ESTIMATION OF VISCERAL ADIPOSE TISSUE AND BODY FAT IN MIDDLE AGED MEN

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE in

THE FACULTY OF GRADUATE STUDIES (HUMAN KINETICS)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

July, 1999

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ABSTRACT

BACKGROUND: Human obesity is associated with a large number or diseases and metabolic complications such as heart disease, diabetes mellitus, hypertentension, gallbladder disease and some types of cancer (Bjorntorp, 1990; Depres et al., 1991). Though a causative relationship has not been established between adipose tissue (AT) distribution and these metabolic disturbances, both prospective and epidemiological studies have demonstrated that measures of visceral adipose tissue (VAT) stores are strong predictors of coronary heart disease, diabetes and stroke. Thus the ability to measure abdominal AT, especially VAT, may be important in epidemiological and clinical research. Advanced imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) offer new promise for the visualization and quantification of abdominal AT masses. However, these imaging techniques are expensive, not generally available, and in the case of CT, expose the subjects to ionizing radiation.

OBJECTIVE: The aim of this study was to offer a less expensive, quick, safe and accurate method to predict VAT and body fat (BF) using measurements from dual-energy X-ray absorptiometry (DXA) and anthropometry. Eleven men over 50 (50-69 y) participated in this study with a wide range of Body Mass Index’s. VAT, subcutaneous abdominal adipose tissue (SAAT) and total abdominal adipose tissue (TAAT) was measured from the 1st to the 5th lumbar vertebrae using MRI as the criterion measure. BF and trunk fat (TF) were also measured using DXA.
RESULTS: Measurements of TF by DXA explained about 70% of the variation in the VAT_{L1-L5} measured by MRI. TF measured by DXA (x_1) in combination with the subscapular (x_2) and sum of seven skinfolds (x_3) could accurately predict the VAT_{L1-L5} mass measured by MRI ($r^2 = 0.95$, SEE = 8.00 %): VAT (kg) = 0.304(x_1) - 0.0526(x_2) - 0.00707(x_3) + 0.414. A single MRI scan at the L2-L3 intervertebral disk region was found to be the best predictor of VAT_{L1-L5} mass ($r = 0.95$). The waist-to-hip ratio was highly correlated with VAT_{L1-L5} ($r = 0.83$). This study also found that BF and TF measured by DXA could be accurately predicted with the suprailiac skinfold (x_4) and waist girth (x_5): BF (kg) = 0.341(x_4) + 0.274(x_5) - 15.5 ($r^2 = 0.96$, SEE = 7.18 %). TF (kg) = 0.150(x_4) + 0.199(x_5) - 9.01 ($r^2 = 0.95$, SEE = 8.80 %).

CONCLUSION: DXA combined with anthropometry can accurately predict VAT mass from the L1-L5 vertebrae, and anthropometry alone can accurately predict BF and TF in men over 50 y.

KEY WORDS: BODY COMPOSITION, VISCERAL ADIPOSE TISSUE, BODY FAT, MRI, DXA, ANTHROPOMETRY, CARDIOVASCULAR DISEASE
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<td>Sagittal diameter</td>
<td>SAGITDIA</td>
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<td>Body mass index</td>
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<td>Waist-to-hip-ratio</td>
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<td>Sum of three skinfolds</td>
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<td>Sum of four skinfolds</td>
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<td>Tesla (magnetic field strength)</td>
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<td>Standard error of estimation</td>
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ACKNOWLEDGEMENT

Many people contributed towards this study and without their support this work would have not been completed. Special thanks are due to my research supervisor Dr. Alan Martin, and the committee members Dr. T. Rhodes and Dr. G. Anderson for providing invaluable assistance and guidance with this project. I also wish to thank the DC Magnetic Resonance Centre in Richmond and North Vancouver for allowing us to use their MRI, DXA machines, scanner and computers and supplying us with their technical expertise. Also, I wish to express my appreciation to all participants of this study for their cooperation, interest and volunteering their time.

Last but not least, special thanks are due to my girlfriend, Linda Vekic, for her tolerance, patience and assistance with this study. Words cannot describe her support and understanding; for without her this paper would not have been possible.
CHAPTER 1: INTRODUCTION

Obesity and Cardiovascular Disease (CVD) Risk

Obesity is associated with numerous metabolic complications such as hypertension, diabetes, and hyperlipidemia, but its association with cardiovascular disease (CVD) remains controversial (Larsson et al., 1981). Longitudinal studies that followed subjects over prolonged periods of time reported a moderate but significant and independent association between obesity and cardiovascular disease (Kissebah et al., 1989).

The first and second National Health, and Nutrition Examination Surveys (NHANES-I and NHANES-II), conducted in the United States from 1971-1974 (National Center for Health Statistics 1973) and 1976-1980 (National Center for Health Statistics 1981), respectively, provided much of the overwhelming evidence of the adverse effects of obesity on health presented at the National Institutes of Health (NIH) Consensus Development Conference on the Health Implications of Obesity (National Institutes of Health Consensus Development Panel 1985). The adverse effect of obesity, defined as excess body fat, include an association with cancers and several of the major risk factors for coronary heart disease (CHD), including hypertension, hypercholesterolemia, and noninsulin-dependent diabetes mellitus, which has made it difficult to determine a direct relationship between obesity and CHD. Severe obesity, defined as being more than 30% above ideal body weight, was identified as an independent risk factor for CHD in the National Cholesterol Education Program (NCEP) Adult Treatment Guidelines released by the National Heart, Lung and Blood Institute in 1987 (National Cholesterol Education Program 1987). Even mild-to-moderate obesity was associated with a substantial elevation in the risk of CHD, after
controlling for other CHD risk factors, in over 100,000 middle-aged women followed for 8 years in the Nurses’ Health study (Manson et al., 1990). Results from other longitudinal studies suggest that only a subsample of obese subjects carry the cardiovascular risk associated with excess body adiposity.

**Abdominal Adipose Tissue and Cardiovascular Disease (CVD) Risk**

One of the confounding factors in the relationship of obesity to the differences in risk for CVD is attributed to the regional distribution of excess body fat. Excess accumulation of fat in the abdominal region is associated with a greater incidence of metabolic abnormalities and risk for CVD, than deposition in the gluteal and/or femoral region in both men and women, as determined by the ratio of waist girth to hip and/or thigh circumference, particularly with increasing obesity (Krotkiewski, et al., 1983; Lapidus et al., 1984; Larsson et al., 1984; Terry et al., 1991; Stokes et al., 1985; Ducimetiere et al., 1986; Donahue et al., 1987). Obese individuals with an accumulation of body fat (BF) in the extremities display no increase in the risk of CVD, whereas subjects with an excessive deposition of fat in the abdominal region display a clear increase in the incidence of coronary heart disease. This effect of body fat distribution has been consistently reported in both sexes and is independent from the amount of obesity itself (Despres et al., 1991; Lemieux et al., 1993; Despres, 1997).

