76 - 0.87	0.82**	0.77 - 0.87
	Trunk lean	0.23**	0.18 - 0.29	0.19**	0.14 - 0.25
Hip circumference	Leg fat	0.68**	0.62 - 0.74	0.67**	0.59 - 0.74
	Leg lean	0.41**	0.35 - 0.53	0.24**	0.17 - 0.32
WHR	Trunk fat	0.78**	0.63 - 0.93	0.69**	0.60 - 0.81
	Trunk lean	0.31**	0.14 - 0.48	0.21*	0.05 - 0.37
	Leg fat	-0.22*	-0.270.07	-0.42**	-0.540.29
	Leg lean	-0.40**	-0.56 – -0.23	-0.06	-0.23 - 0.10
BMI	Trunk fat	0.57**	0.48 - 0.66	0.67**	0.61 - 0.73
	Trunk lean	0.19**	0.09 - 0.29	0.02	-0.06 - 0.10
	Leg fat	0.24**	0.14 - 0.33	0.28**	0.22 - 0.34
	Leg lean	0.09	-0.01 - 0.19	0.08*	0.00 - 0.16

B, standardised beta; CI, confidence interval; WHR, waist-to-hip ratio; BMI, body mass index

^{*}p < 0.05, **p < 0.001

lean and fat mass in the legs. In contrast to the WHR, BMI was mainly associated with fat mass, especially trunk fat. When age was added to these models, the associations did not change substantially (data not shown). There was no significant effect modification by glucose tolerance status or age.

Glucose metabolism

Table 3 shows the results of multiple linear regression analyses involving fat and lean tissue mass in the trunk and legs in relation to fasting and postload glucose levels and HOMA-IR. After adjustment for age, leg lean and leg fat mass, and each other, larger trunk fat mass as well as larger trunk lean mass were associated with higher fasting glucose (model 1). However, in the same regression model, larger leg fat and leg lean mass were associated with lower fasting glucose. In both sexes only larger trunk fat mass, and not trunk lean mass, was significantly associated with higher postload glucose levels (model 2). In contrast, larger leg fat mass was associated with lower postload glucose, but was only statistically significant in women. Similar results were found for In-transformed HOMA-IR (model 3). Trunk fat mass was the strongest independent predictor of glucose levels and HOMA-IR. Adjustment for height did not change the associations (data not shown). In addition, adjustment for physical activity, alcohol intake, and smoking did not change the results (data not shown). There was no statistically significant effect modification by glucose tolerance status, except that the associations of trunk fat and trunk lean mass with fasting glucose was stronger in diabetic subjects (data not shown). Among the four

Table 3. Independent associations of trunk fat, trunk lean, leg fat, and leg lean mass (adjusted for each other) with fasting glucose levels (model 1), with postload glucose levels (model 2), and with HOMA-IR (model 3), additionally adjusted for age

	<u> </u>	Men			Women	
	В	95% CI	р	В	95% CI	р
Model 1: fasting glucose						
Trunk fat mass	0.44	0.25 - 0.64	0.00	0.49	0.35 - 0.63	0.00
Trunk lean mass	0.26	0.05 - 0.46	0.02	0.27	0.09 - 0.45	0.00
Leg fat mass	-0.24	-0.430.05	0.01	-0.24	-0.370.10	0.00
Leg lean mass	-0.27	-0.480.06	0.01	-0.15	-0.34 - 0.03	0.11
Model 2: postload glucose						
Trunk fat mass	0.41	0.22 - 0.60	0.00	0.47	0.33 - 0.61	0.00
Trunk lean mass	-0.02	-0.22 - 0.19	0.89	0.08	-0.10 - 0.25	0.38
Leg fat mass	-0.12	-0.31 - 0.07	0.23	-0.27	-0.400.13	0.00
Leg lean mass	-0.12	-0.33 - 0.10	0.28	-0.04	-0.22 - 0.14	0.69
Model 3: In-HOMA-IR						
Trunk fat mass	0.59	0.41 - 0.76	0.00	0.65	0.52 - 0.77	0.00
Trunk lean mass	0.16	-0.03 - 0.36	0.10	0.19	0.03 - 0.34	0.02
Leg fat mass	-0.18	-0.35 - 0.00	0.05	-0.19	-0.300.06	0.00
Leg lean mass	-0.18	-0.38 - 0.01	0.07	0.00	-0.16 - 0.16	0.98

B, standardised beta; CI, confidence interval

independent variables used in the linear regression models, the highest correlation (Pearson) was between lean mass in the trunk and lean mass in the legs (0.825 in men and 0.815 in women). The stability of the regression models, however, was not disturbed by multi-colinearity. Residual plots were not disturbed, and similar results were found when glucose levels were In-transformed and included as study outcome (data not shown).

Conclusions

In this study, we show that waist or hip circumference does not simply represent the amount of fat at that region. Although the waist circumference is mainly associated with fat mass in the trunk, the hip circumference is strongly associated with both fat mass and lean mass in the legs, particularly in men. Furthermore, our study shows that, as expected, larger trunk fat mass is associated with higher glucose levels. In contrast, accumulation of fat in the legs was associated with lower glucose levels. In men, large lean mass in the legs seems to have an additional independent protective effect.

The recognition of the importance of upper body versus lower body obesity in diabetes and cardiovascular disease by Vague in 1956 led to the development of the WHR and WTR measurements ¹³. Since it was found in the 1980s that the WHR was more closely associated with intra-abdominal fat than with subcutaneous fat, associations of WHR with disease have generally been interpreted as caused by the increased accumulation of visceral fat ^{14,15}. Whether WHR also represents other body tissues was unclear. Data on direct comparison between anthropometrically measured circumferences, particularly hip circumference and WHR, and regional body composition, including both fat and lean tissue, are limited, and little is known about the influence of sex and age on this relationship. Only one previous study in 24 men showed a negative association between WHR and the thigh muscle area by computed tomography, suggesting that high WHR may also result from low muscle mass in the legs ¹⁶. In our study we show that the waist and hip circumferences are not only associated with fat mass at the respective body regions. The hip circumference, a surrogate marker of lower body obesity, was also strongly associated with leg lean mass, particularly in men. WHR was positively associated with trunk fat mass but also negatively associated with both lean and fat mass at the legs.

Because our study showed that the anthropometric measures seemed to represent different tissues, we examined which tissue would be responsible for the earlier observed associations between anthropometric measures and glucose tolerance. Larger waist but smaller hip circumference has been associated with worse glucose tolerance status or diabetes in several studies ⁴⁻⁸. From the present study, we can conclude that, particularly in the trunk, fat tissue is associated with unfavourable glucose metabolism. This could be consistent with the established role of visceral fat, which releases free fatty acids (FFA) directly into the portal vein, causing disturbances in glucose metabolism ¹⁻³. Some studies, however, found that subcutaneous abdominal fat is more closely related to insulin resistance than is visceral fat ^{17,18}.

In contrast to fat mass in the trunk, fat mass in the legs was negatively associated with glucose levels and HOMA-IR. These data are consistent with recently published data on the contributions of regional fat mass by DXA to several cardiovascular risk factors in women, such as

insulin resistance and dyslipidemia 19,20 . It has been suggested that fat tissue in the gluteal or femoral region plays a protective role, because adipocytes in these regions are less sensitive to lipolytic stimuli 21,22 . Therefore, these regions are more likely to take up FFA from the circulation and are less likely to release them readily (so called 'FFA trapping') 23 . Hereby increased peripheral fat stores may protect other organs, such as the liver and the pancreas, as well as skeletal muscle from high FFA exposure. Accumulation of fat in the liver has been found to be positively associated with insulin resistance 24,25 , and infiltrating muscle fat in the thigh, which only accounted for $\approx 3\%$ of total thigh fat, was also positively associated with insulin resistance 26 . Recent work also points to a possible role of lipids in beta cell deterioration ("lipotoxicity") associated with type 2 diabetes 27 . The ectopic fat storage hypothesis is also supported by the observation that adipose tissue deficiency is accompanied by ectopic fat storage and related to insulin resistance and diabetes 28,29 . Furthermore, transplantation of adipose tissue back in lipoatrophic animals reverses the elevated glucose levels and leptin-replacement therapy in women with lipodystrophy improved glycaemic control 30,31 .

Adipose tissue is an endocrine gland that secretes many proteins. Examples include adiponectin, leptin, resistin, angiotensinogen, interleukin-6, tumor necrosis factor-alpha, adipsin, plasminogen activator inhibitor-1 and probably many other, yet unknown factors. Regional differences in secretion of these factors could also be an alternative or additional explanation for the observed relationships between adipose tissue and glucose levels. There are some known differences in secretion between abdominal subcutaneous and visceral fat ³²⁻³⁴, but less is known about differences between gluteal and abdominal subcutaneous fat.

In men, a significant negative association was found between glucose and HOMA-IR levels and lean mass in the legs, probably because lean mass in the legs measured by DXA reflects mainly skeletal muscle tissue and muscle tissue is one of the sites of insulin resistance and the main target of insulin. In women, this negative association with lean tissue was also found, but was not statistically significant. The lean mass in the trunk was not negatively associated with glucose levels; it was even positively associated with fasting glucose levels. However, these results should be interpreted with caution, because lean mass in the trunk as measured by DXA reflects not only muscle mass but also visceral organ mass (e.g., liver and intestinal organs).

The main limitation of our study is that DXA does not allow separate quantification of intermuscular and subcutaneous fat in the legs and visceral fat and subcutaneous fat in the trunk. The contribution of subcutaneous fat to the total amount of fat in the legs, however, is relatively large (\pm 90%) ²⁶. Therefore, the associations found in our study with fat mass in the legs are probably mainly due to the subcutaneous fat depot. As subcutaneous fat accounts for the largest fat component in the trunk, an important role of subcutaneous trunk fat apart from the visceral fat cannot be excluded. Another possible limitation of DXA is that, particularly in women, gluteal fat and abdominal fat cannot be perfectly distinguished. Possibly, part of the gluteal fat was included in the trunk region and part of the abdominal fat in the leg region. Therefore, we may have underestimated the true associations. Finally, because of the selection of the study population, subjects with an impaired glucose metabolism and diabetes were relatively over-represented. Adjustment for glucose tolerance status, however, did not change the conclusions.

In summary, caution is needed when interpreting waist and hip circumferences. Whereas larger waist circumference mainly reflects higher fat mass in the trunk, the hip circumference and WHR are influenced by both fat and lean mass from the legs. We also conclude that although higher fat mass in the trunk was the strongest determinant of disturbed glucose metabolism, larger fat mass in the legs and larger lean mass in men only have a considerable and opposite association with glucose metabolism. These findings provide a relevant new insight in the association between obesity, body composition, and type 2 diabetes. Further investigation of the underlying pathophysiological mechanism is needed to explain the negative association of leg fat with glucose metabolism.

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Regional body composition as a determinant of arterial stiffness in the elderly. The Hoorn Study

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submitted

Abstract

Objective

To estimate the relation of precisely measured regional body composition with peripheral and central arterial stiffness in the elderly.

Methods

We investigated 648 participants of the Hoorn Study, a population-based cohort study (mean age 69.0 ± 6.0 years). Trunk fat, leg fat, trunk lean and leg lean mass were distinguished by dual-energy x-ray absorptiometry. We used ultrasound to measure the distensibility and compliance of the carotid, femoral and brachial arteries, and carotid Young's elastic modulus, as estimates of peripheral stiffness. As estimates of central stiffness we measured carotid-femoral transit-time, aortic augmentation index and systemic arterial compliance.

Results

After adjustment for sex, age, height, mean arterial pressure, leg lean and leg fat mass, a larger trunk fat mass was consistently associated with higher peripheral arterial stiffness (standardized beta (β) of mean Z-scores of all three large arteries -0.24, p<0.001). In contrast, larger leg fat mass (β = 0.15, p=0.009) and leg lean mass (β = 0.09, p=0.20) were associated with lower peripheral arterial stiffness. Trunk or leg fat mass were not associated with central arterial stiffness. Leg lean mass, however, was consistently associated with lower central arterial stiffness (β = 0.29, p<0.001).

Conclusions

Trunk fat mass may have adverse effects on peripheral, but not on central arterial stiffness, while leg fat may have a protective effect. Larger leg lean mass was the most important determinant of lower central arterial stiffness. These results provide a pathophysiological framework to explain not only the higher cardiovascular risk in individuals with larger trunk fat mass, but also the reduced cardiovascular risk in individuals with larger leg lean and fat mass.

Introduction

Obesity, and in particular abdominal fat accumulation, is an independent risk factor for cardiovascular disease ^{1,2}. In contrast, peripheral fat and muscle may independently contribute to a *lower* risk for cardiovascular disease ³⁻⁹. The mechanisms underlying these contrasting associations are not completely understood.

Increased arterial stiffness may represent a pathway through which obesity may lead to cardiovascular disease. Arterial stiffening impairs the ability of the arterial system to handle the pressure boost at systole, which leads to increased systolic blood pressure, decreased diastolic blood pressure, increased left ventricular mass, and decreased diastolic coronary perfusion ¹⁰. Arterial stiffness is known to increase with aging, hypertension, and with deteriorating glucose tolerance status. Nevertheless, arterial stiffness is not uniform along the arterial tree, and depends on the type of artery (e.g. elastic versus muscular). The response to aging and other risk factors is also different along the arterial tree ¹¹⁻¹⁵.

Several studies have considered obesity or fat distribution as determinants of arterial stiffness ¹⁶⁻²⁴. The results, however, have been inconsistent, which may be due to the limited number of subjects ^{16-19,21,24}, or because stiffness has been estimated in only one specific artery ^{16,18-21,23,24}. In addition, usually only anthropometric measures ^{16,18,22,24} or bio-impedance ²⁰ have been used to assess obesity or body composition, which are relatively inaccurate methods, in particular in obese and elderly individuals ²⁵⁻²⁷.

We hypothesized that abdominal fat may be associated with higher arterial stiffness, and that, in contrast, peripheral fat and lean mass may be associated with lower arterial stiffness. To systematically investigate this, we examined data of a large, population-based study. Body composition was estimated by dual-energy x-ray absorptiometry (DXA), which enables the distinction of central and peripheral fat and lean mass ⁹. Arterial stiffness was estimated from the distensibility and compliance of three large (carotid, femoral and brachial) arteries (as estimates of peripheral arterial stiffness), as well as from carotid-femoral transit-time, aortic augmentation index and systemic arterial compliance (as estimates of central arterial stiffness).

Methods

Subjects

The Hoorn Study is a population-based cohort study of glucose metabolism and its complications, which started in 1989 28 . It consisted of 2484 men and women aged 50-75 years at baseline. In 2000-2001, a third examination was carried out among surviving participants who gave their permission to be re-contacted. We invited all participants who had diabetes, as determined by a 75-g oral glucose tolerance test (OGTT) or by diabetes treatment (n=176), at the second examination of the entire cohort in 1996-1998 29 . We also invited a random sample of participants who had normal glucose tolerance (n=705) or impaired glucose tolerance (n=193) in 1996-1998. Of 1074 individuals invited, 648 (60.3 %) persons participated. The main reasons for not participating in the 2000-2001 follow-up examination were lack of interest (30%) or co-morbidity (23%). Other reasons were high age (7%), unwillingness to travel (6%), participation considered

too time-consuming (6%), and miscellaneous reasons (15%), while 13% gave no reason. For the present study cross-sectional data of this examination were analysed. The Ethical Review Committee of the VU University Medical Center approved the study protocol and all participants gave their written informed consent.

Body composition

Total body fat percent, and fat and lean soft-tissue mass of the trunk and legs were determined by whole body dual-energy x-ray absorptiometry (QDR-2000, software version 7.20D, Hologic, Brussel, Belgium), as previously described⁹. All DXA scans were performed and read by one investigator.

Peripheral arterial stiffness 12

Briefly, we obtained the diameter (D) and distension (ΔD) of the right common carotid, the right common femoral and the right brachial arteries, and the intima-media thickness (IMT) of the right carotid artery by ultrasound. All ultrasound measurements were performed by a single sonographist. Brachial systolic and diastolic pressures were assessed in the left upper arm. Brachial pulse pressure (PP) and brachial mean arterial pressure (MAP) were calculated. PP at the carotid and femoral arteries was calculated by the distension waveform calibration method, which is more accurate than using brachial PP 30,31 .

Distensibility (DC) and compliance (CC) coefficients were calculated from D, ΔD and PP 32 . Distensibility reflects the elastic properties of an artery whereas the compliance reflects the buffering capacity of the artery. From carotid IMT, D and DC, we calculated Young's elastic modulus (E), an estimate of the intrinsic elastic properties of the vessel wall.