In 1947, Jean Vague was the first to suggest that body fat distribution was much more important to consider than AT mass per se in the clinical assessment of obese patients. Vague suggested that individuals with excess abdominal obesity (Vague described as android obesity) were much more prevalent to metabolic complications associated with hypertension, diabetes and CVD, than individuals that he referred to as having gynoid or female obesity.
Since 1947 many authors have supported the notion introduced by Vague in showing that the distribution of adipose tissue (AT) is associated with the metabolic complications considered as risk factors for cardiovascular disease such as: insulin resistance, hyperinsulinemia, diabetes, hypertension, glucose intolerance, hypertriglyceridemia, elevated apo B concentrations, reduced HDL cholesterol concentrations, elevated proportion of small, dense LDL (Kissebah et al., 1982; Hartz et al., 1983; Krotkiewski et al., 1983; Kalkhoff et al., 1983; Evans et al., 1984; Bjortorp et al., 1984; Bjortorp et al., 1985; Vague et al., 1985; Ohlson et al., 1985; Kissebah et al., et al. 1985; Stern et al., 1986; Kissebah et al., 1988; Kissebah et al., 1989; Bjorntorp et al., 1988; Bouchard & Johnston 1988; Bouchard et al., 1990; Despres et al., 1990; Despres et al., 1991; Pouliot et al., (1991); Despres et al., 1993).

Despres et al. (1989b) and Pouliot et al. (1992) have reported that the level of VAT measured by CT is the best correlate of glucose tolerance and plasma insulin levels in both men and women and that these associations are independent of the level of total body fat. To further assess the independent contributions of obesity and of VAT accumulation to disturbances in plasma glucose-insulin homeostasis, these authors compared two groups of subjects individually matched for age and total adiposity, but with either low or high levels of VAT, to a group of lean subjects. They performed these analyses in men and women separately. Obese subjects with low levels of VAT showed marginally higher plasma insulin levels than did lean controls, yet the glycemic response to an oral glucose challenge was similar to the lean controls. Obesity in the presence of elevated levels of VAT was associated, with a higher glycemic response in the presence of marked hyperinsulinemia, indicating the occurrence of insulin resistance. Despres (1993) also performed these group comparisons for plasma lipoprotein-lipid levels and reached similar conclusions. The level of
VAT is strongly associated with the dyslipidemic profile frequently observed in obese patients (Despres et al., 1989a; Despres, 1993; Pouliot et al., 1992). It is clear that the measurement of total adiposity alone would mislead an investigator or clinician interested in assessing the risk factors for CVD associated with obesity.

Studies that have assessed VAT accumulation by MRI have shown that the VAT area at the L1-L5 level is a strong correlate of the metabolic complications of obesity (Gray et al., 1991; Leenen et al. 1992, van der Kooy et al., 1992). Indeed, Leenen et al. (1992) demonstrated that women with large VAT area at the L4-L5 level measured with MRI are characterized by higher triglycerides and lower HDL-cholesterol, even after adjustment for age and percent body fat. In men, however, the association between a large VAT area and a dyslipidemic state disappeared after adjustment for age and percent BF. Since age is positively associated with VAT accumulation, control for age may have eliminated the effect of the accompanying increase in VAT area.

Statement of the Problem

Much of this uncertainty derives from the lack of methodology for accurate determination of adipose tissue mass in the different regions of the body, especially abdominal adipose tissue. There is a need for improved techniques that can accurately and reliably measure total and regional adipose tissue distribution in humans. Traditionally researchers and clinicians have used anthropometry to estimate body composition because it was quick, easy and a low cost. Unfortunately there is a great deal of variability between observers and the anthropometric prediction equations were population specific. Advanced imaging techniques such as Dual-Energy-X-ray Absorptiometry (DXA), Computed
Tomography (CT) and Magnetic Resonance Imaging (MRI) offer new promise for the visualization and quantification of adipose tissue masses in different compartments of the body (Svendsen et al., 1992; Abate et al., 1994; Treuth et al., 1995). Studies conducted in the 1980's have indicated that CT is a suitable technique for the measurement of body composition, since cross sectional areas of AT, muscle, and bone can easily be measured in CT scans at any body site (Borkan et al., 1983; Tokunaga et al., 1983; Sjostrom et al., 1986).

CT and MRI are sophisticated imaging procedures that have also been used to quantify abdominal adipose tissue. Both techniques are capable of measuring subcutaneous abdominal adipose tissue (SAAT), visceral adipose tissue (VAT) and total abdominal adipose tissue (TAAT) with a high degree of accuracy (Ross et al., 1991; Seidell et al., 1990). However, both techniques are very expensive and require highly trained operators, limiting their use to specialized centres and are thus not very readily available. An additional disadvantage of CT scanning is the exposure of subjects to ionizing radiation. Therefore, there is a need to develop accurate, simple, less expensive and more readily available techniques to measure body fat and different compartments of abdominal adipose tissue (i.e. VAT, SAAT, TAAT). The most important application in the field of body composition is the measurement of VAT, in order to investigate VAT's association with metabolic complications and the risk factors for cardiovascular disease.

**Purpose of the study**

The aim of this study was to develop a quick, and simple method to estimate VAT and BF through more readily available and less expensive means than CT or MRI. These means were anthropometry and DXA, which are now becoming more widely available and
are not too expensive relative to MRI and CT. The abdominal AT mass measured in different compartments by MRI was used as the criterion measure in this study.

This study developed prediction equations for estimating: 1) VAT mass using anthropometric combined with DXA measurements; 2) TAAT using DXA measurements alone; 3) BF and trunk fat (TF) using anthropometric measurements alone.

**Significance of the study**

A review of the literature has found that using DXA combined with anthropometry to estimate VAT mass measured by MRI in middle aged men has never been done before. The following 5 points emphasize how this study is different from other similar studies:

1) **Criterion Method**

Most previous studies have used adipose tissue measured by CT as the criterion measurement. This study used the areas of different abdominal compartments (i.e. SAAT, TAAT, and VAT) measured by MRI (as the criterion measure).

2) **Method of data collection**

Most previous studies have only used one scan as a reference (Seidell et al., 1987; Svendsen et al., 1993; Treuth et al., 1995; Goran et al., 1998). From these studies the major dependent variable was the cross-sectional adipose area of VAT from a single scan. Total VAT volume could be estimated by multiple scan CT, but this would involve a much higher radiation dose. CT involves radiation and MRI involves no radiation and thus many MRI scans can be made without harm to the subjects. This present study used eleven cross-
sectional MRI scans for the area of interest and had VAT mass as the dependent variable. The area of interest in this study was from the superior plate of the L1 vertebra to the inferior plate of the L5 vertebra, which corresponds approximately to the top of the kidneys to the anterior superior iliac crest.