Central arterial stiffness 15

The carotid-femoral transit time (TT) is the travelling time of a pressure wave from the common carotid to the femoral artery, a measure of the aortic (thoracic-abdominal) compliance. It is closely related to the carotid-femoral pulse wave velocity ^{33,34}, as measured by the length of the carotid-femoral arterial segment divided by carotid-femoral TT. However, as non-invasive measurement of this length may introduce error, in particular in obese ³² and older patients ³⁴, we chose to use the carotid-femoral TT, and adjust for height in the statistical analyses. We determined the carotid-femoral TT by continuous measurement of the diameter (distension curves) of the right carotid artery and the right femoral artery ¹². We then determined the average time delay (mean of 3 recordings of 4 seconds per artery) from the ECG trigger to 10% of the ascending slope of the distension curve and subtracted the carotid value from the femoral value to obtain the femoral-carotid TT ³⁵.

Radial applanation tonometry was used to obtain the aortic augmentation index (AI), and was performed with a piezo-resistive pressure transducer (Millar SPT-301, Millar Instruments Inc. Houston Texas, USA) connected to an arterial waveform analysis device (Sphygmocor, AtCor Medical Ltd., Moreton-in-March, UK). The AI represents the extra pressure boost with which the left ventricle must cope due to (early) wave reflection. The AI was calculated as augmented pressure divided by (tonometrically derived) central pulse pressure.

Systemic arterial compliance reflects the overall buffering capacity of the arterial system, but mainly of the proximal aorta ^{33,36}. Systemic arterial compliance (SAC) in ml/mmHg was determined according to two methods. The first method (SAC1) was the time-decay method based on the Windkessel model ³⁷ and used data obtained by applanation tonometry (see above). The second method used the ratio of stroke volume to aortic pulse pressure to estimate systemic arterial compliance (SAC2) ³⁸. Here we chose to estimate the aortic pulse pressure by calibration of carotid pulse pressure ¹², because studies have suggested that this may be the most accurate estimate ^{31,39,40}, and data on this estimate were available for a larger number of persons in our study. Stroke volume was calculated as the ratio of cardiac output and heart rate. Cardiac output (ml/s) was measured by pulse wave doppler echocardiography (3.5 MHz tranducer, HP 5500, Massachusetts, USA) of the left ventricular outflow tract.

Additional measurements

We determined fasting glucose, insulin, postload glucose after a 75-g OGTT, high-density (HDL) and low-density (LDL) lipoprotein cholesterol, triglycerides, serum creatinine, body mass index (BMI), waist circumference, and prior cardiovascular disease as described elsewhere ^{9,41,42}. We obtained self-reported information on health status, medical history, current medication use, physical activity (min/week), alcohol intake (g/day), macronutrient intake (energy %) and current smoking (yes/no) by questionnaires.

Statistical methods

Multiple linear regression analyses were performed to investigate the association between body composition (determinants) and estimates of arterial stiffness (outcomes). First we considered trunk fat, trunk lean, leg fat and leg lean mass together as central determinants of peripheral and central arterial stiffness, adjusted for age, gender, height and MAP. In a second model we additionally adjusted for glucose tolerance status. Next, we adjusted for other potential confounders by adding these variables to the regression models. Effect modification by gender was tested by adding product terms to the models. Effect modification was considered statistically significant if p<0.05. We considered the stability of the regression models to be disturbed by multicolinearity if the tolerance was <0.1. Standardized betas are reported. A standardized beta of 0.1 indicates that when the independent variable increases by 1 SD, the dependent variable increases by 0.1 SD.

A summarizing peripheral stiffness variable was constructed by means of Z-scores. We calculated (sex-specific) Z-scores for each peripheral stiffness measure (DC, CC and E) of each artery, and multiplied the Z-score of E by -1. A Z-score is calculated as the individual value minus the mean value in the study population, divided by the standard deviation. We then performed regression analyses using the mean of the seven Z-scores as dependent variable. Similarly, we constructed a summarizing score for central stiffness measures (AI, SAC1 and SAC2). The Z-scores were multiplied by -1, except for AI. Because carotid-femoral transit-time was available in fewer persons, we did not include this measure in the mean Z-score for central arterial stiffness. All statistical analyses were performed using SPSS for Windows (version 10.1.0; Chicago, IL).

Table 1. Characteristics of the study population

	Men (n=244)	Women (n=240)	Р
Age (years)	69.1 ± 5.9	69.0 ± 6.3	0.98
Anthropometry			
Height (cm)	175.9 ± 6.1	163.5 ± 6.4	< 0.01
Weight (kg)	82.1 ± 10.1	70.4 ± 9.7	< 0.01
BMI (kg/m2)	26.5 ± 3.0	26.3 ± 3.2	0.48
Waist circumference (cm)	97.6 ± 9.1	87.9 ± 10.0	< 0.01
DXA			
Total body fat percent (%)	27.5 ± 5.9	40.0 ± 6.1	< 0.01
Total fat mass (kg)	22.5 ± 6.7	28.2 ± 7.4	< 0.01
Total lean mass (kg)	55.3 ± 5.8	39.2 ± 4.5	< 0.01
Trunk fat mass (kg)	12.2 ± 4.6	13.4 ± 4.7	< 0.01
Trunk lean mass (kg)	27.9 ± 2.8	20.1 ± 2.3	< 0.01
Leg fat mass (kg)	6.3 ± 1.8	9.9 ± 2.8	< 0.01
Leg lean mass (kg)	17.5 ± 2.3	12.4 ± 1.8	< 0.01
Metabolic variables			
Systolic blood pressure (mmHg)	138.9 ± 16.6	142.6 ± 21.1	0.03
Diastolic blood pressure (mmHg)	77.1 ± 8.2	74.9 ± 9.3	< 0.01
Pulse pressure (mmHg)	61.8 ± 12.4	67.7 ± 16.3	< 0.01
Mean arterial pressure (mmHg)	97.7 ± 10.2	97.5 ± 12.1	0.84
Hypertension (%)	64.3	65.3	0.83
LDL-cholesterol (mmol/L)	3.50 ± 0.85	3.87 ± 0.92	< 0.01
HDL-cholesterol (mmol/L)	1.26 ± 0.31	1.62 ± 0.43	< 0.01
Triglycerides (mmol/L)	1.3(1.0 - 1.7)	1.2 (0.9 – 1.7)	0.40
Fasting glucose (mmol/L)	6.15 ± 1.21	6.09 ± 1.44	0.64
Postload glucose (mmol/L) ^a	6.92 ± 2.59	7.14 ± 2.46	0.36
Fasting insulin (pmol/L)	57.0 (40.3 – 75.0)	53.0 (39.0 – 79.5)	0.76
Glycated haemoglobin (%)	5.91 ± 0.72	6.01 ± 0.67	0.15
IGM (%)	28.8	24.5	0.28
DM (%)	22.2	21.9	0.94
Prior cardiovascular disease (%)	44.3	44.0	0.95
(Micro-) albuminuria (%)	16.8	8.8	< 0.01
Serum creatinine (µmol/l)	130.3 ± 17.1	86.8 ± 9.4	< 0.01
Lifestyle			
Current smokers (% yes)	18.0	12.5	0.09
Physical activity (min/week)	1110 (609 – 1300)	1328 (840 – 2040)	0.02
Alcohol drinker (%)	97.9	91.5	< 0.01
Alcohol intake (g/day)	12.7 (3.7 – 28.7)	4.2 (0.7 – 12.8)	< 0.01
Fat intake (% energy intake)	35.2 ± 5.6	34.0 ± 5.7	0.03
Carbohydrate intake (% energy intake)	44.1 ± 6.3	46.6 ± 6.5	< 0.01

Data are presented as mean \pm SD, percentage, or median (interquartile range)

^a Postload glucose was determined in 216 men and 211 women

Results

Table 1 shows characteristics of the study population. Of the 648 participants, 25 persons were excluded because of missing DXA data. Another 139 participants did not take part in the ultrasound examination and were also excluded from all analyses. The main reason for missing ultrasound data was poor definition of the arterial wall due to obesity ¹².

Table 2 shows estimates of peripheral and central arterial stiffness of the study population. Data on central arterial stiffness were mainly missing due to device availability. Nevertheless, subjects with missing data were statistically significantly older, had higher BMI and total fat percentage, and were more likely to have diabetes (data not shown).

Table 2. Estimates of peripheral and central arterial stiffness (mean \pm SD)

	Men	Women	Р
Peripheral arterial stiffness ^a			
Carotid artery			
Distensibility coefficient (10 ⁻³ /kPa)	12.30 ± 4.47	11.42 ± 4.55	0.03
Compliance coefficient (mm²/kPa)	0.65 ± 0.25	0.48 ± 0.18	< 0.01
Young's elastic modulus (kPa)	0.92 ± 0.49	0.98 ± 0.57	0.28
Distension (µm)	373 ± 123	331 ± 92	< 0.01
Diameter (mm)	8.35 ± 1.10	7.48 ± 0.97	< 0.01
Pulse pressure (mmHg)	58.7 ± 13.3	65.1 ± 17.9	< 0.01
Intima-media thickness (mm)	0.88 ± 0.18	0.83 ± 0.15	< 0.01
Femoral artery			
Distensibility coefficient (10 ⁻³ /kPa)	4.67 ± 2.08	4.86 ± 2.22	0.32
Compliance coefficient (mm²/kPa)	0.42 ± 0.21	0.35 ± 0.18	< 0.01
Distension (µm)	207 ± 75	209 ± 74	0.78
Diameter (mm)	10.76 ± 1.87	9.51 ± 1.24	< 0.01
Pulse pressure (mmHg)	66.6 ± 14.0	74.3 ± 20.4	< 0.01
Brachial artery			
Distensibility coefficient (10 ⁻³ /kPa)	7.73 ± 3.89	8.09 ± 4.50	0.35
Compliance coefficient (mm²/kPa)	0.15 ± 0.07	0.11 ± 0.06	< 0.01
Distension (µm)	152 ± 69	142 ± 66	0.10
Diameter (mm)	5.04 ± 0.62	4.23 ± 0.59	< 0.01
Pulse pressure (mmHg)	61.8 ± 12.4	67.7 ± 16.3	< 0.01
Central arterial stiffness			
Carotid-femoral transit time (ms) ^b	55.8 ± 16.8	52.0 ± 16.1	0.08
Aortic augmentation index (%) ^c	144.9 ± 19.1	156.5 ± 18.5	< 0.01
Systemic arterial compliance (ml/mmHg) ^d	0.83 ± 0.33	0.64 ± 0.25	< 0.01
Systemic arterial compliance (ml/mmHg) ^e	1.19 ± 0.34	0.93 ± 0.30	< 0.01

a 244 men and 240 women

^b 114 men and 121 women

^c 272 men and 270 women

^d 238 men and 253 women, estimated by time-decay method

^e 229 men and 245 women, estimated by stroke volume-to-pulse pressure ratio

Associations of body composition with peripheral arterial stiffness (Table 3)

After adjustment for the other body composition variables, trunk fat mass was consistently associated with larger arterial stiffness as estimated from DC, CC and E (Model 1). Addition of trunk lean mass to this model did not change the results of the other variables, and because of the strong correlation between trunk lean and leg lean mass (Pearson's r=0.93), the model including trunk lean mass became disturbed by multi-colinearity. Therefore, Model 1 is shown without adjustment for trunk lean mass. In contrast to trunk fat mass, larger leg fat mass was associated with *lower* femoral stiffness. Larger leg lean mass was also associated with higher compliance in the femoral and brachial arteries. Associations with fat mass were generally stronger in men than in women, and associations with lean mass were stronger in women, but there was no statistically significant effect modification by gender, except for the association between leg fat mass and carotid E (p interaction=0.03). The independent associations of trunk fat, leg fat and leg lean mass with peripheral arterial stiffness are further summarized and illustrated in Figure 1A. We performed regression analyses using the mean of the seven Z-scores as dependent variable (similar to models in Table 3) and plotted the standardized betas in Figure 1A.

Additional adjustment of Model 1 for lifestyle (physical activity, smoking, alcohol or nutrient intake), LDL- and HDL-cholesterol, triglycerides, hypertension, (micro-) albuminuria, serum creatinine and prior cardiovascular disease did not materially change the associations (data not shown). Adjustment for HbA1c, insulin, fasting or postload glucose attenuated the associations, in particular in the femoral and brachial arteries (data not shown). After adjustment for insulin and fasting and postload glucose levels together all associations weakened and most became non-significant, except for the associations with carotid E, and femoral CC (data not shown). Model 2 shows the independent associations of trunk fat, leg fat and leg lean mass with peripheral stiffness after adjustment for glucose tolerance status.

Table 4 provides insight into which elements of the peripheral arterial stiffness estimates (i.e. D, ΔD , PP or IMT) contributed to the associations shown in Table 3. The associations were mainly determined by D or ΔD , or by both in the femoral artery, but not by PP.

There was no statistically significant effect modification by gender. None of the regression models were disturbed by multi-colinearity.

Associations of body composition with central arterial stiffness (Table 5)

Trunk fat mass was not associated with higher central arterial stiffness, except for SAC2. Leg lean mass was consistently associated with lesser central arterial stiffness. There was no statistically significant effect modification by gender. The independent associations of trunk fat, leg fat and leg lean mass with central arterial stiffness are further illustrated in Figure 1B, with the mean Z-scores of each central stiffness estimate as dependent variable. Because carotid-femoral transit-time was available in fewer persons (see Table 2), we did not include this measure in the mean Z-score presented in Figure 1. Results including this measure yielded similar results (data not shown). Additional adjustment for heart rate did not influence the association between body composition and carotid-femoral TT (data not shown). Adjustment for lifestyle measures did not affect any of the associations, nor did adjustment for LDL- and HDL-cholesterol, fasting and postload glucose,

In-transformed insulin, triglycerides, hypertension, (micro-) albuminuria, serum creatinine and

Table 3. Associations (standardized betas) of body composition by DXA with peripheral arterial stiffness estimates

		Carotid artery	\		Femoral artery	tery	Brachial artery	
		DC	8	ш	DC DC	8	DC	8
Model 1	Trunk fat mass -0.15**	3 -0.15**	*80.0-	0.15**	-0.18**	-0.26**	-0.14**	-0.16**
	Leg fat mass	0.07	0.04	-0.08	0.13**	0.22**	0.05	0.08
	Leg lean mass	-0.07	0.00	0.00	0.09	0.36**	-0.06	0.14*
Model 2	Trunk fat mass	5 -0.13**	-0.07	0.14**	-0.02	-0.13**	-0.05	-0.09
	Leg fat mass	0.04	0.02	-0.07	0.02	0.13**	-0.02	0.04
	Leg lean mass	-0.08	-0.01	0.01	0.03	0.31**	-0.09	0.12
All models are	All models are adjusted for age, height, sex, mean arterial pressure and the other two body composition variables. Model 2 is Model 1 with additional	height, sex, me	an arterial pressu	re and the othe	r two body cor	nposition variables.	Model 2 is Model	1 with additional

adjustment for glucose tolerance status ** p < 0.05, * p < 0.10 DC, Distensibility coefficient; CC, Compliance coefficient; E, Young's elastic modulus

Table 4. Associations (standardized betas) of body composition measured by DXA with individual elements of the peripheral arterial stiffness estimates

D AD PP 0.12** -0.07 0.06 -0.04 0.00 -0.05 0.12 -0.01 0.01 0.12** -0.07 0.03 -0.04 -0.01 -0.02	Carotid artery	Femo	Femoral artery		Brachial artery	rtery	
Trunk fat mass 0.12** -0.07 0.06 Leg fat mass -0.04 0.00 -0.05 Leg lean mass 0.12 -0.01 0.01 Trunk fat mass 0.12** -0.07 0.03 Leg fat mass -0.04 -0.01 -0.02		IMT D	ΔD	ЬР	D	ΔD	ЬР
Trunk fat mass 0.12** -0.07 0.06 Leg fat mass -0.04 0.00 -0.05 Leg lean mass 0.12 -0.01 0.01 Trunk fat mass 0.12** -0.07 0.03 Leg fat mass -0.04 -0.01 -0.02							
Leg fat mass -0.04 0.00 -0.05 Leg lean mass 0.12 -0.01 0.01 Trunk fat mass 0.12** -0.07 0.03 Leg fat mass -0.04 -0.01 -0.02	-0.07	0.02 -0.17**	** -0.24**	0.04	-0.02	-0.13**	90.0
Leg lean mass 0.12 -0.01 0.01 Trunk fat mass 0.12** -0.07 0.03 Leg fat mass -0.04 -0.01 -0.02	0.00	-0.01 0.17**		-0.03	*60.0	0.05	-0.03
Trunk fat mass 0.12** -0.07 0.03 Leg fat mass -0.04 -0.01 -0.02	-0.01	0.13 0.41**	** 0.32**	-0.01	0.36**	0.03	-0.07
-0.04 -0.01 -0.02	-0.07	-0.04 -0.17**	** -0.05	0.04	-0.05	-0.05	0.03
	-0.01	0.04 0.17**	·* 0.11*	-0.02	0.13**	0.01	-0.00
-0.02 0.01	0.10 -0.02 0.01	0.14 0.41**	·* 0.25**	-0.01	0.37**	-0.00	-0.07

All models are adjusted for age, height, sex, mean arterial pressure and the other two body composition variables. Model 2 is Model 1 with additional adjustment for glucose tolerance status

** p<0.05, * p<0.10

D, Diameter; AD, Distension; PP, pulse pressure; IMT, intima-media thickness

prior CVD. Model 2 shows the association adjusted for glucose tolerance status. None of the regression models were disturbed by multi-colinearity.