3) Adjacent scans

Other researchers who determined volumes from a series of adjacent scans usually had gaps between the scans. For example, Ross et al. (1992) did whole body scans with MRI where the thickness of each scan was 10mm with 50mm centres. SAAT and VAT volumes in previous studies were estimated by assuming truncated pyramidal, conical, or cylindrical geometric configurations between pairs of consecutive slices; these estimated volumes may not be accurate (Abate et al., 1997). Thomas et al. (1998) has shown that there is an increase in measurement uncertainty as slices are removed from a continuous data set. This uncertainty highlights the possible inaccuracies in using single slice data sets. One scan is not a good predictor of AT volume due to large variance between individuals (Thomas et al., 1998). This present study used a series of adjacent MRI scans (i.e. no gaps) to determine VAT and SAAT volumes (mass).

4) Anthropometry and DXA Measurements Combined

Similar studies by other researchers used only anthropometry to estimate adipose tissue (Kvist et al., 1988; Depres et al., 1991). Only a few studies have used anthropometry combined with DXA (Svendsen et al., 1993; Treuth et al., 1995; Jensen et al., 1995; Goran et al, 1998); however, these authors used CT as the criterion measure and looked at different
populations other than the middle aged men as in this present study. Svendsen et al. (1993) used the fat mass from the L1 to L4 vertebrae measured by DXA. Unlike the study by Svendsen et al. (1993) this present study utilized the common and easily distinguishable trunk fat mass measured by DXA combined with anthropometry to derive estimation equations.

5) Subject population

This study used a population of middle aged men over the age of 50 y. This population was chosen because of the prevalence of cardiovascular problems with middle aged men (Larson et al., 1984; Ducimetiere et al., 1986; Donahue et al., 1987). Other researchers who have derived estimation equations to predict VAT have used populations other than middle aged men. Svendsen et al. (1993) and Treuth et al. (1995) estimated VAT in women; Jensen et al. (1995) looked at 6 men and 15 women (38 ± 12 y), and Goran et al. (1998) looked at pre-pubertal children.

Summary

Obesity and VAT has been found to be associated with cardiovascular disease. BF, TF and especially VAT have been difficult to measure accurately in order to investigate this association further. Imaging techniques such MRI and CT are good but the use of these imaging techniques is limited due to factors such as cost, availability and ionizing radiation (for CT). Thus there is a need to develop alternative approaches for estimating BF, TF and VAT which are simple, quick, accurate and inexpensive. The rationale for developing this study is to derive prediction equations to estimate BF, TF and VAT using DXA and
anthropometry in order for future researchers to investigate their association with metabolic complications and the risk factors for cardiovascular disease.
CHAPTER 2: LITERATURE REVIEW

Abdominal Adipose and Cardiovascular Disease (CVD) Risk

There is growing evidence that the distribution of body fat influences metabolic abnormalities and cardiovascular risk factors such as insulin resistance, hyperlipidemia, hypertension, and hyperinsulinemia (Kissebah et al., 1982; Krotkiewski et al., 1983; Modan et al., 1985; Stern & Haffner, 1986; Landsberg, 1986; Reaven & Hoffman, 1987). Specifically, the accumulation of body fat in the abdominal region has been independently associated with diabetes, stroke, coronary heart disease, and related mortality; especially in elderly men (Larsson et al., 1984; Ohlson et al., 1985; Ducimetiere et al., 1986; Donahue et al., 1987). However, not all investigators agree that abdominal obesity has a more detrimental effect on these metabolic abnormalities than excess fat in other body locations. Furthermore, the distribution of fat to the gluteal femoral area does not appear to increase the risk of metabolic abnormalities (Depres et al., 1990). These researchers have also suggested that these metabolic abnormalities may be particularly associated with the accumulation of visceral rather than subcutaneous abdominal tissue (Depres, 1992). Much of this uncertainty derives from the lack of methodology for accurate determination of adipose tissue mass in the different regions of the body, especially adipose tissue in different compartments in the abdominal region.

Visceral AT and Cardiovascular Disease (CVD) Risk

Methods for assessing BF, abdominal, and VAT range from simple anthropometry to complex and expensive imaging techniques like magnetic resonance imaging (MRI) and
computed tomography (CT) (Borkan et al., 1982; van der Kooy and Seidell, 1993). Waist-to-hip girth ratio (WHR), while simple to take, is a complex measure that includes many other tissues along with SAAT and VAT. Advanced imaging techniques such as DXA and MRI offer new promise for the visualization and quantification of adipose tissue masses in different compartments (Svendsen et al., 1992; Abate et al., 1994; Treuth et al., 1995). MRI is particularly promising because of its lack of radiation exposure and its superior imaging of internal adipose tissue. The estimation of adipose tissue mass by MRI has also been validated against dissection of human cadavers (Abate et al., 1994). Unfortunately MRI is expensive, not readily available and requires an experienced radiologist.

Dual energy X-ray absorptiometry (DXA) measures abdominal fat mass, but because of the planar nature of the scanning process, it cannot distinguish between visceral fat and the subcutaneous fat of the trunk (Goulding et al., 1996; Treuth et al., 1995). However, by adding anthropometric measures such as sagittal diameter to DXA measurements, it is possible to generate regression equations to predict CT- or MRI-measured VAT volume (Svendsen et al., 1993; Bonora et al., 1995; Jensen et al., 1995; Kekés-Szabo et al., 1996; Goran et al., 1998).

This study was conducted to determine whether 1) the combination of DXA and anthropometric measures could be used to predict VAT mass measured by MRI in middle aged men; 2) if anthropometry alone could predict BF and TF measured by DXA. Men over 50y are at high risk for cardiovascular disease and the ability to estimate VAT may be very beneficial in the study of the association between VAT and cardiovascular risk factors.
Levels of Body Composition

The distinction of levels of measurements of body composition (i.e. tissue, cellular, molecular or atomic level) is an important point and has been discussed by Wang et al. (1992). DXA measures fat and MRI measures AT. AT and fat are two separate entities and should not be used interchangeably. According to Wang et al, (1992) adipose is part of the tissue level of body composition.

1) Adipose tissue is composed of 80-85% fat, 12-18% water, and 2-3% proteins, with negligible minerals (Thomas, 1962; Garrow 1974) – tissue level

2) Fat is made up of triglycerides - molecular (chemical) level.

Cadaver Analysis

Direct measurement of abdominal adipose tissue is the 'gold standard' for other techniques; however, the only true direct method of measuring AT is by dissection. But cadaver analysis is usually not an option for research purposes. In addition, dissection seems to be the only available way to clearly distinguish between intraperitoneal (mesenteric and omental) and retroperitoneal AT. This distinction is important because only the intraperitoneal AT depots can be considered as 'portal' tissues which are associated with the risk factors for cardiovascular disease (Bjorntorp et al., 1990; Depres, 1992).

Computerized Tomography (CT)

Computed tomography (CT) is a radiological technique that is commonly used for diagnostic purposes in medicine. The CT system consists of an X-ray tube and detectors aligned at opposite poles of a circular apparatus. The X-ray beam is rotated around a subject
located in the centre of and perpendicular to the apparatus, and information about the intensity of the attenuated X-ray beams is recorded and stored. The scanner’s computer reconstructs the information to give cross sectional images. Clear differences in attenuation intervals between bone, adipose and adipose-free tissue make this technique appropriate for quantification of separate adipose compartments and whole body composition (Borkan et al., 1982; Tokunaga et al., 1983; Sjostrom et al., 1986). CT has the advantage of being able to provide a visual image and differentiate between VAT and SAAT (Sjostrom et al., 1986; Kvist et al., 1988; Baumgartner et al., 1988).

Quantification of CT images

A CT scan can be compared to a black and white newspaper photograph constructed from dots with varying shades of gray in a range from white to black. A typical scanner creates images that contain 256 X 256 “dots” (pixels in CT jargon) where the signal intensity from each pixel is translated into shades of gray and scaled as the CT number in Hounsfield units (HU). The number of pixels (256 X 256) combined with a field of view of say 400mm implies that one pixel covers 2.4 mm². The number of pixels in relation to the field of view determines the resolution of the method. Hounsfield units are defined as ranging from -1000 (air: all signals pass without absorption) to +1000 (dense bone: no signal gets through) with zero representing the density of water. Determination of the range of Hounsfield units which represent pixels with adipose tissue is generally carried out by inspection of histograms of pixel intensities in regions of interest specified by the investigator. Regions of interest are usually some representative areas containing both adipose tissue and bordering tissues or air.
The degree of separation of "fat" peaks from "organ and muscle" peaks is usually determined by the amount of "partial volume effects". Partial volume phenomena reflect that some pixels contain both fat and non-fat tissues and they exhibit a signal strength in Hounsfield units somewhere between those of pure adipose tissue and non-adipose tissue. If there are many pixels that contain mixtures of tissues, the peaks will show considerable overlap and misclassification of tissue will occur. By choosing a point midway between the peaks it is usually assumed that the misclassification of adipose tissue and non-adipose tissue will cancel each other out. The assessment of visceral fat is in particular affected by partial volume effects because this fat is not only bordering muscle tissue, but it is also aligned to irregular boundaries of the intestines.

Different ranges of Hounsfield units have been determined for quantification of adipose tissue (Borkan et al., 1982; Grauer et al., 1984; Kvist et al., 1986) and it may be that the limits vary with the type of scanner used and that they differ in different individuals (Seidell et al., 1990). The most commonly used intervals are -190 to -30 HU and -150 to -50 HU. Fortunately, varying the upper and lower limits does not have a major effect on the assessment of fat areas. Varying the upper limit (-30 HU) by +/- 10 HU or varying the lower limit (-190 HU) by +/- 10 HU resulted in a change in area determinations of 5 - 6 % and <1% respectively (Rossner et al., 1990).

**CT artifacts**

One of the technical problems that one may encounter when using CT for measurement of AT areas is beam hardening and scatter radiation caused by bone tissue. This is especially a problem in scans of the hips and thighs. A method to correct for these
artifacts has been described by Kvist et al. (1988) but this is a tedious procedure introducing unknown errors. It is also difficult to make an accurate distinction between retroperitoneal and intra-peritoneal AT areas using CT because the peritoneum is not visible (Rossner et al., 1990; Seidell et al., 1990). Ashwell et al. (1987) developed a method by drawing straight lines from the mid-point between the abdominal aorta and the inferior vena cava through the centres of the ascending and descending colon and defined fat located posterior to these lines as "retroperitoneal fat". It is unclear, however, how accurate and reproducible this procedure is.

Accuracy and precision of CT images

Perhaps due to its high cost and the associated ionizing radiation, few validation studies of CT have been performed. Only a few studies have used cadavers for the validation of adipose tissue measured by CT (Rossner et al., 1990). Rossner et al. (1990) compared the AT cross-sectional areas from 21 abdominal CT scans on two cadavers with the corresponding values obtained using direct planimetry on frozen sections from cadavers. High correlation coefficients were found between CT and planimetry for both total (r = 0.94) and visceral (r = 0.83) AT areas. Rossner et al. (1990) also stated that the percentage of visceral fat as intraperitoneal (portal) fat was 59% and 75% in the two cadavers.

Janssens et al. (1994) also attempted to assess the relationships between AT volumes measured by CT and corresponding volumes obtained by dissection of unembalmed frozen cadavers. Unfortunately, problems inherent to the CT imaging of frozen cadavers limited the observations of AT, but the CT and dissection values for muscle volumes did not differ. Ross et al. (1991) reported that whole body AT mass from CT correlated highly (r = 0.98)
with chemically extracted lipid mass in a group of rats varying in adiposity. Therefore, the precision of CT measurements of AT is very good, but volume or mass of the adipose tissue in humans has not been validated using CT (Abate et al., 1994). Therefore, the accuracy of CT to estimate adipose tissue “mass” in various compartments is not known.

**Coefficient of variation of CT**

CT has also been proven to be highly reproducible. Kvist et al. (1988a) reported that the difference between repeated measurements of total AT volume in eight subjects was about 0.6%. The variability found for integrated total fat areas from multiple scans ranged from 0.6% (Kvist et al., 1986) to 1.4% (Shuman et al., 1986). The reproducibility of adipose tissue areas solely, calculated from single or multiple scans, has been reported by Ross et al. (1993). Reliability of the MRI model used was assessed by repeated measurements (same day) of transverse images obtained at the L4-L5 level on 12 subjects. The segmentation of AT for all subjects was performed by using a single threshold, above which pixels were considered AT. For subcutaneous AT, the mean difference between test 1 and 2 was 10.6% (range 1.9 -22.9%); for VAT the mean difference was 10.2% (range 0.8-44.0%) (Ross et al., 1993).

The inter-observer error for segmenting subcutaneous and VAT was tested by comparing the segmentation results, for both area (L4-L5) and volume measurements, obtained on 10 subjects by two individuals (Ross et al., 1993). For subcutaneous AT area, the mean difference was 1.1%, with a range of 0.1-2.8%; for subcutaneous AT volume the, mean difference was 1.5% with a range of 0.1-5.6%. For VAT area the mean difference was
5.5%, with a range of 0.1-13%; for VAT volume the mean difference was 10.1%, with a range of 3.1-18% (Ross et al., 1993).

CT can give an estimate of the area occupied by adipose tissue in each compartment of a given "scan" of abdomen. The precision of the CT measurement is very high (coefficient of variation 0.5-1.4%) (Sjostrom, et al., 1986; Van der Kooy & Seidell, 1993a), but the accuracy may depend on the range of Hounsfield units (Heymsfield, et al., 1979).