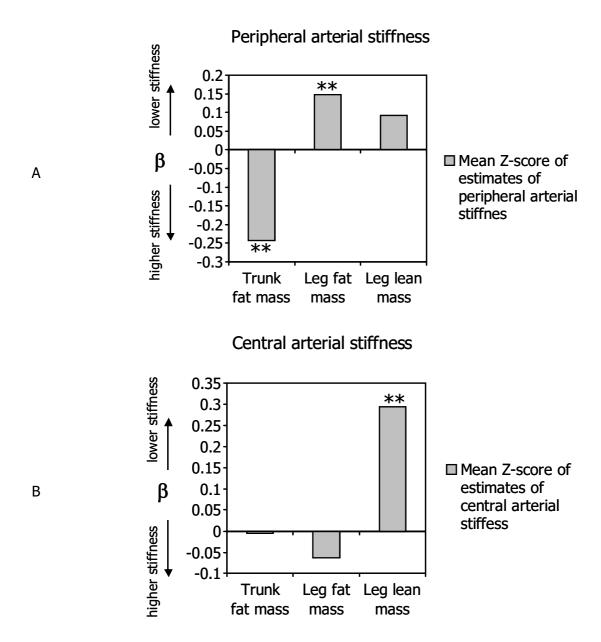


Figure 1. Independent associations (standardized betas, β) of body composition variables, adjusted for each other, age, height, sex and mean arterial pressure, with the mean Z-scores of measures for peripheral (A)^a and central (B)^b arterial stiffness (** p < 0.01)

^a distensibility and compliance of the carotid, femoral and brachial arteries and carotid Young's elastic modulus

^b aortic augmentation index, systemic arterial compliance by time decay method and systemic arterial compliance by stroke-to-volume pressure ratio

Table 5. Associations (standardized betas) of body composition by DXA with central arterial stiffness estimates

		Car-fem TT	AI	SAC1	SAC2
Model 1	Trunk fat mass	0.06	-0.06	0.05	-0.08*
	Leg fat mass	0.14	-0.06	0.08	0.09*
	Leg lean mass	0.23**	-0.15**	0.17**	0.30**
Model 2	Trunk fat mass	0.10	-0.08	0.06	-0.03
	Leg fat mass	0.12	-0.05	0.07	0.05
	Leg lean mass	0.21*	-0.15**	0.17**	0.29**

All models are adjusted for age, height, sex, mean arterial pressure and the other two body composition variables. Model 2 is Model 1 with additional adjustment for glucose tolerance status **p < 0.05, *p < 0.10

Car-fem TT, carotid-femoral transit time; AI, augmentation index; SAC1, systemic arterial compliance by time-decay method; SAC2 systemic arterial compliance by stroke volume-to-pulse pressure ratio

Discussion

This study, in men and women aged 60 to 86 years, had three major findings. First, larger trunk fat mass was associated with higher peripheral, but not central, arterial stiffness. Second, and in contrast, larger leg fat mass was not associated with higher peripheral arterial stiffness, but was associated with *lower* peripheral arterial stiffness, notably of the femoral artery. Third, larger leg lean mass was consistently, and independently of trunk and leg fat mass, strongly associated with lower central and less femoral and brachial arterial stiffness. These results provide a pathophysiological framework for understanding how abdominal obesity may contribute to cardiovascular disease, and how leg fat and lean mass may protect against cardiovascular disease.

Results of previous studies on body composition and arterial stiffness have not shown consistent results. Most studies were performed with a relatively small number of individuals (24 to 75) ^{16-19,21,24} and some were restricted to children ^{20,21} or men only ¹⁶. In some studies measures of obesity (usually BMI) were associated with higher arterial stiffness ^{16,19-23}, while other studies found the opposite ^{17,18,24}. The present study has important advantages, because it was large and population-based, and because comprehensive measures of both peripheral and central arterial stiffness were determined, as well as a very accurate and precise measurement of body composition. The latter is important because a higher BMI represents larger fat as well as larger lean mass ⁹. Because trunk fat mass and leg (or trunk) lean mass have opposite associations with arterial stiffness, the association of BMI with arterial stiffness will depend on the extent to which BMI reflects fat versus lean mass, and this may explain the divergent results reported in previous studies.

Larger trunk fat mass was associated with higher peripheral arterial stiffness in the elastic carotid artery and the muscular femoral and brachial arteries. Adjustment for glucose tolerance status did not materially affect the associations with carotid artery stiffness, but decreased those with femoral and brachial artery stiffness (Table 3). This finding may indicate that the association of trunk fat with femoral and brachial artery stiffness is, in part, mediated by trunk-fat-induced glucose intolerance. However, we cannot exclude confounding, because our study population was

a sample stratified for glucose tolerance status and individuals who are glucose intolerant on average have more trunk fat. Nevertheless, the concept that intra-abdominal fat in particular contributes to hyperglycaemia and hyperinsulinaemia, possibly due to an increased secretion of free fatty acids (FFA) ¹, is generally accepted. Because DXA cannot distinguish between visceral and subcutaneous trunk fat, additional studies are needed to investigate the effect of subcutaneous, which is the largest component of trunk fat, versus visceral fat on peripheral arterial stiffness.

Several mechanisms can explain the relation between abdominal obesity and arterial stiffness. Obesity is related to insulin resistance and subsequently increased insulin levels may promote endothelial dysfunction, oxidative stress, vascular smooth muscle cell growth, and stimulation of the sympathetic nervous system ⁴³, all of which may contribute to arterial stiffness. Advanced glycation end products can form cross-links in collagen fibers, thereby decreasing the distensibility of the arterial wall ⁴⁴. Both insulin and glucose levels attenuated some of the associations we found in the present study, which supports these mechanisms. In addition, inflammatory mediators, which are increased in obesity, have been shown to relate to endothelial dysfunction ^{45,46}. Finally, several other proteins secreted by adipose tissue have been shown to be a possible link between obesity and vascular structure and function, such as resistin ⁴⁷, adiponectin ⁴⁸, and leptin ^{20,49}.

Our finding that storage of fat in the legs may be favourable for peripheral arterial stiffness provides a potential explanation for the inverse relationship between hip circumference and cardiovascular risk ³⁻⁶. The underlying mechanisms linking leg fat to (lower) arterial stiffness remain to be identified. However, it is becoming increasingly clear that leg fat is metabolically different from trunk fat, and is associated with a more favourable metabolic profile ⁷⁻⁹. Leg fat has greater lipolytic activity than fat in the abdominal region ^{50,51}, thus being able efficiently to take up FFA from the circulation, thereby protecting against the development of hyperglycaemia and hyperinsulinaemia ^{9,52}. In addition, leg fat and trunk fat may differ in secretion of adipokines that influence vascular function and structure. There are some known differences in secretion of leptin, adiponectin, and interleukin-6 between abdominal subcutaneous and visceral fat ⁵³⁻⁵⁶, but less is known about differences between abdominal and femoral subcutaneous adipose tissue. Taken together, we speculate that metabolic differences between leg and trunk fat may be responsible for their opposite associations with peripheral arterial stiffness. More work in this area is clearly needed.

We found that leg lean (or muscle) mass was a more important determinant of central arterial stiffness than was fat mass. As muscle mass increases, so will the requirements for blood supply, resulting in a higher cardiac output and stroke volume and size adaptation of the arteries. This is also demonstrated by the larger diameter and distension of both femoral and brachial arteries in people with more leg lean mass in our study.

The differences in the impact of body composition on the various arterial stiffness estimates suggests that, like the influence of aging and other risk factors ^{11,14}, the impact of body composition is not uniform along the arterial tree. Local differences in physiological or mechanical mechanisms (e.g. between proximal elastic vs. peripheral muscular arteries) may play a role ^{11,14}.

The present study has some limitations. First, we cannot exclude the possibility that the associations between fat distribution and arterial stiffness are caused by an unmeasured common underlying factor. Adjustment, however, for many alternative determinants, such as renal function, hypertension, and lifestyle variables, did not change our findings. Prospective studies are needed further to address this issue. Second, because we investigated an older Caucasian population, it is unclear whether these results apply to younger subjects or other ethnic populations. Finally, because of selective mortality and loss of follow-up of the unhealthiest subjects (who are likely to have had increased fatness and increased arterial stiffness), we probably have investigated a relatively healthy population and therefore may have underestimated the true associations (healthy survivor effect).

We conclude that trunk fat has adverse effects on peripheral, but not central, arterial stiffness. These adverse effects are partly, but not completely, explained by hyperglycaemia and hyperinsulinaemia. In contrast, peripheral fat mass may have a protective effect on peripheral arterial stiffness. In addition, larger lean mass was strongly associated with lower central arterial stiffness and lower peripheral stiffness in the muscular arteries. These results provide a pathophysiological framework to explain not only the higher cardiovascular risk in individuals with larger trunk fat mass, but also the reduced cardiovascular risk in individuals with larger leg lean and fat mass.

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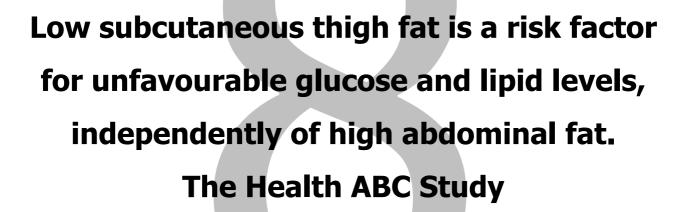
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submitted

Abstract

Objectives

We investigated whether low subcutaneous thigh fat is an independent risk factor for unfavourable glucose and lipid levels, and whether these associations differ between sexes, and between white and black people. Our secondary aim was to investigate which body composition characteristics (lean tissue, fat tissue) are reflected by anthropometric measures (waist and thigh circumferences).

Methods

Anthropometric measurements and computed tomography of the abdomen and of the thigh were performed in all participants of the Health, Aging and Body Composition Study who were aged 70-79 years. Fasting glucose, triglycerides and HDL-cholesterol, and 2-hr postload glucose, were determined.

Results

After exclusion of those already diagnosed with diabetes or dyslipidemia, analyses were performed in 2106 participants. After adjustment for abdominal subcutaneous and visceral fat, and intermuscular thigh fat, larger thigh subcutaneous fat area was statistically significantly (p<0.05) associated with lower In-transformed triglycerides (standardised beta (95% CI) -0.12 (-0.20 to -0.04) in men and -0.13 (-0.21 to -0.05) in women) and higher In-HDL-cholesterol (0.10 (0.02 to 0.19) and 0.09 (0.01 to 0.18), respectively). The associations with lower glucose levels were strong in men (-0.11 (-0.20 to -0.02) for fasting and -0.14 (-0.23 to -0.05) for postload glucose), but not statistically significant in women (-0.02 (-0.10 to 0.07) and -0.04 (-0.13 to 0.05), respectively). There were no differences in the associations between white and black persons. Waist circumference was more strongly associated with abdominal subcutaneous fat, and this association became stronger with increasing BMI, whereas the association with visceral fat became weaker. Thigh circumference was equally dependent on thigh fat and thigh muscle in men, whereas in women the fat component was the main contributor.

Conclusion

Larger subcutaneous thigh fat is independently associated with more favourable glucose (in men) and lipid levels (in both sexes), after accounting for abdominal fat depots, which are associated with unfavourable metabolic levels. Anthropometric measures reflect different fat depots at different levels of BMI at the abdomen, and reflect both fat and lean tissue at the thigh. These results underline the importance of accurate measures of regional body composition when investigating potential health risks.

Introduction

Waist and hip circumferences have been shown to have independent and opposite associations with cardiovascular risk factors in Caucasian men and women. Larger waist circumference was associated with unfavourable glucose and lipid levels, whereas larger hip or thigh circumference was associated with more favourable levels ¹⁻⁶. Although visceral fat is thought to be involved ⁷, the exact underlying pathophysiological mechanisms remain unclear. This may partly be due to the fact that not many studies include accurate assessments of body composition. By use of dual-energy x-ray absorptiometry (DXA) it has been shown that a larger waist circumference mainly represents more trunk fat rather than trunk lean mass, but a larger hip circumference represents both leg fat mass as well as leg lean mass ⁸. In addition, larger leg fat mass as well as larger leg lean mass was associated with lower insulin and glucose levels, after adjustment for trunk fat and lean mass ^{8,9}.

Since abdominal and femoral fat depots have different lipolytic activity ^{10,11}, femoral fat tissue may be more likely to take up free fatty acids (FFA) from the circulation. As a result, the gluteal-femoral fat depots protect the liver, pancreas, and skeletal muscle from high FFA exposure and accumulation, which is related to the development of insulin resistance and beta-cell dysfunction ¹²⁻¹⁸. Unfortunately, DXA does not allow separate quantification of intermuscular and subcutaneous fat in the legs, and of visceral and subcutaneous fat in the trunk.

In the Health, Aging and Body Composition Study investigators have found that more intermuscular fat in the thigh determined by computed tomography (CT) was significantly associated with a worse glucose tolerance status, whereas subcutaneous fat in the thigh was not ¹⁹. In these analyses, however, abdominal fat was not taken into account and the independent role of thigh fat depots was not studied. Potential differences between races in the relation of the different fat depots with glucose metabolism were not reported.

The aim of the present study was to investigate independent associations of CT measured body composition characteristics with glucose and lipid levels in subjects without known diabetes or dyslipidemia. In addition, we investigated whether these associations differed between men and women, and between black and white people. We hypothesised that low subcutaneous thigh fat would be independently associated with unfavourable glucose and lipid levels. Our secondary aim was to investigate what body composition characteristics (lean tissue, different fat depots) are reflected by anthropometric measures (waist and thigh circumferences) in the elderly.

Methods

Subjects

The study population consisted of 3075 well-functioning black and white men and women, aged 70-79 years, participating in the Health, Aging, and Body Composition (Health ABC) Study. White participants were recruited from a random sample of Medicare beneficiaries residing in zip codes from the metropolitan areas surrounding Pittsburgh, PA and Memphis, TN. Black participants were recruited from all age-eligible residents in these geographic areas. Eligibility criteria included: age 70-79 years in the recruitment period from March 1997 to July 1998, self-report of no difficulty

walking one-quarter of a mile or climbing 10 steps without resting, no difficulty performing basic activities of daily living, no reported use of a cane, walker, crutches or other special equipment to get around, no history of active treatment for cancer in the prior 3 years, and no plan to move out of the area in the next 3 years. The population comprised 41.7% African-American, 58.3% Caucasian, and 48.5% male. The experimental procedures were approved by the Human Investigation and Review Boards at the University of Pittsburgh at Pittsburgh and the University of Tennessee at Memphis. Written informed consent was obtained from all subjects.

For this study, subjects who had missing fasting glucose (n=29) or missing abdominal or thigh CT data (n=154) were excluded from all analyses. Also, persons who reported previous diabetes diagnosis (n=468, of which 385 persons were using glucose lowering medication) or who were using lipid-lowering medication (n=437) were excluded, because treatment (either diet or medication) could possibly influence the associations under consideration. Finally, 2106 subjects were studied in the analyses.