**CT and body composition**

Two groups of investigators have used CT to assess whole-body composition. Tokunaga et al. (1983) measured the areas in multiple scans and the distances between scans. From these data Tokunaga et al. (1983) calculated total and regional AT volumes. Sjostrom and Kvist (1988) made 22 consecutive scans covering the whole body. They found that the AT volume from CT is highly correlated with fat mass estimated by the tritiated water, 40K, or hydrostatic weighing techniques (Kvist et al., 1988b; Sjostrom et al., 1986). Thus, it appears that CT is an accurate technique for the assessment of body composition, and it does not rely on assumptions such as the constancy of either the density or the water content of fat-free mass. A clear distinction between AT and lean tissue can be obtained with CT, but, since this method involves exposure to radiation, scans at many levels cannot be used in serial studies. It has been shown, however, that nine scans can provide essentially the same information as 22 scans for the assessment of total AT volume (Sjostrom, 1988). Furthermore, Ferland et al. (1989) reported that a partial AT volume obtained from the measurement of three scans (TH8-TH9, L4-L5, and mid-thigh), and the assumption of cylindrical shapes, is highly correlated with the fat mass determined by hydrostatic weighing.
in premenopausal obese women.

The number of scans performed determines the duration of the procedure. One CT scan usually takes less than 10 seconds. A pre-scan "scout" view allows the investigator to precisely define the anatomical location of each slice. An increasing number of scans is undoubtedly associated with higher precision of the visceral fat volume but also with increased exposure to ionizing radiation (Kvist et al., 1986).

The most important application of CT in the field of body composition has been to the measurement of abdominal VAT. Fortunately, visceral adipose areas from a single scan taken at the level of L3-L4 or L4-L5 (approximately umbilicus level) have been shown to be highly correlated to the total VAT volume (r > 0.95) (Kvist et al., 1988; Ross et al., 1992). By drawing a line within the muscle wall of the abdominal cavity at L4-L5 and by providing the computer with the attenuation values of AT (-190 to -30 HU), the VAT area can be calculated. The cross-sectional VAT area at L4-L5 is highly correlated with the VAT volume from multiple abdominal scans (Kvist et al., 1988a). Furthermore, others have shown that the total cross-sectional AT area at the abdominal (L4-L5) level is highly correlated with total AT mass in men and women (Despres et al., 1991; Ferland et al., 1989; Koester et al., 1992; Lemieux et al., 1993). Thus, a single abdominal scan can be used not only to obtain critical information concerning the amount of VAT at one level, but also to estimate total VAT mass. Thus, the use of one abdominal scan appears to be sufficient to assess VAT accumulation and to identify those individuals who are at risk for the development of diabetes mellitus and some cardiovascular diseases. It is not clear, however, whether more than one abdominal scan should be recommended in intervention studies in which the effects of diet and/or exercise would be examined.
CT also has the major drawback of being expensive, time consuming (10-20 minutes for whole body scans), and requiring a relatively high radiation dose of about 800 mrem.

**Magnetic Resonance Imaging (MRI)**

Magnetic resonance imaging (MRI) is another imaging technique similar to CT but without the ionizing radiation. MRI is based on the interaction between the nuclei of hydrogen atoms, which occur abundantly in all biological tissues, and the magnetic fields generated and controlled by the MRI system's instrumentation. Hydrogen nuclei, or protons, have a non-zero magnetic moment, which causes them to behave like tiny magnets. In the weak magnetic field of the Earth, these magnetic moments are oriented randomly and thus tend to cancel each other. When a subject is placed inside the magnet of a magnetic resonance imager, where the field strength is typically 10,000 times stronger than that of the Earth, the magnetic moments of the protons tend to align themselves with the magnet's field. The strength of the magnetic field is measured in Tesla (T). T1 is equal to about 42.6 MHZ.

Having aligned the hydrogen protons in a known direction, a pulsed radio frequency (RF) field is then applied to the body tissues, causing a number of the hydrogen protons to flip or absorb energy. When the RF field is turned off, the protons gradually return to their previous positions, releasing in the process the energy that they absorbed in the form of an RF signal. It is this signal that is used to generate the MRI images by computer.

To increase the contrast between AT and skeletal muscle, MRI data acquisition can be programmed to take advantage of the specific proton density and relaxation times (the rate at which absorbed energy is released) of the various types of tissues. This is accomplished by varying what are known as time parameters. These are the time-to-repeat (TR) and the
time-to-echo (TE) of the RF pulse. Manipulation of the TR and TE times varies the RF pulse sequence. When using one such sequence called spin-echo, the TR parameter can be adjusted to exploit the difference in T1 relaxation times of AT and muscle, which provides the tissue contrast required for high quality MRI images. With few exceptions, the spin-echo pulse sequence has been selected to acquire AT data from MRI. For a detailed description of MRI principles, see Mansfield et al. (1982).

In order to distinguish adipose tissue from non-adipose tissue, MRI experiments have been designed to give an optimal contrast between the tissues. (Seidell et al., 1990; Ross et al., 1992; Sobol et al., 1991; Gray et al., 1991) These experiments are characterized by specific sequences of radio pulses (Bushong, 1988).

Sobol et al. (1991) compared their sequence to the one used by Seidell et al (1990) and found that the results were very similar and highly correlated. In addition, useful results can be achieved with different magnetic field strengths. A recent paper by Ohsuzu et al. (1998) compared CT and MRI and found very good correlations (r > 0.91) for measuring adipose tissues in the abdomen using 1.5 Tesla (T) magnetic resonance spin-echo imaging TR/TE 200 ms/15ms).

The acquisition time for one scan depends mainly on the chosen sequence. The strength of the magnetic field also has some influence. For example, a spin-echo experiment may take about 1 minute by 0.5T whereas an inversion-recovery sequence may take 10 minutes by 1.5T (Seidell et al., 1990). For the scan sequences with a relatively long acquisition time, movement artifacts of the abdomen due to breathing and intestinal movements are a source of error, particularly for the assessment of visceral fat (increasing the partial volume effects).
Fast MRI imaging

As mentioned earlier, one limitation associated with the acquisition of body composition data from MRI is the time required to obtain quality images. For example, for the spin-echo pulse sequences, approximately 8 minutes are required to obtain quality MR images of the abdominal region (Ross et al., 1992). As a result, motion artifacts caused by respiration and cardiac motion tend to decrease the image quality. Consequently, MR pulse sequences have been developed that require less time. Known as FLASH (Fast Low Angle Shot) or GRASS (Gradient Recalled Acquisition at Steady State), these RF pulse sequences manipulate the time parameters (TR and TE) so that quality MR images can be obtained in seconds. Using these procedures, MRI data from the abdominal region can now be obtained during a normal breath-hold (i.e., less than 20 seconds). While it remains to be determined whether the accuracy or precision of MRI will be improved by using fast imaging techniques, there is little doubt that they will allow the acquisition of MRI data in relatively short periods of time.

Quantification of MRI data

Once an MR image is acquired, the next step involves quantifying the tissue of interest by subjecting the MRI data to various segmentation techniques similar to those described for CT. Unfortunately, the quantification of AT data from MRI is not as straightforward. Usually the MR image matrix consists of 256 rows by 256 columns; each resulting square is termed a pixel.

In a study by Abate et al. (1994), the entire abdominal region was scanned using
adjacent axial scans. Adipose tissue volume was measured in each scan by mapping various adipose tissue compartments on the computer screen using a highlighting technique with the mouse. The number of pixels corresponding to adipose tissue were counted in each compartment (i.e. VAT or SAAT) and converted into a volume (multiplying the number of pixels by 0.04 cm$^3$). Assuming that adipose tissue was composed of 84.67% fat, 12.67% water, and 2.66% proteins the density of adipose tissue was calculated to be 0.9196 g/cm$^3$. Therefore, adipose tissue mass could be calculated in grams for each scan. The masses obtained for each scan could then be summed to calculate the total adipose tissue mass for each identified compartment.

**MRI artifacts**

Unlike CT, where pixel values consistently represent specific tissues regardless of slice position or individual being assessed, MRI pixel values for a given tissue (the emitted RF-signal of the protons within a pixel) may vary from slice to slice or between individuals. This is partially due to the fact that pixel values in MR images are dependent on the excitation pulse sequences and a combination of proton density and tissue relaxation values, which may vary between individuals. More important, however, is the variation in signal intensities that may occur for the same tissue within a single study or acquisition. This variation may result from heterogeneities in the magnetic field or other system imperfections (e.g., variations in slice profile, main or gradient field heterogeneities) (Ross et al., 1992).

The principal result of heterogeneities in the magnetic field is the occurrence of random variations in pixel intensities for a given tissue within and/or between images. This term is called “ghosting” and it must be corrected for interactively. This randomly occurring MRI
artifact may appear as a shadow in the SAAT region. An example of this phenomenon is illustrated in Appendix C. The pixels in this "ghost shadow" region have a lower intensity value than the other subcutaneous AT (SAT) pixels. Thus, when the SAT is quantified by using a single threshold for AT, the pixels in the affected area will not be counted, resulting in an underestimation of the true AT area. As described by Ross et al. (1992), this problem can be corrected by using a straightforward image-processing technique called signal averaging (Ross et al., 1992).

Recent improvements to MRI hardware and computer software have improved the quality of MR images and substantially reduced the frequency of MRI artifacts. Nevertheless, their occurrence suggests that quantification of a given tissue on MR images requires image analysis software that permits visual verification of the segmentation result. Regardless of the segmentation procedure, until MRI acquisition procedures improve, visual verification of all segmented MRI images may be required. In other words, the researcher may have to manually correct for AT areas that are not counted by the initial threshold selection. While these procedures can be accomplished using straightforward image analysis techniques, they add a degree of subjectivity to the quantification of tissues on an MR image that is not required when quantifying a given tissue using CT.

**Accuracy of MRI images**

Foster et al. (1984) were among the first to demonstrate that MRI could measure AT accurately. They reported that a T1 weighted inversion recovery pulse sequence yielded a very high contrast between adipose tissue and adjacent muscle on the MRI image and, as a consequence, any AT thickness could be obtained by MRI. These observations were
validated using both carcass and cadaver data. In the same study, it was observed that the tissue thicknesses determined by MRI did not differ from those determined by direct physical measurement.

Using a rat model, Ross et al. (1991) found that whole-carcass chemically extracted lipid was highly correlated with AT mass from MRI \( (r = 0.97, p < 0.01) \) and that the standard error of estimate was 10.5%. In another validation study of MRI, (Fowler et al., 1992) dissection and chemical analysis of cross-sectional slices of 12 pigs were compared to the percentage fat assessed in the same slices by MRI. Although pigs do not really have visceral fat in a similar way to humans this study illustrated that there is a distinction between expressing visceral fat in terms of tissue or stored lipid. Fowler et al. (1992) compared AT from MRI measurements to those obtained by dissection in lean and obese pigs. The authors observed that the AT from MRI correlated strongly with AT by dissection \( (r = 0.98) \), and that the mean square error was 2.1%.

At this point, there is only one report that has compared MRI measurements of AT with those derived by dissection of human cadavers. A study by Abate et al. (1994) estimated adipose tissue mass by MRI and validated these estimations against dissection in human cadavers. For the various compartments, the mean of the difference between dissection and MRI estimation was only \( 0.076 \pm 0.071 \) kg and found to have an average coefficient of variation of less than 9%. The “limits of agreement” (mean of the differences ± SD) between the dissection and MRI measurements was \( -0.066 \) kg and \( +0.218 \) kg. This study found that MRI is an accurate technique for estimating adipose tissue in the abdomen with MRI slightly overestimating VAT by less than 5%. Abate et al. (1994) feel that a shorter duration for MRI acquisitions can also minimize the potential effects of respiratory movements and intestinal
peristalsis and thus make it a potentially valuable method for the *in vivo* estimation of adipose tissue mass. In this study, the authors subdivided visceral AT into intraperitoneal and retroperitoneal depots. Thus, although additional data comparing MRI and human cadaver derived estimates of AT would be useful, these preliminary observations provide evidence in support of the accuracy of MRI estimates of human adiposity.

In summary, by comparison to limited cadaver and animal data, it appears that MRI measures TAAT with a standard error of estimate in the range of 2 to 10%. Comparison with CT suggest that MRI provides images of the abdomen with similar anatomic detail, with CV for SAT and VAT in the order of 5 and 15%, respectively.

**Precision of MRI images**

Several studies have evaluated whether MRI measurements of SAT and VAT are reproducible (Staten et al., 1989; Seidell et al., 1990; Gerard et al., 1991; Ross et al., 1991; Ross et al., 1993; Sohlstrom et al., 1993). The results show that, for a single MR image in the abdominal region, the CV for repeated measures of SAT ranges from 1.1 to 10.1%. For VAT, the CV for repeated measurements ranges from 5.3 to 10.6%. Taken together, these data suggest that when MRI is used the expected error for measurement of VAT areas is approximately 10%, but it is lower for SAAT areas. The reproducibility of visceral fat areas measured by MRI (coefficient of variation (CV%) of about 10-15% (Seidell et al., 1990) is due to the potential measurement errors mentioned above, which are larger compared to measurement errors by CT.

As described previously, MRI can discriminate between lean tissue and AT. This is particularly true in the limbs because the principal tissues (skeletal muscle and AT) are
discriminated easily based upon pixel intensity values. In the abdominal region, however, discrimination between them is ambiguous because the pixel intensity values of all lean tissues (skeletal muscle, organs) fall within a small range. Therefore measurements of lean tissue by MRI, in particular skeletal muscle, are usually restricted to limbs.

Unfortunately there is little evidence regarding the precision of MRI measures of lean tissue. Ross et al. (1994) reported that, for a single MR image of the proximal thigh, the CV for repeated measurements of lean tissue (skeletal muscle and bone combined) is 1.2%. In addition, these authors reported that the CV is 3.9% for lean tissue volume in the leg derived from 15 images. While the precision of MRI measures of lean tissues requires further investigation, these preliminary results are encouraging. Given the importance of skeletal muscle in the development of insulin resistance and glucose intolerance, the availability of a noninvasive method for measuring lean tissue, without the approximations of anthropometric techniques, would be very beneficial.