Body composition

Body weight was measured with a standard balance beam scale to the nearest 0.1 kg. Height was measured barefoot to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd., UK). BMI was calculated as weight divided by height squared (kg/m²). Abdominal circumference (cm) was measured with a flexible plastic tape measure to the nearest 0.1 cm at the level of the largest circumference at the end of expiration, while subjects were standing with their weight equally distributed on both feet, arms at their sides, and head facing straight forward. Thigh circumference (cm) was measured at the mid-thigh between the inguinal crease and the proximal border of the patella. A total body DXA scan was performed to measure total body fat using the fan beam technology (Hologic QDR4500A, software version 8.21). CT scans of the abdomen and thighs were acquired in Memphis using a Somatom Plus 4 (Siemens, Erlangen, Germany) or a Picker PQ 2000S (Marconi Medical Systems, Cleveland, OH), and in Pittsburgh using a 9800 Advantage (General Electric, Milwaukee, WI) as described previously ^{20,21}. Briefly, the scans were completed at 120 kVp, 200-250 mAs and slice thickness was set at 10 mm. For the scan at the abdomen at L4/L5 level, subjects were placed in the supine position with their arms above their head and legs elevated with a cushion to reduce the curve in the back. The scan at mid-thigh level was performed at one-half of the distance between the medial edge of the greater trochanter and the intercondyloid fossa. The images were transferred to a SUN workstation (SPARCstation II, Sun Microsystems, Mountainview, CA) for determination of adipose and muscle tissue areas using IDL (RSI Systems, Boulder, CO) based software developed at the reading centre. Fat tissue, muscle tissue and bone were distinguished by their particular range of tissue density in Hounsfield Units (HU). The determination of soft tissue type was made using the bimodal image distribution histogram resulting from the distribution of HU values in fat tissue and muscle tissue ²². Visceral fat tissue was manually distinguished from the subcutaneous fat tissue by tracing along the fascial plane defining the internal abdominal wall. For the muscle area of the abdomen, areas of the left and right psoas, rectus, and lateral abdominal muscles were added. In the thighs, intermuscular and visible intramuscular fat tissue were separated from subcutaneous adipose tissue by drawing a line along the deep fascial plane surrounding the thigh muscles. The total area of non-adipose,

non-bone tissue within the deep fascial plane was used as a measure of muscle area. Areas of the left and right thigh were added.

Metabolic variables and lifestyle

Serum triglycerides and HDL-cholesterol after an overnight fast were determined on a Vitros 950 analyser (Johnson & Johnson). A 75-g oral glucose tolerance test (OGTT) was performed in subjects without previously diagnosed diabetes. Participants were considered to have previously diagnosed diabetes if they reported to have diabetes or were using glucose-lowering medication. Fasting and 2-hr postload plasma glucose was measured using an automated glucose oxidase reaction (YSI 2300 Glucose Analyser, Yellow Springs, OH). Systolic and diastolic blood pressures were measured in duplicate with a conventional mercury sphygmomanometer after at least 5 minutes rest in a seated position.

Smoking status (never, current, former), pack-years exposure to cigarettes, drinking history (never, current, former) and current drinking status (no, less than once a week, 1-7 times a week, more than 1 a day) and physical activity (in the past 7 days) were assessed by an interviewer-administered questionnaire. The time spent on gardening, heavy chores, light house work, grocery shopping, laundry, climbing stairs, walking for exercise, walking for other purposes, aerobics, weight or circuit training, high intensity exercise activities and moderate intensity exercise activities was obtained as well as information on the intensity level at which each activity was performed. A metabolic equivalent value was assigned to each activity/intensity combination and was used to calculate the number of kilocalories per week per kilogram of body weight spent on that activity ²³. For each participant the scores of all performed activities were summed and multiplied by body weight to create an overall physical activity score in kilocalories per week. Similarly, a summary score of performed exercise activities was created, which included aerobics, weight training, medium and high intensity exercise activities and walking for exercise.

Statistical methods

All analyses were performed with the use of SPSS for Windows version 10.1 (SPSS Inc., Chicago, IL). Multiple linear regression analyses were performed to study independent associations between body composition measures by CT (independent variables), and glucose and lipid levels (dependent variables). Effect modification by sex and race was evaluated by adding product terms to the regression models. Effect modification by BMI was evaluated by stratification, because in this case adding product terms disturbed the models due to multi-colinearity. Confounding by lifestyle factors and muscle area was evaluated by adding these variables to the regression models.

In addition, similar regression analyses were performed to study independent associations between body composition measures by CT (independent variables) and anthropometrically derived waist and hip circumferences (dependent variables).

To facilitate direct comparisons, results of the regression analyses are reported as standardized betas. A standardized beta of 0.1 indicates that when the independent variable increases by 1 SD, the dependent variable increases by 0.1 SD. We considered the stability of the regression models to be disturbed by multi-colinearity if the tolerance was <0.1. The tolerance is a

statistic used to determine how much the independent variables are linearly related to one another. It is calculated as 1 minus R squared for an independent variable when it is predicted by the other independent variables already included in the model.

Results

Characteristics are shown in Table 1, by gender and race. In spite of higher BMI and subcutaneous fat, black adults have lower visceral fat compared to white adults. Table 2 shows univariate associations of body composition variables with fasting and 2-hour glucose levels, and with Intransformed triglycerides and HDL-cholesterol, whereas Table 3 shows the associations additionally adjusted for other body composition variables. Larger total abdominal fat area was associated with unfavourable levels of all these metabolic variables, whereas larger total thigh fat area was associated with more favourable levels after adjustment for total abdominal fat (Model 1), in both men and women. Subdivision of the fat areas revealed that the associations with total thigh fat

Table 1. Characteristics of the study population, stratified by gender and race

	М	en	Wo	men
	White	Black	White	Black
	N=660	N=359	N=615	N=472
Age, y	73.9 ± 2.9	73.3 ± 2.8	73.6 ± 2.8	73.5 ± 3.0
BMI, kg/m ²	26.9 ± 3.8	26.7 ± 4.4	25.6 ± 4.3	29.0 ± 5.6
Waist circumference (cm)	101.5 ± 13.0	98.3 ± 12.1	94.7 ± 12.1	99.4 \pm 14.9
Thigh circumference (cm)	49.7 ± 4.7	51.3 ± 5.8	50.0 ± 6.1	55.2 ± 8.0
Total fat by DXA, %	28.6 ± 4.9	26.5 ± 5.5	38.7 ± 5.6	39.7 ± 6.2
Abdomen				
Total area, cm ²	673.6 ± 152.0	641.7 ± 167.4	642.9 ± 151.3	725.0 ± 190.9
Fat area, cm ²	394.4 ± 135.0	352.6 ± 147.4	425.1 ± 144.9	483.8 ± 174.9
Visceral fat area, cm ²	165.3 ± 70.5	122.6 ± 63.2	122.7 ± 56.4	119.2 ± 53.5
Subcutaneous fat area, cm ²	229.1 ± 87.1	229.9 ± 104.0	302.4 ± 108.5	364.6 ± 143.6
Muscle area, cm ²	81.2 ± 15.7	83.5 ± 18.4	53.8 ± 10.8	61.4 ± 12.7
Thigh				
Total area, cm ²	394.6 ± 71.8	420.7 ± 90.2	394.8 ± 92.9	483.9 ± 129.0
Fat area, cm ²	115.1 ± 47.7	119.6 ± 51.6	209.6 ± 78.6	263.7 ± 107.6
Subcutaneous fat area, cm ²	96.5 ± 41.2	99.0 ± 43.3	192.7 ± 74.6	239.4 ± 101.0
Intermuscular fat area, cm ²	18.6 ± 10.8	20.6 ± 13.6	$\textbf{16.8} \pm \textbf{8.4}$	24.3 ± 13.6
Muscle area, cm ²	255.0 ± 38.9	276.0 ± 50.9	167.8 ± 27.1	199.1 ± 34.5
Fasting glucose, mmol/l	5.48 ± 1.11	5.43 ± 1.12	5.06 ± 0.66	5.32 ± 1.02
2 hour glucose, mmol/l	7.21 ± 3.14	7.42 ± 3.08	7.18 ± 2.54	7.38 ± 2.64
Systolic blood pressure, mmHg	132.9 ± 19.9	137.8 ± 21.9	133.1 ± 19.7	139.2 ± 23.0
Diastolic blood pressure, mmHg	71.8 ± 10.5	76.3 ± 12.3	68.6 ± 11.0	73.1 ± 12.5
Triglycerides, mmol/l	1.39	1.15	1.44	1.13
	(1.00-1.93)	(0.89-1.46)	(1.10-1.95)	(0.90-1.49)
HDL-cholesterol, mmol/l	1.11	1.27	1.53	1.55
	(0.96-1.37)	(1.09-1.55)	(1.27-1.86)	(1.29-1.84)

Data are presented as mean \pm standard deviation, or median (interquartile range)

area were mainly determined by the subcutaneous thigh fat area (Model 2), whereas the associations of total abdominal fat were mainly determined by visceral fat area (Model 3). However, if visceral fat is accounted for then abdominal subcutaneous fat is also associated with a poorer metabolic profile. If all four fat depots were included into one model (Model 4), larger thigh subcutaneous fat area was independently associated with more favourable glucose and lipid levels, except for glucose levels in women. We also added abdominal and thigh subcutaneous fat areas to calculate total subcutaneous fat. If total subcutaneous fat was added to the regression model which already included visceral fat and intermuscular thigh fat, it was not significantly related to glucose and lipid levels (data not shown), confirming opposite associations of different regional subcutaneous fat depots with metabolic variables. There was no statistically significant effect modification by race, except for the association of thigh subcutaneous fat area with HDLcholesterol in men in Model 2 and Model 4, which was present in white men, but not in black men (standardised beta of 0.20 in white and -0.04 in black men in Model 4). No consistent associations were found with each of the fat depots when systolic or diastolic blood pressure (other components of the metabolic syndrome) was studied as outcome, and most associations were non-significant (data not shown). In all models of Table 3, additional adjustment for abdominal or thigh muscle area did not materially change the results, and a larger muscle area was not associated with glucose and lipid levels, or associated with less favourable glucose and lipid levels. Also adjustment for height and lifestyle variables (smoking, alcohol intake, and physical activity) did not change the results. When we only selected subjects younger than 75 years old, the associations remained similar. Stratification by BMI-group did not show clear trends of increasing or decreasing associations with higher BMI-group. None of the regression models showed evidence of multi-colinearity by statistical testing.

To visualize the meaning of the opposite associations that we found in Table 3, as an example, mean fasting glucose levels in men are shown in Figure 1 after stratification of the male population in (race-specific) tertiles of visceral and subcutaneous thigh fat, adjusted for site, age, race, abdominal subcutaneous fat and intermuscular thigh fat. It is clearly shown that with a larger visceral fat area, glucose levels are higher. In contrast, within each tertile of visceral fat, with a larger subcutaneous thigh fat area, glucose levels are lower.

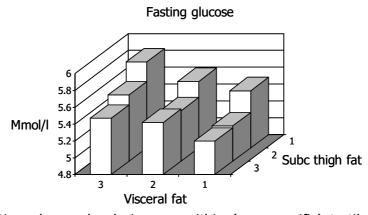


Figure 1. Mean fasting glucose levels in men within (race-specific) tertiles of visceral fat and subcutaneous thigh fat, adjusted for site, age, race, abdominal subcutaneous fat and intermuscular thigh fat

Table 2. Univariate associations (standardized betas) of fat areas with fasting and postload glucose levels, In-transformed triglyceride levels and HDL-cholesterol, adjusted for age, site, and race

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	Fasting glucose	cose	Postload glucose	ncose	Ln-triglycerides	rides	Ln-HDL-cholesterol	lesterol
	Men	Women	Men	Women	Men	Women	Men	Women
Total abdominal fat area	0.17**	0.23**	0.17**	0.19**	0.28**	0.24**	-0.22**	-0.24**
Visceral fat area	0.19**	0.27**	0.19**	0.28**	0.31**	0.36**	-0.23**	-0.30**
Abdominal subcutaneous fat area	0.12**	0.18**	0.12**	0.12**	0.19**	0.15**	-0.17**	-0.17**
Total thigh fat area	0.07**	0.12**	0.05	**20.0	0.12**	0.04	**60.0-	-0.08**
Thigh subcutaneous fat area	0.04	0.12**	0.03	*90.0	**60.0	0.03	**80 · 0-	-0.07**
Thigh intermuscular fat area	0.14**	0.13**	0.11**	0.12**	0.18**	0.11**	-0.12**	-0.13**

** p < 0.05, * p < 0.10

Table 3. Independent associations (standardized betas) of fat areas (adjusted for each other) with fasting and postload glucose levels, In-

		Facting of	9303	Postload ulicosa	93031	I n-trialycaridae	ridac	I n-HDI -cholestarol	plecterni
		ו מארוווט אור	giacose	rustioau y	นเบรต	LII-UIGIYCEI	Incs	LII-I IDE-CII	الحاددان
		Men	Women	Men	Women	Men	Women	Men	Women
Model 1	Model 1 Total abdominal fat area	0.25	0.30**	0.28**	0.29	0.40**	0.43**	-0.32**	-0.37**
	Total thigh fat area	-0.11**	**60.0-	-0.15**	-0.14**	-0.17*	-0.27**	0.14**	0.18**
Model 2	Total abdominal fat area	0.22**	0.29**	0.26**	0.27**	0.38**	0.42**	-0.32**	-0.36**
	Thigh subcutaneous fat area	-0.13**	*60.0-	-0.16**	-0.14**	-0.17**	-0.25**	0.12**	0.18**
	Thigh intermuscular fat area	90.0	-0.01	0.03	0.02	0.02	-0.03	0.02	-0.00
Model 3	Model 3 Visceral fat area	0.18**	0.23**	0.18**	0.29**	0.30**	0.38**	-0.20**	-0.29**
	Abdominal subcutaneous fat area	0.11**	*80.0	0.14**	0.02	0.14**	0.08*	-0.16**	-0.11**
	Total thigh fat area	*60.0-	-0.02	-0.13**	-0.05	-0.12**	-0.15**	0.12**	0.10**
Model 4	Visceral fat area	0.15**	0.24**	0.16**	0.29**	0.29**	0.39**	-0.20**	-0.29**
	Abdominal subcutaneous fat area	0.10**	*60.0	0.13**	0.01	0.14**	0.08*	-0.16**	-0.11**
	Thigh subcutaneous fat area	-0.11**	-0.02	-0.14**	-0.04	-0.12**	-0.13**	0.10**	0.09
	Thigh intermuscular fat area	90.0	-0.02	0.02	-0.00	0.02	*90.0-	0.02	0.02

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In both sexes waist circumference was more strongly associated with abdominal fat area compared to abdominal muscle area (Table 4). When visceral fat and subcutaneous fat from the abdomen were examined separately, the subcutaneous compartment appeared the strongest determinant of the waist circumference. Stratification by (gender- and race-specific) BMI tertiles revealed that the association of waist circumference with abdominal subcutaneous fat became stronger, and with visceral fat became weaker with higher BMI-group, particularly in men. The thigh circumference was equally dependent on thigh fat and thigh muscle area in men, whereas in women the fat component was the main contributor. Intermuscular fat area had a relative small contribution. In particular the association of the subcutaneous thigh fat area, not the intermuscular fat area, with thigh circumference strengthened with higher BMI-group. There was no statistically significant (p < 0.05) effect modification by race, except for the associations of waist circumference with abdominal subcutaneous fat and with muscle area, which were stronger in black men compared to white men (standardised betas were 0.61 and 0.41 for subcutaneous fat and 0.15 and 0.07 for muscle, respectively).

Table 4. Independent associations (standardised betas) of CT abdominal fat and muscle areas with anthropometrically derived waist circumference, and of CT thigh fat and muscle areas with thigh circumference, adjusted for age, site, and race

		Men	Women
Dependent	Independent	Beta	Beta
Waist circumference	Abdominal total fat area	0.71	0.73
	Abdominal muscle area	0.10	0.11
Waist circumference	Visceral fat area	0.34	0.27
	Abdominal subcutaneous fat area	0.48	0.57
	Abdominal muscle area	0.10	0.10
Thigh circumference	Thigh total fat area	0.51	0.78
	Thigh muscle area	0.57	0.26
Thigh circumference	Thigh subcutaneous fat area	0.40	0.72
	Thigh intermuscular fat area	0.17	0.11
	Thigh muscle area	0.56	0.26

Beta, standardized beta; All p<0.001

Discussion

In the present study, we found that in particular visceral fat was an important independent determinant of unfavourable glucose and lipid levels, in both men and women, and both white and black people. In contrast, subcutaneous thigh fat was independently related to more favourable levels of glucose and lipids, except to glucose levels in women. This paper also showed that waist circumference was related to both visceral fat and subcutaneous fat, and that waist circumference better represented visceral fat at a low BMI. Apart from reflecting subcutaneous thigh fat, thigh circumference also represented muscle, in particular in men.

Visceral fat was the strongest independent correlate of unfavourable metabolic variables, which supports the hypothesis that particularly visceral fat contributes to the higher free fatty acid (FFA) levels, which are directly released in the portal vein leading to the liver. In the liver FFA play an important role in the development of insulin resistance, by reducing hepatic insulin clearance, increasing gluconeogenesis and increasing dyslipidemia ^{7,24}. In addition, due to increased FFA levels, FFA also accumulate in non-adipose tissue (i.e. ectopic fat storage) such as muscle, the pancreas and the liver, which contributes to and exacerbates insulin resistance and type 2 diabetes ¹³⁻¹⁸. We found that abdominal subcutaneous fat was also related to unfavourable metabolic variables, even after adjustment for visceral fat area. This may indicate that also abdominal subcutaneous fat contributes to higher FFA levels.