**MRI vs. CT**

Further attempts to validate MRI have been made by comparing MRI measurements of AT to those obtained by CT using both animal (Ross et al., 1991) and human (Seidell et al., 1990) equations. In human beings, evaluation of the relationships between the two methods has generally been performed by comparing measurements of SAT and VAT areas obtained from a single abdominal image. The data reveal that, in general, the correlations obtained between MRI and CT areas are quite good for SAT ($r = 0.79 - 0.98$) and slightly lower for VAT ($r = 0.79 - 0.93$) (Seidell et al., 1990, Sobel et al., 1991). Furthermore, given that the measurements of the extremities are unaffected by the motion artifacts previously
described, it is likely that the coefficient of variation (CV) between MRI and CT measures of SAT in the limbs is substantially less than the 5 to 12% reported for the abdominal region.

While the differences between measures from CT and MRI are generally low for SAT, the CV for VAT is higher, ranging from 13 to 20%. In addition, it has been observed that the relationship between MRI and CT measures of VAT improves with increasing visceral adiposity (Seidell et al., 1990). This is probably due to the increased signal-to-noise (motion artifacts) ratio associated with increasing quantities of VAT. Thus the ability of MRI to predict CT measures of VAT in lean subjects is suspect due to the decreased signal-to-noise ratio. Unfortunately, data are not available that would indicate the lower limit of values for VAT areas below which substantial differences occur between MRI and CT measures. The use of fast imaging protocols that avoid the problems associated with motion artifacts may enhance tissue contrast and, thus, improve the ability of MRI to measure VAT in lean subjects.

In other studies, MRI was validated by comparing measurements of AT areas by MRI with those obtained by CT in human volunteers (Seidell et al., 1990; Sobol et al., 1991). Although in both studies CT and MRI yield different absolute values for abdominal AT areas, the ranking of individuals on the basis of their AT areas was similar with both methods. Ohsuzu et al. (1998) found that the correlation between adipose area obtained with CT and MRI for SAAT, TAAT and VAT vs. SAAT ratio were highly significant (r = 0.93, 0.91, and 0.94, respectively; p < 0.01) and the standard errors of estimation were 9.99, 23.87, and 0.0047 cm². These authors suggested that 1.5 magnetic resonance spin-echo imaging is a practical approach to evaluate body AT distribution without the exposure to radiation. But as
for CT, it is also difficult in MRI images to distinguish between retroperitoneal and intraperitoneal AT (Rossner et al., 1990; Seidell et al., 1990).

MRI and body composition

Because multiple images can be obtained without any known health risks to the subject, MRI is well suited for assessment of whole-body AT distribution. One principal benefit of multislice acquisitions is that the volumes of various AT depots can be derived. A single MRI or CT scan yields the cross-sectional area of AT at that particular scan level. The AT areas of a continuous series of such scans can be integrated to give an estimate of VAT volume (Jacobi et al., 1997; Ross et al., 1992), which can then be converted to VAT weight by assuming a density for AT. Therefore, regional or segmental analysis of AT is possible and permits evaluation of regional AT distribution. (Ross et al. 1994). This protocol is particularly useful when evaluating the efficacy of interventions that may induce changes in AT distribution.

Four groups have employed a multislice MRI protocol to evaluate whole body AT distribution in human subjects. Fowler et al. (1991) acquired 28 MR images over the entire body, while Ross et al. (1992, 1993) used 41 images to measure AT and lean tissue distribution in normal male and obese female subjects. Sohlstrom et al. (1993) have described AT distribution in normal, healthy women using a 30-image protocol.

One principal concern with multislice protocols is the high cost associated with obtaining multiple images. As a result, attempts have been made to establish the minimal number of images required for accurate measurements of the whole-body AT. For example, Fowler et al. (1991) reported that as few as four properly positioned MR slices can accurately
predict whole-body AT volume derived using 28 slices. Furthermore, Ross et al. (1992) have shown that cross-sectional AT area measured by MRI at the L4-L5 level is highly correlated \((r = 0.95)\) with the total AT volume obtained from 41 images. These results suggest that in cross-sectional studies, where a rapid estimate of total adipose tissue is required, the measurement of AT area at the L4-L5 level could be useful.

It is important to note, however, that the practical or physiological value of substantially reducing the number of images required to derive whole-body AT volume from MRI is limited. For CT, a reduction in the number of images acquired to calculate AT volume has the practical advantage of reducing the exposure of the subjects to ionizing radiation. Since MRI is not subject to such restrictions, a reduction in data acquisition has no practical advantage. Furthermore, a reduction in the number of images acquired would not substantially decrease the time required to gather the data since most MRI systems can acquire data for several body slices in the same time it takes to acquire a single slice. Therefore, if MRI is to be used as a reference method to assess AT distribution, it is recommended that, within feasible and reasonable limits, the maximum number of images be acquired.

MRI is also useful for discriminating between VAT and SAT accumulation. To obtain a volume of VAT using MRI, Ross et al. (1992) performed seven abdominal scans (two distal to L4-L5, one at L4-L5, and four proximal to L4-L5) in a sample of men. Their results indicated that the VAT area at L4-L5 is the strongest correlate of total VAT volume from seven scans \((r = 0.95)\). Therefore, one image at the L4-L5 level can adequately identify subjects with excess VAT accumulation. Since, however, multiple slices are possible with MRI, the measurement of VAT volume using multiple images is suggested, especially in
intervention studies where small changes in VAT volume are expected. Finally, as with CT, efforts to discriminate between retroperitoneal and intraperitoneal AT must be viewed with caution since the relevant methods have not been fully validated (Rossner et al., 1990; Seidell et al., 1990; Ross et al., 1994).

Studies that have assessed VAT accumulation by MRI have shown that the VAT area at the L4-L5 level is a strong correlate of the metabolic complications of obesity (Gray et al., 1991; Leenen et al., 1992). Leenen et al. (1992) demonstrated that women with large VAT area at the L4-L5 level measured with MRI are characterized by higher triglycerides and lower HDL-cholesterol, even after adjustment for age and percent body fat. In men, however, the association between a large VAT area and the dyslipidemic state disappeared after adjustment for age and percent body fat. Since age is positively associated with VAT accumulation, control for age may have eliminated the effect of the concomitant increase in VAT (Leenen et al., 1992).