In contrast to abdominal subcutaneous fat, subcutaneous thigh fat was independently related to more favourable levels of metabolic variables, which confirmed our hypothesis of low subcutaneous fat as a risk factor for unfavourable glucose and lipid levels. It has been proposed that subcutaneous thigh fat acts as a 'metabolic sink' for circulating FFA ¹². Due to differences in lipolytic activity between abdominal subcutaneous fat and subcutaneous thigh fat ^{10,11}, subcutaneous thigh fat is more likely to take up FFA from the circulation, and therefore protects other organs against high FFA exposure. In this manner, ectopic fat storage is prevented which leads to a lower risk of insulin resistance ^{12,13}.

Fat cells are known to secrete many signalling factors, of which some may be involved in the development of insulin resistance ²⁵. Examples include leptin, adiponectin, interleukin-6, tumor necrosis factor-alpha, plasminogen activator inhibitor-1, and many more. There are some known regional differences in the secretion of leptin, adiponectin and interleukin-6 between visceral and abdominal subcutaneous fat ²⁶⁻²⁸. It might be possible that there are also regional differences in secretion of these adipokines between abdominal subcutaneous and subcutaneous thigh fat, which could contribute to the different associations of these fat depots with metabolic variables. More research in this area is clearly needed.

The independent relation of larger leg fat mass measured by DXA with more favourable metabolic variables has been found in a number studies, in both middle-aged and elderly subjects ^{1,8,9}. When we used the DXA measurements from the Health ABC Study, we observed similar associations (unpublished results). The limitation of DXA is that it cannot distinguish between visceral and abdominal subcutaneous fat in the trunk, and between intermuscular and subcutaneous fat in the legs. By the use of CT, we found that the protective association of DXA leg fat with metabolic variables was due to the subcutaneous thigh fat, and not intermuscular thigh fat. Previously, in a small group of obese men (aged 29-42 years), femoral adipose tissue by CT was also found to be negatively associated with triglycerides and positively with HDL-cholesterol ²⁹, and similar results were found in women ³⁰. Also in a small sample of black women, leg fat appeared to be independently related to better lipid levels ³¹. In the present paper, we confirmed these observations using CT measurements in a large elderly population, including both black and white people, and extended the observations by including glucose levels as outcome measures.

Previously, we found larger leg lean mass by DXA (which is mainly muscle mass) to be related with lower glucose levels, independent of trunk fat and leg fat mass ⁸, which could also be confirmed in the Health ABC Study when we used DXA measurements (unpublished results).

However, we did not observe any relationship with thigh muscle area if using CT data, after adjustment for abdominal and thigh fat depots. CT and DXA generally agree in measuring muscle and fat mass, if similar regions are compared ^{32,33}. However, it is possible that the thigh muscle area from a single slice CT scan is not representative of the muscle mass of the total leg as is measured by DXA. Similarly, this could also be the reason for not finding a relation with intermuscular fat. More detailed measurements of total muscle might be needed to appropriately assess the relation with the metabolic profile.

There was a difference between black and white participants in body composition in our study, which is also known from previous studies ³⁴⁻³⁶. Black persons have generally less visceral fat compared to white persons, whereas the subcutaneous fat (either abdominal or at the thigh) is higher, for any level of total body fat. Black people had a better lipid profile in our data, which has also been found previously ^{36,37}. It has been shown that in both sexes black persons have a higher LPL activity and lower HL activity compared to white persons ³⁶, which may explain these differences in lipid profile and fat distribution. It has been suggested that due to these differences in enzyme activities, black persons are more likely to store their lipids in subcutaneous fat depots, which in turn will lead to a better lipid profile, as explained above. There were no clear race differences observed in glucose levels, which may only become apparent after appropriate adjustment for other characteristics such as BMI. We did not find a statistically significant difference between races in the relation of different fat depots with metabolic variables. Therefore, the difference between black and white people in metabolic profile might be explained by the relative amounts of different fat depots, rather than by the relation between these fat depots and metabolic variables per se.

WHR is generally used as measure of abdominal fat distribution, presumably reflecting visceral fat ^{38,39}. A higher WHR, however, can also be caused by a smaller hip circumference. With the use of DXA measurements, it has recently been shown that the hip circumference not only represents fat accumulation in the legs, but is also related to the lean mass in the legs ⁸. In the same study, the waist circumference mainly reflected fat mass in the trunk. It remained unclear whether this was mainly caused by the visceral or subcutaneous fat depot. In the present paper we show that abdominal subcutaneous fat depot mainly determines the waist circumference, particularly in persons with a higher BMI. Clearly, the WHR does not simply represent visceral fat accumulation only. This might be important in the context of operationalising the metabolic syndrome, but also in research that studies body composition in relation to health risks. It has been suggested that increased abdominal fat accumulation may be less hazardous in older than in younger persons, because anthropometric measures of abdominal obesity were not related to (cardiovascular) mortality in the elderly, and it has been shown that lipolysis in visceral fat, which causes free fatty acid flux, is reduced with aging ⁴⁰. Our study shows, however, that if more precise measures of body composition are taken, visceral fat is still hazardous in older adults.

This study has some limitations. First, because we have investigated a relatively healthy and well-functioning population, we may have underestimated the true associations. Second, because of the cross-sectional design of the study, causality cannot be assumed. Longitudinal analyses, however, are also limited because body composition values are not stable over time, particularly in elderly. Finally, the anthropometric analyses were performed in people without

known diabetes or dyslipidemia, which could create a bias when generalizing to the total population. Performing analyses in the total study population, however, gave similar results.

In conclusion, high visceral fat and high abdominal subcutaneous fat are both independently associated with unfavourable glucose and lipid levels. In contrast, high subcutaneous thigh fat is independently associated with more favourable glucose (in men) and lipid levels (in both sexes), if the abdominal fat depots are taken into account. These results underline the importance of accurate measures of regional body composition in health risk research. Further research is needed to elucidate underlying pathophysiological mechanisms. Waist circumference reflects different fat depots at different levels of BMI, and thigh circumference reflects both fat and muscle tissue. Caution is needed when interpreting anthropometric measurements.

Acknowledgement

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The prediction of visceral fat by DXA in the elderly: a comparison with computed tomography and anthropometry

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Abstract

Introduction

Effective methods for assessing visceral fat are important to investigate the role of visceral fat for the increased health risks in obesity. Techniques for direct measurement of soft tissue composition such as CT or MRI are expensive, time-consuming or require a relatively high radiation dose. Simple anthropometric methods, such as waist-to-hip ratio, waist circumference or sagittal diameter are widely used. However, these methods cannot differentiate between visceral and subcutaneous fat and are less accurate. The aim of the present study is to investigate whether the DXA method, possibly combined with anthropometry, offers a good alternative to CT for the prediction of visceral fat in the elderly.

Methods

Subjects were participants in the Health ABC-study, a cohort study of black and white men and women aged 70-79, investigating the effect of weight related health conditions on disablement. Total body fat and trunk fat were measured by dual energy x-ray absorptiometry (DXA) using a Hologic QDR 1500. A 10-mm CT scan at the L4-L5 level was acquired to measure visceral fat and total abdominal fat. Weight, height, sagittal diameter and waist circumference were measured using standard methods. Fat in a manually defined DXA sub-region (4 cm slice at the top of iliac crest) at the abdomen was calculated in a sub-group of participants (n=150; 50% male; 45,3% Afro-American / 54,7% Caucasian, age 70-79 y). This sub-region, the standard trunk region and total fat were used as indicators of visceral fat.

Results

Total abdominal fat by DXA (sub-region) was strongly correlated with total abdominal fat by CT (r ranging from 0.87 in white men to 0.98 in black women). The DXA sub-region underestimated total abdominal fat by 10% compared to the CT slice. The underestimation by DXA was seen especially in people with less abdominal fat. The association of visceral fat by CT with the DXA sub-region (r = 0.66, 0.78, 0.79 and 0.65 for white and black men and women respectively) was comparable with the association of the CT measure with the sagittal diameter (r = 0.74, 0.70, 0.84 and 0.68). Combining DXA measurements with anthropometry gave only limited improvement for the prediction of visceral fat by CT compared to univariate models (maximal increase of R^2 4%).

Conclusion

DXA is a good alternative to CT for predicting total abdominal fat in an elderly population. For the prediction of visceral fat the sagittal diameter, which has a practical advantage compared to DXA, is just as effective.

Introduction

The association between obesity, especially android obesity, and health risks has been recognized for many decades. In the 1940s Jean Vague already characterized android obesity, showing differences in the prevalence of diabetes and heart disease in comparison with gynoid obesity. Vague proposed that the upper body fat distribution in android obesity was a causal factor in determining the adverse health consequences of obesity and, in particular, its metabolic and cardiovascular consequences ¹. More recent studies indicate that intra-abdominal or visceral fat, especially, is strongly associated with metabolic disturbances and cardiovascular disease ²⁻⁴. Numerous techniques have been developed to assess visceral fat ⁵.

Computerized tomography (CT) has been demonstrated to be an accurate and precise technique for measuring soft tissue composition. It permits differentiation between visceral and subcutaneous fat in a cross-section of the body ^{5,6}. Magnetic Resonance Imaging (MRI) compares well with CT-measured fat and both techniques have a similar accuracy in comparison with chemical analysis ⁷⁻⁹. Therefore CT and MRI are both used as a gold standard for measuring visceral fat. However, both methods suffer the major drawbacks of being expensive and time-consuming. In addition, CT-scans require a relatively high radiation dose ⁶. Therefore alternative ways to estimate visceral fat continue to be developed and tested.

Simple anthropometric measurements are often used as indirect measurements of visceral fat. Most widely used are waist-to-hip ratio, waist circumference, sagittal diameter and skin fold thickness. These indirect measurements cannot differentiate between visceral and subcutaneous fat, but they are more strongly correlated with visceral fat than subcutaneous fat and can therefore be used as a marker for visceral fat ^{5,10}. However, several studies found that these correlations are weaker in obese or older subjects ¹¹⁻¹³. The decreasing correlation is thought to reflect problems in anthropometric measurements in these populations, as these methods are subject to considerable between and within-examiner variation ^{5,14}. But it may also represent factors that systematically alter the relationship between visceral and subcutaneous fat such as physical inactivity or lifetime cigarette smoking.

An alternative method for measuring total body soft tissue composition is dual energy X-ray absorptiometry (DXA). The principle of absorptiometry is based on the exponential attenuation of X-rays at two energies as they pass through body tissues. DXA is accurate, precise, and the radiation dose is minimal ¹⁵⁻¹⁷. It can provide measurements of fat, bone mineral content, and fatfree soft tissue in the total body and in specifically defined regions. However, as it measures two-dimensionally, DXA cannot differentiate between visceral and subcutaneous fat directly. In spite of this limitation, DXA has been used to estimate visceral fat by using the standard trunk region or by manually defining a sub-region at the abdomen. These sub-regions correlate strongly with visceral fat ^{18,19} and it is assumed that the between and within-examiner variation is smaller. A combination of DXA and anthropometry has been suggested to further improve the prediction of visceral fat by DXA ²⁰⁻²². Currently, comparisons between DXA and anthropometry with CT or MRI as the gold standard are performed in small groups with a wide age range. As the interest in body composition in old age increases, and fat accumulation in the abdomen increases with age, validation studies in older age groups are becoming increasingly important.

The aims of the present study are firstly to investigate the agreement between DXA and CT for measuring total body fat at the abdominal region (visceral + subcutaneous fat) in older men and women. Secondly, this study examines whether a DXA region would be a better marker of visceral fat compared to the generally used anthropometric measurements in this population. Finally, the study investigates whether visceral fat as measured with CT can be optimally estimated by DXA regions in combination with anthropometric measures.

Methods

Subjects

Participants in this study were enrolled in the Health and Body Composition (Health ABC) Study, a longitudinal study of 3,075 non-disabled men and women aged 70-79 years residing in Pittsburgh, PA and Memphis, TN. The population comprised 48.5% male, 41.7% African-American / 58.3% Caucasian, mean age 73.9 years. Participants were recruited from a random sample of Medicare beneficiaries from a list provided by the Health Care Financing Administration. Eligibility criteria included age 70-79, self-report of no difficulty walking ¼ mile and no difficulty walking up to 10 steps without resting, no difficulty with basic activities of daily living, no history of active cancer in the 3 years prior to the study, and no plan to move in the next three years. In a convenience sample of 150 participants, with gender and race equally distributed (the first 150 suitable HABC participants enrolled in the study), extended analyses of DXA measurements were performed, and these participants (50% male, 45,3% African American) were included in the present study. The experimental procedures were approved by the Human Investigation and Review Boards at the University of Pittsburgh at Pittsburgh and the University of Tennessee at Memphis. Written informed consent was obtained from all the subjects.

Anthropometry

Body weight was measured at a standard balance beam scale to the nearest 0.1 kg. Height was measured barefoot to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd., UK). BMI was calculated as weight divided by height squared (kg/m²). Abdominal sagittal diameter was measured by a Holtain-Kahn abdominal calliper (Holtain Ltd., UK). While supine, participants were asked to bend their knees to 45° angle with their feet flat on the examination table. The left and right iliac crests were marked, the lower blade of the calliper was placed under the small of the back, and the upper blade of the calliper was lowered to a mark midway between the iliac crests. Sagittal diameter, the distance between the abdomen and back, was measured as the distance between the blades of the calliper at the end of normal expiration. Abdominal circumference was measured with a flexible plastic tape measure to the nearest 0.1 cm at the level of the largest circumference at the end of expiration, while subjects were standing with their weight equally distributed on both feet, arms at their sides, and head facing straight forward.

Dual energy X-ray Absorptiometry

A total body DXA scan was performed using the pencil beam technology (QDR 1500, Hologic, Waltham, MA; enhanced whole body, software version 5.71). A standard soft tissue examination

includes total body and regional measurements of trunk, arms and legs to analyse body composition according to a three-compartment model including fat mass, lean tissue and bone mineral content. Besides the standard regions, a manually defined sub-region was examined, which consisted of 3 pixels (3.96 high, the smallest sub-region possible to approach the thickness of CT). The sub-region was placed at the abdomen with the bottom side of the sub-region on top of the iliac crests (Figure 1). This sub-region, the standard trunk region and total fat were used as indicators of visceral fat.

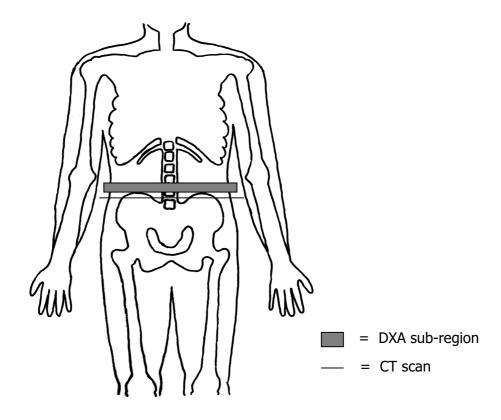


Figure 1. Location of the abdominal sub-region by DXA and by CT to measure total abdominal fat

Computerized tomography

The CT scan of the abdomen was acquired in Memphis using a Somatom Plus 4 (Siemens, Erlangen, Germany) or a Picker PQ 2000S (Marconi Medical Systems, Cleveland, OH), and in Pittsburgh using a 9800 Advantage (General Electric, Milwaukee, WI). The scans were completed at 120 kVp, 200-250 mAs. Slice thickness was set at 10 mm. For the scan at the abdomen at L4/L5 level, subjects were placed in the supine position with their arms above their head and legs elevated with a cushion to reduce the curve in the back. The images were transferred to a SUN workstation (SPARCstation II, Sun Microsystems, Mountainview, CA) for determination using IDL development software (RSI Systems, Boulder, CO). Areas were calculated by multiplying the number of pixels of a given tissue type by the pixel area. Density values were determined by averaging the CT number (pixel density) values of the regions outlined on the images. CT numbers were defined on a Hounsfield Unit (HU) scale where 0 equals the HU of water and -1000 equals the HU of air. The external contours of the waist were determined using a threshold of -224 HU

and the external bone contours were derived at 150 HU. For each participant the determination of soft tissue type was made using the bimodal image distribution histogram resulting from the distribution numbers in adipose tissue and non-adipose soft tissue ²³. Visceral fat tissue was manually distinguished from the subcutaneous fat tissue by tracing along the fascial plane defining the internal abdominal wall.

Comparison of DXA and CT

The DXA method distinguishes between fat mass and lean mass in soft tissues, whereas the CT provides muscle tissue volume and adipose tissue volume. These methodological differences were taken into account when comparing DXA to CT. All adipose tissue volumes from the 10 mm CT slices were multiplied by 3.96 to estimate the same area used for the DXA sub-region. Abdominal adipose tissue volumes were then multiplied by 0.923 to calculate adipose tissue mass (grams), where 0.923 is the assumed constant density of adipose tissue ²⁴. Adipose tissue by CT consists of 80% fat and a lean compartment of 20% water, proteins and minerals ²⁵. This lean compartment within adipose tissue is measured as lean tissue by DXA. Therefore, the lean compartment within adipose tissue was subtracted from adipose tissue by CT before comparison with DXA.

Statistical analysis

Data were analysed stratified by gender and race using SPSS for Windows version 9.0.1 (SPSS Inc., Chicago, USA). All values are expressed as mean \pm SD. To validate the DXA absolute measurements of total fat mass in the abdomen, sub-regions of DXA and CT scans were compared using the Students t-test. The method of Bland and Altman ²⁶ was used to compare DXA and CT measurements. In this method the difference between fat by CT and by DXA is plotted against the mean of CT and DXA measurements. Pearson's correlation coefficients and linear regression analysis were used to examine the association of anthropometric and DXA measurements with visceral fat by CT. Subsequently DXA was combined with one or more anthropometric measurements to improve the explained variance, using multiple linear regression with visceral fat by CT as dependent variable. Multi-colinearity between DXA measurements and anthropometric measurements was investigated by studying the tolerance and variance inflation factor (VIF). The stability of estimated parameters was not influenced by multi-colinearity.

Results

The participants' characteristics are shown in Table 1. Two subjects were excluded because of artefacts in the DXA scans that affected the soft tissue calculation of the trunk. Men were taller and heavier with a greater sagittal diameter, waist circumference and visceral fat by CT, but women had a higher fat percentage by DXA. No differences between sexes were found for BMI and total abdominal sub-region fat by DXA and total abdominal fat by CT. In men only visceral fat differed between races, with black persons having less visceral fat than white persons. Black women had a higher weight, BMI, sagittal diameter, waist circumference, total body fat by DXA, and abdominal fat by DXA and CT than white women. There was no difference in weight and

Table 1. Characteristics of the study participants (mean \pm SD)

	Ma	ale	Fem	ale
	White	Black	White	Black
n	41	33	40	34
Age (years)	73.5 ± 3.2	73.2 ± 3.1	74.1 ± 2.9	72.9 ± 3.4
Weight (kg) [§]	78.7 ± 9.3	82.2 ± 14.7	$61.8 \pm 10.5*$	73.7 ± 15.0
Height (m) §	172.0 ± 6.2	172.7 ± 6.3	159.8 ± 6.9	158.6 ± 6.6
Body mass index (kg/m²)	26.6 ± 3.0	27.5 ± 4.5	$24.2 \pm 3.8*$	29.2 ± 5.0
Sagittal diameter (cm) §	22.6 ± 2.5	23.4 ± 2.9	$19.7 \pm 2.7*$	23.1 ± 3.0
Waist circumference (cm) §	101.6 ± 8.1	100.1 ± 11.1	$93.4 \pm 11.5*$	100.1 ± 12.4
DXA total fat percent (%) §	30.3 ± 5.3	28.1 ± 7.8	$39.1 \pm 7.4*$	43.3 ± 7.7
DXA abdominal sub-region fat (g)	1046 ± 361	992 ± 467	944 ± 456*	1367 ± 553
DXA trunk fat (g)	13244 ± 4132	12067 ± 5500	11107 ± 4827*	14873 ± 6023
Total abdominal fat by CT (g)	1166 ± 317	1089 ± 426	$1098 \pm 420*$	1458 ± 483
Visceral abdominal fat by CT (g) §	520 ± 227*	371 ± 172	340 ± 166	362 ± 180

^{*} Significant difference between races within gender (p < 0.05)

visceral fat by CT between black and white women. In comparison with the total HABC cohort, the convenience sample was representative. Only white women in the convenience sample weighted slightly less and tended to have less abdominal fat compared to white women in the total cohort.

Validation of total abdominal fat by DXA

For the validation of DXA, total abdominal sub-region fat estimates were compared to CT (Table 2, Figure 2A). The two measurements were positively associated with a correlation coefficient (r) of 0.96 (0.87 for white men, 0.97 for black men, 0.97 for white women and 0.98 for black women; all p<0.001). Mean total abdominal sub-region fat measured by DXA was lower than by CT, the difference ranging from -91 \pm 128g in black women to -154 \pm 116 g in white women. The difference between DXA and CT for measuring total abdominal fat was approximately 10%. With the use of the Bland and Altman procedure a negative association was observed between the

Table 2. Validation of pencil beam DXA technique for measuring total fat mass in a sub-region at the abdomen against the CT method (mean \pm SD)

_	=					
		n	Fat by CT (g)	Fat by DXA (g)	Difference (g)	r
Abdominal fat	Men	74	1132 ± 369	1022 ± 410	-110 ± 156*	0.93
	White	41	1166 ± 317	1046 ± 361	-120 ± 177*	0.87
	Black	33	1089 ± 426	992 ± 467	-97 ± 125*	0.97
	Women	74	1264 ± 482	1139 ± 543	-125 ± 125*	0.98
	White	40	1098 ± 420	944 ± 456	-154 ± 116*	0.97
	Black	34	1458 ± 483	1367 ± 553	-91 ± 128*	0.98
	Total	148	1198 ±433	1080 ± 483	-117 ± 141*	0.96

^{*} p < 0.05 (Student's t-test)

[§] Significant difference between sexes, regardless of race (p < 0.05)

r, Pearson correlation coefficient

mean and the difference of the two methods (Figure 2B). Pearson correlation coefficients for this association were -0.33 (p=0.062) and -0.26 (p=0.105) for white and black men, and -0.31 (p=0.049) and -0.55 (p=0.001) for white and black women respectively. Exclusion of outliers (defined as those subjects for whom the difference between the two methods was greater then 2 SDs from the mean) did not change this association. To study whether it was the obesity level causing this association we corrected for total body fat mass by DXA. After adjustment for total body fat, the negative correlations were not significant anymore (correlation coefficient -0.12 and -0.13 for white and black men, and -0.11 and -0.24 for white and black women).

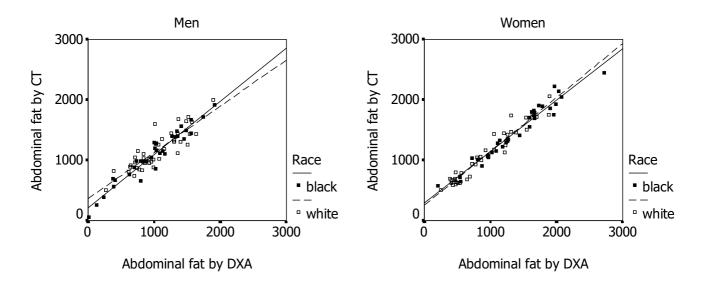


Figure 2A. Correlation between total fat measurements at the abdomen by DXA and by CT

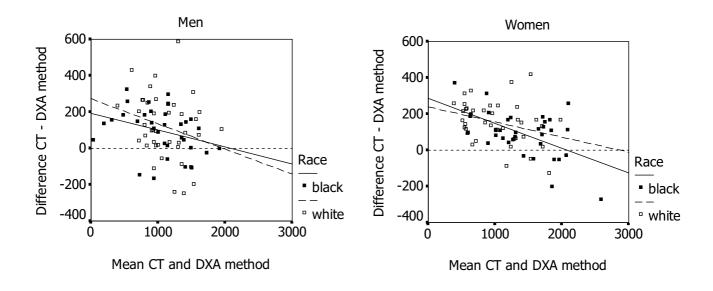


Figure 2B. Difference in total fat measurements between DXA and CT plotted against the mean

Estimation of visceral fat by DXA

Pearson's correlation coefficients of DXA fat and anthropometric measurements with visceral fat measurements by CT are given in Table 3. All DXA and anthropometric measurements were strongly correlated with visceral fat (p<0.001). The weakest relationships were observed for body weight and, in white persons, BMI. For white men and white and black women, the sagittal diameter had the strongest association with visceral fat. The strength of the association with DXA abdominal sub-region fat was comparable to that of the anthropometric measures; for black men it was slightly stronger. Of the DXA measurements, DXA abdominal sub-region fat had the strongest correlation with visceral fat in black persons, in white persons the DXA trunk fat correlated more with visceral fat. Overall there were no very large differences in correlations with visceral fat between DXA and anthropometric measures. DXA total body fat always had a lower correlation with CT visceral fat than the DXA abdominal sub-region fat or DXA trunk fat.

Table 3. Pearson's correlation coefficients between DXA and anthropometric measurements and visceral fat measured by CT

		Visceral fat (CT)			
	_	Male		Female	
	_	White	Black	White	Black
Anthropometry	Weight	0.548	0.648	0.734	0.500
	BMI	0.612	0.712	0.721	0.562
	Abdominal circumference	0.738	0.693	0.751	0.606
	Sagittal diameter	0.743	0.701	0.838	0.682
DXA	DXA abdominal sub-region fat	0.662	0.783	0.794	0.646
	DXA trunk fat	0.679	0.773	0.835	0.606
	DXA total fat	0.586	0.747	0.763	0.514

All coefficients p < 0.001

Regression analysis was performed to determine which measurement, or combination of measurements, was the best predictor for visceral fat by CT. Univariate models are shown in Table 4. Models including weight, BMI and DXA total fat are not shown because of their limited association with CT visceral fat. In accordance with Table 3, the models including sagittal diameter gave the highest explained variance in all groups (R^2 =0.553 for white men, R^2 =0.701 for white women, R^2 =0.465 for black women) except for black men, among whom the DXA abdominal subregion fat gave the highest explained variance (R^2 =0.614). Overall, the explained variance in black women was lower compared to the other groups. The DXA abdominal sub-region fat and the DXA trunk fat were comparable for predicting CT visceral fat.

Multivariate models are shown in Table 5. Because of the comparability of the results for DXA abdominal sub-region fat and DXA trunk fat, only multivariate models including DXA trunk fat are shown. DXA trunk fat is a standard variable provided by the DXA software, and therefore practically easier to obtain than the abdominal sub-region. Multivariate models combining DXA trunk fat with anthropometric measurements only gave limited improvement for prediction of CT visceral fat compared to the best univariate model. The largest improvement was found in white women. A combination of DXA trunk fat with sagittal diameter gave an explained variance of 0.744

Table 4. Univariate models including either an anthropometric or DXA body composition measure for prediction of visceral fat by CT

·			2	
	Model	$\beta \pm SE(g)$	R ²	SEE (g)
White men	abdominal circumference	20.8 ± 3.0	0.545	155.3
	sagittal diameter	66.4 ± 9.6	0.553	154.1
	DXA sub-region	0.4 ± 0.8	0.439	172.6
	DXA trunk fat	0.04 ± 0.01	0.462	169.0
Black men	abdominal circumference	10.7 ± 2.0	0.480	125.9
	sagittal diameter	42.1 ± 7.7	0.492	124.6
	DXA sub-region	0.29 ± 0.04	0.614	108.6
	DXA trunk fat	0.024 ± 0.004	0.597	110.9
White women	abdominal circumference	10.8 ± 1.5	0.563	110.8
	sagittal diameter	52.3 ± 5.6	0.701	92.9
	DXA sub-region	0.29 ± 0.04	0.631	101.9
	DXA trunk fat	0.029 ± 0.003	0.698	92.2
Black women	abdominal circumference	8.8 ± 2.0	0.367	145.8
	sagittal diameter	40.7 ± 7.7	0.465	134.0
	DXA sub-region	0.21 ± 0.04	0.417	139.9
	DXA trunk fat	0.018 ± 0.004	0.368	145.7
	C '' ' '' '' CC' '	0.004		

SEE, standard error of estimate; all coefficients p<0.001

compared to 0.701 when using sagittal diameter alone (increase of 4%). Overall, sagittal diameter explained slightly more than the abdominal circumference did. We also tested whether a combination of anthropometric measures would improve the prediction of CT visceral fat. A combination of sagittal diameter and abdominal circumference was not superior compared to the other univariate or multivariate models including DXA trunk fat, except in white men ($R^2 = 0.598$) (data not shown). Combining more than two variables did not improve the prediction of visceral fat significantly in any group; therefore these models are not shown.

Discussion

The results of the present study show that DXA is a good method to measure total abdominal fat in elderly men and women when a single slice CT is used as a gold standard, although DXA seems to underestimate total abdominal fat, especially in older people with less abdominal fat. This underestimation was found in both black and white subjects, in spite of differences in body composition. This study also shows that neither of the regional DXA measurements of abdominal fat (standard trunk region or manually defined abdominal slice) is superior to anthropometric measurements (waist circumference, sagittal diameter) in prediction of visceral fat by CT. A combination of DXA with anthropometry gave only limited improvement in the prediction of visceral fat by CT compared to univariate models.

Despite the higher fat percentage in women, men had a higher waist circumference and abdominal diameter in this study. This is in agreement with the greater tendency of men to accumulate excess fat in the abdominal region relative to the gluteal-femoral region. However, there was no difference in total abdominal fat between sexes. Considering the high correlation between abdominal fat measured by CT and DXA, it could be concluded that DXA measures total abdominal fat just as well as CT does. Especially in obese subjects, who are subjects of high interest, absolute differences in abdominal fat between DXA and CT were small. When the difference between the two measurements is plotted against the mean, a negative relationship was observed. This means that in subjects with less abdominal fat, DXA underestimates the amount of total abdominal fat compared to CT, or CT overestimates the amount of total abdominal fat compared to DXA. This phenomenon has been found in other studies. Tothill et al ²⁷ showed that pencil beam (Hologic QDR-1000W) underestimated total body fat and trunk fat at low degrees of phantom adiposity. Prior et al ²⁸ found that this pencil beam underestimated percent body fat in women with low BMI compared to a four-compartment model. However, as we used an Hologic 1500 and measured in a very different group of subjects we cannot assume that these conclusions hold for our data. There are also some other possible explanations for the discrepancy between DXA and CT. For the comparison between the two methods several assumptions were made. First, it was assumed that 80% of adipose tissue measured by CT consists of fat ²⁵. This percentage is based on several references representing a wide range of values for fat content of adipose tissue

Table 5. Multivariate models including DXA trunk fat and one anthropometric measure for prediction of visceral fat by CT

	М	odel	$\beta \pm SE(g)$	p value	R^2	SEE (g)
White men	1	DXA trunk fat	0.006 ± 0.013	0.665	0.555	155.7
		sagittal diameter	58.4 ± 20.7	0.008		
	2	DXA trunk fat	0.01 ± 0.01	0.298	0.558	155.1
		abdominal circumference	16.0 ± 5.5	0.006		
Black men	1	DXA trunk fat	0.021 ± 0.007	0.008	0.601	112.2
		sagittal diameter	7.2 ± 14.0	0.612		
	2	DXA trunk fat	0.021 ± 0.007	0.005	0.601	112.2
		abdominal circumference	1.9 ± 3.4	0.589		
White women	1	DXA trunk fat	0.02 ± 0.01	0.020	0.744	87.2
		sagittal diameter	25.7 ± 12.1	0.041		
	2	DXA trunk fat	0.02 ± 0.01	0.000	0.707	92.0
		abdominal circumference	2.5 ± 2.3	0.290		
Black women	1	DXA trunk fat	0.007 ± 0.006	0.228	0.490	133.0
		sagittal diameter	30.6 ± 11.2	0.011		
	2	DXA trunk fat	0.01 ± 0.01	0.120	0.415	142.3
		abdominal circumference	5.0 ± 3.1	0.122		

SEE, standard error of estimate

(62 to 91%). Whether this variation is caused by differences between races, or by changes in age or obesity level is not known, although some studies have suggested that the fraction of lipid in adipose tissue varies with obesity level ²⁹. The variation might also reflect differences in fat content (and proportion) between subcutaneous and visceral adipose tissue. This has not been studied yet. Secondly, 0.923 g/ml is the assumed constant density of adipose tissue ²⁴. The literature reveals a considerable range of reported densities. It is not known whether these differences result from technical reasons (source of study tissue; handling of the tissue specimens), or whether they are due to subject factors such as age, sex, race, or total body fatness, which might also influence tissue densities. Again, it is not known whether there are differences in densities between subcutaneous and visceral fat. To study the importance of the assumed fat content and fat density we performed a sensitivity analysis by varying these factors. When we compared total abdominal fat by DXA and by CT lowering the assumed fat content or density, the mean absolute difference between the two measures became smaller. For example when we lowered the fat content of CT fat to 74% (as suggested by Wang and Pierson 30) instead of 80%, the mean difference in total abdominal fat was 27.4 ± 151 g for the total group (p = 0.03). However, the correlations between the mean and the difference (Bland and Altman plot) became higher (-0.39 and -0.54 in white and black male, -0.54 and -0.72 for white and black female), indicating that individual differences between DXA and CT became bigger and were depended on abdominal obesity level. When we increased the fat content or density, the mean difference total abdominal fat became bigger and the correlations between the mean and the difference approached zero. It can be concluded from this analysis that absolute differences in abdominal fat between CT and DXA are influenced by the assumed values of densities. However, our regression analyses to predict visceral fat are not influenced by these assumed factors. Thirdly, the CT scan is located at the L4-L5 disk space. We used the iliac crest as a reproducible landmark to locate the DXA abdominal sub-region. This area was relatively free of bone and solid organs and therefore should more closely reflect the true abdominal fat without having to rely on algorithmic interpolations to compute the fat. As can be observed in Figure 1, the DXA sub-region is likely to be placed a little more cranially than the L4-L5 disk space on same patients. Differences in the placement of the DXA vs. CT scans may have caused differences in the amount of total abdominal fat because of the (convex) shape of the abdomen. In addition, the subjects' position during scanning differs for CT and DXA. For the CT scan the subjects lay their arms above their heads and their legs are elevated with a cushion, while for DXA scanning the subjects lay supine with their arms along their body and legs stretched. The last explanation could be found in the CT scan analyses. The pixels in a CT scan are classified as either adipose or non-adipose, although some may contain both tissues. In lean subjects relatively more pixels with combined tissues may be present causing potential misclassification (the so-called partial volume phenomenon ⁵).

Simple anthropometric measurements have been used to estimate visceral fat. A traditional measurement to identify subjects at increased health risk due to the accumulation of visceral fat is the waist-to-hip ratio (WHR). More recently, several studies have indicated that waist circumference is more strongly related to health risks associated with obesity than WHR and that it correlates more with visceral fat by CT ^{31,32}. A relatively new anthropometric measurement to estimate visceral fat and to predict cardiovascular risks is the sagittal abdominal diameter. Kahn et

al. showed possible associations between the sagittal diameter and ischemic heart disease, and Pouliot et al. demonstrated that the sagittal diameter predicts cardiovascular risk just as well as waist circumference ^{33,34}. In a study by Turcato et al., it was shown that the waist and sagittal diameter are the anthropometric measures that are most closely related to CVD risk factors in old age ³². It has been suggested that the assessment of the amount of visceral fat by the sagittal diameter could be improved by extraction of the subcutaneous fat layer measured by skin fold thickness ³⁵. However, especially these measurements are inaccurate in obese and older subjects, who are the subjects of interest considering their increased health risks. Our results confirm the prediction qualities of the abdominal circumference and the sagittal diameter for visceral fat by CT in older subjects. An abdominal sub-region from a total body DXA-scan, not subject to the methodological drawbacks of anthropometry, was not superior or could not improve the prediction of visceral fat.

The variability of the abdominal DXA sub-region has never been reported in the literature. However, the coefficient of variation (CV) for a similar DXA sub-region at the mid-thigh, based on repeated reading of the same scan, was 4.3% for lean soft mass ³⁶ and 7.8% for fat mass ³⁷. We calculated the variability of the abdominal DXA sub-region based on a single reading of duplicate scans of 11 individuals aged 61-85 years and found a CV of 3.9% for total abdominal fat (unpublished data). This variation is similar to reported CV of about 2% for abdominal circumferences in the general population, which is likely to be higher in elderly persons ^{5,14}. In the HABC study CVs of 0.79% for sagittal diameter and 2.31% for abdominal circumference were found (unpublished data).

Consistent with our results, Kamel et al. did not find a considerable difference between anthropometry and DXA sub-regions to predict visceral fat, in a group of non-obese subjects ¹⁸. Only in women the DXA was just slightly better than anthropometry to predict visceral fat. However, the univariate correlations of DXA and anthropometric measures with visceral fat were all higher than in our study, and a combination of abdominal fat by DXA and waist to predict visceral fat gave a R² of 0.77 for women and 0.81 for men. Kamel did the same analysis in a group of obese subjects ²⁰. The correlations between visceral fat and prediction measures were about the same in women (0.75 with abdominal circumference, 0.74 with DXA sub-region) and much lower in men (NS with abdominal circumference, 0.46 with DXA sub-region) compared to our study. In contrast to the analysis in non-obese, DXA was just slightly better than anthropometry to predict visceral fat in men, not in women. In a study by Svendsen et al. measurements of abdominal fat by DXA explained about 80% of the variation of visceral fat, while the sagittal diameter only explained 60% ³⁸. Bertin et al. found the opposite result ²¹: in a group very obese subjects the univariate correlations with visceral fat were highest for the sagittal diameter (0.74 in women, 0.67 in men) compared to abdominal fat mass by DXA (0.57 in women, 0.51 in men). Results from earlier studies concerning the prediction of visceral fat by DXA and anthropometry are not very consistent and seem to depend on obesity level. But they are all conducted in small groups with very large age ranges and no study performed measurements in subjects above 70 years of age.

In conclusion, DXA is a good alternative to CT for predicting total abdominal fat in an elderly population. For the prediction of visceral fat, the sagittal diameter, which has a practical advantage compared to DXA, is just as effective.

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Diab Care 2003 May; 26:1656-7 (Letter)



Letter to the Editor, response to Han et al.¹

We read with great interest the paper recently published in the journal by Han et al. who studied the association between baseline levels of C-reactive protein (CRP) and the 6-year incidence of the metabolic syndrome and type 2 diabetes in Mexican subjects 1 . They found an odds ratio (OR) of 4.1 (95% CI 2 .1 – 8.0) for developing the metabolic syndrome and an OR of 5.4 (2 .2 – 13.4) for incident type 2 diabetes, among women in the highest compared to the lowest tertile of CRP. ORs were adjusted for age, smoking, physical activity and alcohol intake. In men no such association was found. Additional adjustment for body mass index (BMI) slightly lowered the ORs, whereas adjustment for waist-to-hip ratio (WHR) did not.

We have studied baseline CRP levels and its relation to incident type 2 diabetes in an age-, sex- and glucose-stratified sample of the Hoorn Study, a population-based cohort study of glucose tolerance among Caucasian people 2 . The study methods and follow-up duration are closely similar to the Mexico City Diabetes Study (MCDS) described by Han et al., except that our subjects were about 15 years older at baseline. Glucose tolerance status was assessed by a 75-g oral glucose tolerance test at baseline and after 6.4 years of follow-up. CRP was measured in plasma by high sensitive ELISA, while information was available on BMI, WHR, smoking and physical activity. Of the 140 men and 139 women who had follow-up measurements and were free of diabetes at baseline, 17.8% of the men and 20.9% of the women developed diabetes. In contrast to the findings of Han et al. we did not observe an association between baseline CRP levels and incident diabetes in women, while in men we found an OR of 3.0 (1.0 – 9.3) in the highest compared to the lowest tertile of CRP, after adjustment for age (Table).

Table. Relative risk (odds ratios with 95% confidence intervals) associated with high C-reactive protein (second and third tertile compared to first tertile) for developing type 2 diabetes after 6.4 years of follow-up in the Hoorn Study

		M	en	Women		
Model		2nd tertile	3rd tertile	2nd tertile	3rd tertile	
1	Age	1.4 (0.4 – 4.8)	3.0 (1.0 – 9.3)	0.7 (0.3 – 2.1)	1.1 (0.4 – 3.0)	
2	Model 1 + BMI	1.4 (0.4 – 4.8)	3.0 (1.0 – 9.3)	0.6 (0.2 – 1.8)	0.9 (0.3 – 2.5)	
3	Model 1 + WHR	1.4 (0.4 – 4.8)	1.9 (0.6 – 6.4)	0.6 (0.2 – 1.7)	0.9(0.3 - 2.5)	
4	Model 1 + smoking	1.3 (0.4 – 4.7)	2.9 (0.9 – 9.2)	0.7 (0.3 – 2.1)	1.2 (0.4 – 3.2)	
5	Model 1 + physical activity	1.4 (0.4 – 4.7)	3.0 (1.0 – 9.3)	0.7 (0.3 – 2.1)	1.1 (0.4 – 3.0)	

BMI, body mass index; WHR, waist-to-hip ratio

Further adjustment for BMI, smoking or physical activity did not materially change these results, while adding WHR to the model substantially lowered the OR in men. Thus, in the Hoorn Study CRP is not a very strong determinant of the development of type 2 diabetes, in contrast to WHR and impaired glucose metabolism 3 . We realise that our study sample of 279 subjects is much smaller than the study presented by Han et al. (n=1244). The relationship in men, however, was strong, whereas in the MCDS it was strong only in women. In the previous prospective study by Barzilay et al. an OR of 2.0 (1.4 – 2.9) was found when extreme quartiles of CRP were compared 4 . They did not analyse men and women, or black and white subjects separately. Pradhan et al.

found an OR of 4.2 (1.5 - 12.0) in women 5 . Both studies did not take WHR into account. Ethnicity may also play an important role explaining the inconsistent results.

In conclusion, we suggest caution in the interpretation of the results because of inconsistent findings, in particular between sexes. It is unclear why inflammation would be important in the pathogenesis of type 2 diabetes only in women or only in men.

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Summary

Due to the increased availability of food and the greater energy density of food in combination with a more sedentary lifestyle there is a worldwide increase in the prevalence of obesity (severe overweight). Type 2 diabetes mellitus is the most apparent health consequence of obesity. Type 2 diabetes is associated with microvascular and cardiovascular complications. Therefore, type 2 diabetes has a large and increasing impact on the health care system and on health related quality of life.

Type 2 diabetes is characterized by chronically elevated blood glucose concentrations due to relative lack of insulin. It mostly develops gradually, in contrast to type 1 diabetes, which usually becomes apparent in childhood. Type 2 diabetes is most prevalent among middle-aged and elderly people, however, due to the alarming increase of obesity among people of all ages, type 2 diabetes is increasingly being diagnosed at younger ages and even in children.

Apart from overall obesity, the distribution of body fat influences health risk. An abdominal (upper body) fat distribution is associated with increased risk of type 2 diabetes and cardiovascular disease. In particular intra-abdominal fat (also referred to as 'visceral fat'), is thought to play an important role in the aetiology of insulin resistance and type 2 diabetes, whereas abdominal subcutaneous fat seems less important. In addition to high glucose levels, type 2 diabetes is also characterised by an adverse cardiovascular risk profile including high levels of triglycerides, low levels of high-density lipoprotein cholesterol, hypertension and hyperinsulinaemia. Together with abdominal obesity, the clustering of these risk factors is referred to as 'the metabolic syndrome'.

Waist circumference is the strongest correlate of visceral fat. Waist circumference, however, is not always a stronger predictor of type 2 diabetes and other cardiovascular risk than is waist-to-hip ratio (WHR). A higher WHR can be the result of a larger waist as well as smaller hips. This suggests that smaller hips (representing either lower muscle mass and/or lower fat mass at the hips) may be involved in determining the increased risk associated with an increased WHR.

The aim of the present thesis was to investigate different aspects of body composition (the role of different fat depots and lean tissue) in relation to type 2 diabetes and cardiovascular risk. Specifically, we formulated the following main research questions:

- 1. What are the separate associations of waist and hip circumferences, independently of each other and of overall obesity (BMI), with glucose metabolism and other components of the metabolic syndrome?
- 2. Do these associations differ between men and women, between different age groups, and between different ethnic populations?
- 3. Which tissues (lean or fat tissue) are reflected by the waist circumference and by the hip circumference?
- 4. Are these tissues independently associated with glucose levels and other components of the metabolic syndrome?
- 5. What are the independent relations of visceral fat, abdominal subcutaneous fat, subcutaneous thigh fat, intermuscular thigh fat with glucose levels and other components of the metabolic syndrome?

In Chapter 2, cross-sectional associations of waist and hip circumferences with measures of glucose metabolism were investigated in the Hoorn Study (age participants 50 to 75 years). We found that, as expected, a larger waist circumference was associated with higher levels of fasting glucose, insulin, HbA1c, and postload glucose. After adjustment for waist circumference, age, and BMI, however, a larger hip circumference was associated with lower levels of fasting glucose, insulin, HbA1c, and postload glucose, in both sexes. For thigh circumference similar results were found, but these associations were statistically significant in women only.

In Chapter 3 the associations of waist and hip circumference with glucose metabolism were studied longitudinally. In 1996 all surviving cohort members of the Hoorn Study were invited to participate in a second health examination. We observed that among persons free of diabetes at baseline, those with a larger hip or thigh circumference at baseline were at lower risk to develop diabetes after 6 years, taking baseline age, BMI and waist circumference into account.

In a larger Australian population-based study, the AusDiab Study, we confirmed the cross-sectional results of the Hoorn Study by showing that a larger hip circumference was independently associated with lower glucose levels (Chapter 4). Furthermore, we extended our observations by considering additional components of the metabolic syndrome as outcome, i.e. hypertension and dyslipidemia. The AusDiab Study covers a wide age range (all ages above 25 years), and the independent association of larger hip circumference with more favourable levels of glucose, triglycerides and HDL-cholesterol, was apparent across all age groups, except in persons aged 75 years or older. The associations with blood pressure were weak.

In population studies at Mauritius, Rodrigues, Nauru, and Papua New Guinea we found that the independent associations of waist circumference with unfavourable glucose and lipid levels, and of hip circumferences with more favourable glucose and lipid levels, were consistent in different ethnic populations, despite considerable differences in body composition and susceptibility to diabetes (Chapter 5). The association with blood pressure, again, were weak.

Use of dual energy x-ray absorptiometry (DXA) enabled us to distinguish between fat mass and lean mass in the total body, but also in specific regions of the body. In the third health examination of the Hoorn Study in 2001, when participants were aged 60 to 85 years, we found that waist circumference mainly reflects trunk fat mass, and is hardly influenced by the amount of lean mass in the trunk, in both sexes (Chapter 6). The variability of hip circumference was mostly determined by fat mass in the legs, but was also largely dependent of the amount of leg lean mass, particularly in men. Accumulation of fat mass in the legs as well as a larger lean (muscle) mass in the legs was associated with a better glucose tolerance status, after adjustment for trunk fat mass.

In Chapter 7 we investigated whether different regional fat depots or lean tissue were determinants of arterial stiffness, a marker of impaired vascular function which is related to cardiovascular disease. Larger trunk fat mass by DXA was associated with higher peripheral arterial stiffness, but not with central arterial stiffness. Leg fat had opposite, although weaker, associations with peripheral arterial stiffness. Larger leg lean mass was the most important determinant of lower central arterial stiffness.

Use of computed tomography (CT) enables distinction between visceral fat and subcutaneous fat in the abdomen, and distinction between subcutaneous and intermuscular fat in

the legs. In Chapter 8 we related all these separate fat depots to components of the metabolic syndrome. In participants of the Health ABC Study (aged 70 to 79 years) the CT results showed that waist circumference was mainly determined by subcutaneous fat from the abdomen, and to a lesser extent to visceral fat (Chapter 8). Thigh circumference reflected mainly subcutaneous fat in women, whereas muscle and subcutaneous fat both contributed considerably to thigh circumference in men. Furthermore, visceral fat was the strongest independent determinant of unfavourable glucose and lipid levels, although abdominal subcutaneous fat also significantly contributed to unfavourable levels. In contrast, thigh subcutaneous fat was independently associated with more favourable lipid levels in both sexes, and with lower glucose levels in men.

Because visceral fat is probably the most important culprit for the metabolic syndrome and cardiovascular disease, but often too expensive and time-consuming to measure in clinical practice and epidemiological studies, it is important to assess this fat depot by more practical measures. In a sub-sample of 150 subjects of the Health ABC Study population, we found that measurement of total abdominal fat in a 4 cm slice at the abdomen by DXA (a sub-region comparable to the CT slice used) was highly correlated with total abdominal fat measured by CT. However, DXA tended to underestimate abdominal fat in relatively older people with less abdominal fat (Chapter 9). We also showed that regional DXA measurements (both total trunk fat and 4 cm slice sub-region) of abdominal fat were not stronger associated to visceral fat than anthropometric measures (waist circumference and sagittal diameter).

In conclusion, apart from overall obesity, body fat distribution and muscle mass are important determinants of insulin resistance, the metabolic syndrome, diabetes and cardiovascular risk.

In addition to the well-known detrimental effects of visceral fat, it was shown that abdominal and femoral-gluteal subcutaneous fat depots are differently related to the metabolic profile. The abdominal subcutaneous fat depot also has a detrimental association, in addition to visceral fat, whereas the fat depot in the femoral-gluteal region has no detrimental effect and may even be beneficial. Larger skeletal muscle mass is related to a more favourable metabolic profile.

It is suggested that, due to lower lipolytic activity compared to abdominal fat, femoral-gluteal fat is more likely to take up free fatty acids (FFA) from the circulation. Hereby, it protects other organs against high FFA exposure, which is associated insulin resistance. Also differences between abdominal and femoral-gluteal fat depots in secretion of adipokines, which are involved in the glucose metabolism, may be involved in the pathophysiological mechanism. More pathophysiological research, however, is needed.

Anthropometric measures, such as waist and hip circumferences, sagittal diameter, or BMI, cannot accurately distinguish between body composition characteristics (different fat depots and lean tissue). This should be kept in mind when interpreting results of studies on body composition in relation to health risk.

This thesis provides important insight in the relation between body composition, type 2 diabetes and cardiovascular disease risk. This insight and future additional knowledge of underlying mechanisms may contribute to the development of prevention strategies targeted on nutrition, lifestyle, and drugs to stop the increase in prevalence and incidence of type 2 diabetes and cardiovascular disease.





Type 2 diabetes en vetverdeling

Een grotere beschikbaarheid van voedsel, een grotere energiedichtheid van het voedsel, en ongunstige veranderingen in het activiteitenpatroon, veroorzaken gezamenlijk een wereldwijde toename in het voorkomen van obesitas (ernstig overgewicht). De meest duidelijke gezondheidsconsequentie van obesitas is type 2 diabetes (ouderdomssuikerziekte). Type 2 diabetes wordt gekenmerkt door chronisch verhoogde glucose concentraties (suikerspiegels) in het bloed, veroorzaakt door een relatief tekort aan insuline. Insuline is een hormoon dat zorgt voor de opname van glucose uit het bloed in de weefsels. Bij een relatief tekort aan insuline, doordat de weefsels ongevoelig worden voor dit hormoon, wordt de glucose niet goed opgenomen, wat resulteert in te hoge glucose concentraties in het bloed.

Type 2 diabetes gaat gepaard met een hoger risico op macrovasculaire complicaties, zoals hartinfarcten en beroertes, en met microvasculaire complicaties, zoals beschadigingen aan ogen en nieren,. Daardoor heeft type 2 diabetes een grote impact op het gezondheidszorgsysteem en op de gezondheidsgerelateerde kwaliteit van leven. Type 2 diabetes komt het meest voor op middelbare en oudere leeftijd, maar door de enorme toename van obesitas bij mensen van alle leeftijden wordt type 2 diabetes ook steeds vaker gediagnosticeerd op jongere leeftijd en zelfs bij kinderen.

Naast het totale gewicht is ook de verdeling van het vet over het lichaam van invloed op het gezondheidsrisico. Meer vetopslag in de buik (appelvorm) gaat samen met een verhoogd risico op type 2 diabetes en hart- en vaatziekten, in tegenstelling tot meer vetopslag in de heupen en benen (peervorm). Met name het viscerale vet (vet binnenin de buikholte, tussen de organen) lijkt een belangrijke rol te spelen in het onderliggende mechanisme, terwijl het subcutaan vet (het oppervlakkige vet, onder de huid) op de buik minder belangrijk lijkt. (Voor het onderscheid tussen visceraal en subcutaan buikvet zie Figuur pagina 168.)

Naast hoge glucose concentraties in het bloed komen bij mensen met type 2 diabetes ook vaak andere ongunstige risicofactoren voor hart- en vaatziekten voor, zoals hoge concentraties van triglyceriden en LDL-cholesterol (slechte vetten in het bloed), lage concentraties van HDL-cholesterol (gunstige vetten in het bloed) en een hoge bloeddruk. Samen met meer vetopslag in de buik, wordt de clustering van al deze risicofactoren vaak het 'metabool syndroom' genoemd.

De onderzoeksvragen

Een maat om de hoeveelheid lichaamsvet te meten is de body mass index (BMI), het gewicht gedeeld door de lengte in het kwadraat. Als maat voor vetverdeling hanteert men vaak de middelomtrek, of de middelomtrek gedeeld door de heupomtrek (middel-heupomtrek ratio). Dit zijn echter ruwe schattingen van overmatige vetopslag en vetverdeling, aangezien er geen onderscheid gemaakt kan worden tussen vet en vetvrij weefsel. In bepaalde groepen mensen kunnen deze ruwe schattingen daardoor geen goede maten zijn. Bijvoorbeeld bij ouderen, omdat de verhouding vet weefsel en vetvrij weefsel verandert bij het ouder worden.

Van deze simpele methoden is de middelomtrek het sterkst gerelateerd met visceraal vet (het meest schadelijke vet binnenin de buik, tussen de organen). De middelomtrek is echter niet altijd een sterkere voorspeller voor het krijgen van type 2 diabetes en hart- en vaatziekten dan de middel-heupomtrek ratio. Dit zou kunnen betekenen dat de heupomtrek ook iets toevoegt bij het voorspellen van deze ziekten. Een hogere middel-heupomtrek ratio kan namelijk zowel het resultaat zijn van een grotere middelomtrek als van een kleinere heupomtrek. Smallere heupen spelen dus mogelijk ook een rol bij het verhoogde risico bij mensen met een hogere middelheupomtrek ratio.

Het wordt over het algemeen aangenomen dat we met het meten van de middel- en heupomtrekken de mate van vetopslag op deze plaatsen meten. Het is echter onduidelijk of de hoeveelheid van vetvrij weefsel (bijvoorbeeld spiermassa) mogelijk ook van invloed is op het risico op type 2 diabetes.

Het doel van dit proefschrift is om de verschillende aspecten van de lichaamssamenstelling (de rol van vet op verschillende plaatsen en de rol van vetvrij weefsel) te bestuderen in relatie met het risico op hoge glucose concentraties en diabetes, en op andere componenten van het metabool syndroom (ongunstige vetten in het bloed, hoge bloeddruk). We hebben de volgende onderzoeksvragen geformuleerd:

- 1. Wat zijn de relaties van de middelomtrek en de heupomtrek -onafhankelijk van elkaar en onafhankelijk van totaal gewicht (BMI)- met glucose concentraties en andere componenten van het metabool syndroom?
- 2. Zijn deze relaties verschillend tussen mannen en vrouwen, tussen verschillende leeftijdsgroepen en tussen verschillende etnische groepen?
- 3. Welke weefsels (vet of vetvrij weefsel) worden gemeten door de middelomtrek en door de heupomtrek?
- 4. Zijn deze weefsels onafhankelijk gerelateerd met glucose concentraties en andere componenten van het metabool syndroom?
- 5. Wat zijn de onafhankelijke relaties van visceraal vet, subcutaan vet op de buik, subcutaan vet op de benen, en intermusculair vet (vet tussen de spiervezels) met glucose concentraties en andere componenten van het metabool syndroom?

De resultaten

In hoofdstuk 2 zijn de relaties bestudeerd van middel- en heupomtrekken met glucose concentraties bij deelnemers van de Hoornstudie. De Hoornstudie is een populatiestudie die in 1989 is begonnen toen de deelnemers 50 tot 75 jaar oud waren. Zoals verwacht was een grotere middelomvang gerelateerd met hogere glucose concentraties, onafhankelijk van de leeftijd, BMI en heupomtrek. Onafhankelijk van de middelomtrek, leeftijd en BMI, was een grotere heupomtrek echter gerelateerd met lagere glucose concentraties, bij zowel mannen als vrouwen.

In 1996 zijn alle deelnemers van de Hoornstudie die nog in leven waren opnieuw uitgenodigd om deel te nemen aan een onderzoek, waarbij onder andere opnieuw glucose concentraties werden gemeten. In hoofdstuk 3 beschrijven we dat van de mensen die geen diabetes hadden aan het begin van de studie in 1989, de mensen met een grotere heupomtrek

een lager risico hadden om diabetes te ontwikkelen na ongeveer 6 jaar, vergeleken met mensen met een kleinere heupomtrek, onafhankelijk van hun middelomtrek, leeftijd en BMI.

In een grotere Australische populatiestudie, de AusDiab-studie, hebben we de resultaten van de Hoornstudie kunnen bevestigen (hoofdstuk 4). Een grotere heupomtrek was onafhankelijk gerelateerd met lagere glucose concentraties. Eveneens was een grotere heupomtrek ook gerelateerd met gunstigere lipiden (vetten in het bloed). In de AusDiab-studie hebben personen deelgenomen van alle leeftijden boven de 25 jaar. De onafhankelijke relatie tussen een grotere heupomtrek en meer gunstige concentraties van glucose en lipiden in het bloed bestond in alle leeftijdsgroepen, behalve bij mensen boven de 75 jaar.

In populatiestudies uitgevoerd op de eilanden Mauritius, Rodrigues, Nauru en Papua Nieuw Guinea vonden we dezelfde relatie tussen een grotere heupomtrek en gunstigere glucose en lipiden concentraties in verschillende etnische groepen. Deze relatie was weer onafhankelijk van de middelomtrek die geassocieerd was met ongunstige glucose en lipiden concentraties.

Door gebruik te maken van een zogenaamde dual energy x-ray absorptiometry (DEXA) scan kan er onderscheid gemaakt worden tussen vetmassa en vetvrije massa in het totale lichaam, of in specifieke gedeelten van het lichaam (zoals buik of benen). In het derde vervolgonderzoek van de Hoornstudie in 2001, de deelnemers waren toen 60 tot 85 jaar oud, is tijdens het lichamelijk onderzoek een DEXA scan gemaakt. We vonden dat een grotere middelomtrek voornamelijk een grotere vetmassa in de buik reflecteerde, terwijl de vetvrije massa in de buik nauwelijks invloed had op de grootte van de middelomtrek (hoofdstuk 6). De variabiliteit van de heupomtrek werd bepaald door vetmassa in de benen, maar ook door de vetvrije massa in de benen (voornamelijk spieren), vooral bij mannen. Zowel een grotere vetopslag in de benen als een grotere vetvrije massa in de benen was geassocieerd met een betere glucose concentraties, onafhankelijk van de hoeveelheid vet en vetvrije massa in de buik.

In hoofdstuk 7 onderzochten we of verschillend gelokaliseerde vetmassa's (in de buik of in de benen) en vetvrije massa factoren zijn die van invloed zijn op vaatstijfheid. Stijvere vaten zijn een marker voor een gestoorde vaatfunctie, dat weer gerelateerd is met meer hart- en vaatziekten. Een grotere vetmassa in de buik gemeten met een DEXA scan was gerelateerd met meer stijfheid in de perifere vaten (in arm, hals en bovenbeen) maar niet met meer stijfheid in de centrale vaten (aorta). Vetmassa in de benen was gerelateerd met minder stijfheid in de perifere vaten. Een grotere vetvrije massa in de benen was de sterkste determinant van minder stijfheid in de centrale vaten.

Door het gebruik van computer tomography (CT) scans kan er nog verder onderscheid gemaakt worden tussen subcutaan vet en visceraal vet in een dwarsdoorsnede van de buik (Figuur), en tussen subcutaan vet en intermusculair vet in een dwarsdoorsnede van de benen. Gebruik makend van CT gegevens van de Health ABC studie, een Amerikaanse populatiestudie met deelnemers van 70 tot 79 jaar oud, hebben we in hoofdstuk 8 al deze verschillende vet depots gerelateerd aan verschillende componenten van het metabool syndroom. Meer visceraal vet was de belangrijkste factor voor ongunstige glucose en lipiden concentraties, maar ook subcutaan vet in de buik droeg onafhankelijk bij aan ongunstige concentraties. Subcutaan vet in de benen daarentegen was gerelateerd aan meer gunstige lipiden concentraties bij vrouwen, en met meer

Visceraal vet



Subcutaan vet

Figuur. Dwarsdoorsnede van de buik (CT scan) waarmee onderscheid gemaakt kan worden tussen visceraal vet (vet binnenin de buik, tussen de organen) en subcutaan vet (oppervlakkige vet, vlak onder de huid). De lichtergekleurde gebieden zijn spieren, botten en organen.

gunstige lipiden en glucose concentraties bij mannen. Daarnaast bleek dat de middelomtrek met name bepaald werd door de hoeveelheid subcutaan vet op de buik, en niet zozeer door de hoeveelheid visceraal vet. De dijomtrek reflecteerde voornamelijk subcutaan vet bij vrouwen, terwijl bij mannen zowel spier als subcutaan vet in de benen bijdroegen aan het bepalen van de dijomtrek.

Omdat visceraal vet de belangrijkste factor is voor ongunstige glucose en lipiden concentraties, maar de methode om dit te meten (CT) erg duur en tijdrovend is, is het belangrijk om dit vet goed te kunnen schatten met een meer praktische methode. Bij 150 personen van de Health ABC studie hebben we onderzocht of een DEXA scan (die goedkoper is dan een CT scan) misschien een meerwaarde heeft boven de simpele omtrekmaten zoals de middelomtrek of de sagittale diameter (de afstand tussen de rug en de buik). Dit bleek echter niet het geval.

De conclusies

We kunnen concluderen dat behalve het totale gewicht de verdeling van het vet over het lichaam en de vetvrije massa (spiermassa) belangrijke factoren zijn van glucose concentraties, type 2 diabetes en andere componenten van het metabool syndroom.

Naast het bekende ongunstige effect van visceraal vet, vonden we dat ook subcutaan vet op de buik een ongunstige relatie heeft met het metabool syndroom. Het vet in de benen heeft geen negatieve relatie en lijkt zelfs gunstig. Ook een grotere spiermassa is gunstig voor een beter metabool profiel.

Door verschillen in fysiologische activiteit tussen het vet in de buik en het vet in de benen is het laatste meer geneigd om vrije vetzuren op te nemen uit het bloed. Op deze manier beschermt dit vet mogelijk andere organen en weefsels tegen een hoge blootstelling aan vrije vetzuren. Een hoge blootstelling aan vrije vetzuren speelt een rol bij het ontstaan van de ongevoeligheid van weefsels voor insuline, wat hoge glucose concentraties tot gevolg heeft. Op

deze manier kan de beschermende relatie van vet in de benen tegen ongunstige glucose en lipiden concentraties worden verklaard.

Vetweefsel scheidt ook veel eiwitten en andere stoffen uit, zogenaamde adipokinen, waarvan sommigen mogelijk invloed hebben op de glucosestofwisseling. Het zou kunnen dat het buikvet en het beenvet verschillen in de uitscheiding van bepaalde adipokinen, waardoor de verschillende relaties met de glucose en lipiden concentraties kunnen worden verklaard. Meer onderzoek op dit gebied is echter nodig.

Simpele maten voor gewicht en vetverdeling, zoals BMI, middel- en heupomtrekken, en middel-heupomtrek ratio, kunnen geen goed onderscheid maken tussen vet en vetvrije massa, en tussen verschillende vet depots. Doordat deze componenten van lichaamssamenstelling wel verschillende relaties hebben met gezondheidsrisico's moet hier rekening mee worden gehouden bij de interpretatie van de resultaten van studies die deze simpele maten gebruiken.

Dit proefschrift geeft meer inzicht in de relatie tussen lichaamssamenstelling en type 2 diabetes en cardiovasculair risico. Dit inzicht en toekomstige kennis van onderliggende mechanismen kan mogelijk bijdragen aan de ontwikkeling van preventie op het gebied van voeding, leefgewoonten en medicatie om de toename van type 2 diabetes en hart- en vaatziekten te stoppen.





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Curriculum Vitae

Marieke Snijder was born on December 29th 1975 in Vlissingen, the Netherlands. In 1994 she graduated from secondary school (Stedelijke Scholengemeenschap Middelburg) and started her study Human Nutrition and Health at the Department of Human Nutrition and Epidemiology at Wageningen University. As part of this study, she conducted a research project on the effect of body build on the validity of predicted body fat from body mass index and bioelectrical impedance. She fulfilled two traineeships, at the Department of Nutritional Research of the National Medical Center in Mexico-city, and at the Department of Nutritional Sciences of Numico Research BV in Wageningen. After she graduated in 1999, she started working on the present thesis at the Institute for Research in Extramural Medicine (EMGO Institute) at the VU University Medical Center in Amsterdam. During this PhD period she followed the Postgraduate Epidemiology Program of the EMGO Institute and attended several statistical and methodological courses given by Stanley Lemeshow, David Hosmer, and Kenneth Rothman. In the winter of 2003 she spent three months at the International Diabetes Institute in Melbourne in the group of Paul Zimmet to work on two manuscripts included in this thesis (Chapters 4 and 5). Currently, she is working as a post-doctoral research fellow at the EMGO Institute and at the Department of Nutrition and Health, Faculty of Earth and Life Sciences, at the VU University Amsterdam.

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