Recently Han et al. (1997) have also looked at the predictive ability of one scan to estimate abdominal adipose tissue volumes. These authors did a study to determine at what level between the L1 and L5 vertebrae best predicts VAT volumes. The subjects in this study were 16 men and 7 women with non-insulin-dependent diabetes mellitus, aged 44-74 y. The BMI's for the men and women were 27.9 ± 3.0 and 31.6 ± 4.7 kg/m² respectively. The 20 mm MRI scans were adjacent and covered the L1-L5 area completely. The volumes and masses were calculated from the adipose areas from each of the cross-sectional transverse scans. The VAT was estimated to be 2.3 ± 0.5 kg in the men and 2.5 ±0.6 kg in the women. Han et al. (1997) stated that the scans in the L2-L3 level gave the highest prediction of VAT in both men (r = 0.96, P < 0.001) and women (r = 0.97, P < 0.001). Han et al. (1997)
concluded that in large studies of VAT, using MRI or CT scanning, a single VAT area scan between L2 and L3 vertebrae offers a less expensive, faster and safer method, with a good prediction of VAT volumes and masses. Thus a single axial MRI scan at the L2-L3 intervertebral level is an acceptable and accurate method for estimating the masses of various abdominal adipose tissue compartments (Han et al., 1997a; Han et al., 1997b; Abate et al., 1997). However, both CT and MRI are expensive, time consuming, require highly trained operators, limiting their use to specialized centres. Thus, there is a need to develop simpler, more accessible, less expensive techniques to measure abdominal adipose tissue.

**Dual Energy X-ray Absorptimetry (DXA)**

Dual photon absorptiometry (DPA) and dual energy X-ray absorptiometry (DXA) were originally designed to measure bone mineral content, and are still most widely used for this application. Recently with advances in software analysis it has been used to estimate the composition of soft tissue in the body (Gotfredsen et al., 1986). DXA is now widely available and relatively inexpensive in relation to MRI and CT.

Dual photon (153-Gd) absorptiometry is performed during a 50-90 minute scanning procedure of the entire body or parts of the body by a moving source below and a detector above the subject (Schlemmer et al., 1990). The 153-Gd source emits photons with principal energies at 44 and 100 KeV. The ratio of attenuation at the two energies can be used as an indicator of the fat composition of tissue (Gotfredsen et al., 1986). As DPA gives a direct measurement of both the lean and the fatty component in any pixel of the body, both local and total body composition can be measured.
Dual energy X-ray absorptiometry (DXA) is based on a similar principle to DPA by using dual energy determination for soft tissue. DXA measurements are made with a scanner that uses a constant potential X-ray source at 12.5 keV and a K-edge filter (cerium) to achieve a congruent beam of stable dual energy radiation (Mazess et al., 1990). The effective energies are 6.4 and 11.2 keV. A DXA measurement does, however, involve X-ray exposure of subjects, including the thyroid and gonadal regions when scanning the whole body. Mazess et al. (1990) reported scanning times of 10-20 minutes for the total body determination. In the same report these authors reported that radiation doses were 0.05 and 0.10 μGy for the 10 and 20 minute scanning procedures respectively. A whole body DXA involves a very low amount of radiation; about 1 mRem (i.e. about 1/30th of a chest X-ray).

As with DPA, using DXA the composition of soft tissue is given by the ratio of the beam attenuation at the lower energy relative to that at the higher energy level (the R value). The R values from human scans are compared to a calibration line based on measurements of water (maximal R value), of phantoms containing almost 100% lipid such as lard (minimal R value) and of various other solutions, e.g. mixtures of water and alcohol (intermediate R values). The different attenuation of soft tissue and bone mineral at the two energy levels is detected by a scanner. The measurement of fat is derived from the ratio of the attenuation of the two energy levels. DXA provides a three-compartment equation of body composition: bone mineral, fat, and bone-free lean mass. The fat (lipid) mass derived from DXA is not solely the fat situated inside adipose tissue, but from the sum of all the fatty elements in the body. The bone-mineral free lean mass is a complex entity and represents everything that is not fat or bone mineral and even includes bone protein. Bone-mineral free lean mass is equal to fat free mass minus the bone mineral mass. Thus, the fat (lipid) estimated by DXA and the
adipose tissue by MRI are two different entities (as described by Wang et al., 1992) that may not be directly comparable.

Accuracy and precision of DXA

The reproducibility of the DXA measurements of abdominal fat mass is about 12% (CV%) (Schlemmer et al., 1990). The coefficient of variation of the fat mass and fat percentage of the abdominal region has been found to be 4.3% and 3.4% respectively (Svendsen et al., 1993). The DXA method has been reported to have better reproducibility compared to DPA and is considerably faster (Mazess et al., 1990).

DXA and body composition

The measurements of abdominal fat is affected by movement artifacts and overestimation of fat tissue occurs in subjects with reduced bone mineral because mineral relative to lean tissue is assumed to be constant (van der Kooy & Seidell, 1993a). In addition, a common disadvantage of DXA is that although sections of the body can be evaluated separately, no distinction can be made between visceral and subcutaneous fat (Van der Kooy & Seidell, 1993a). However, an estimate of the subcutaneous adipose issue may be achieved when DXA is combined with anthropometric measurements.

Anthropometry

Anthropometric measurements such as skinfolds, diameters, and circumferences are easy, inexpensive and quick to perform. They are therefore attractive for clinical settings and for use in epidemiological studies. Anthropometric measurements have been used for many
decades to describe fat distribution or fat patterning. Historically, the use of anthropometric measurements for this purpose shifted from skinfold thicknesses (Garn, 1955; Edwards, 1950; Mueller & Stallones, 1981; Mueller & Wohlieb, 1981) to circumferences (Johnstone et al., 1988; Mueller et al., 1987; Mueller et al., 1991; Shimokata et al., 1989) and most recently abdominal diameters (Kvist et al., 1988; Despres et al., 1991; van der Kooy et al., 1993).

Due to the high cost and restricted availability associated with both CT and MRI, attempts have been made to estimate CT and MRI body composition values using anthropometry. Effective predictive equations would be useful in epidemiological and clinical situations. Because it is hypothesized that the VAT depot conveys the greatest health risk (Despres et al., 1990), and it is the parameter uniquely measured by CT and MRI, numerous studies have examined the relationship between VAT and anthropometric variables. Skinfold thicknesses, body circumferences, and diameters have been studied as potential correlates of VAT accumulation. The heterogeneity of body fatness in the samples that have been studied has influenced the strength of the associations reported between anthropometric variables and VAT. In fact, when a sample with large variation in total adiposity was studied, numerous anthropometric variables were significantly associated with abdominal VAT accumulation (Despres et al., 1991).

Among the various skinfold thicknesses assessed, those measured on the trunk, and more specifically the abdomen, were the most closely associated with the accumulation of abdominal VAT (Depres et al., 1991; Koester et al., 1992). The mean adiposity level of the study sample may affect the relationships between skinfold thicknesses and VAT accumulation. It has been demonstrated that the correlation between trunk skinfold thicknesses and VAT is significant in a subsample of men with BMI values less than 28
kg/m². This association was not present in men with BMI values greater than 28 kg/m² (Despres et al., 1991).

The use of these measurements for the prediction of the amount of visceral fat has been evaluated through the construction of equations regressing sets of anthropometric measurements on visceral fat areas or volumes obtained by imaging techniques. Several prediction equations with a high proportion of explained variance of visceral fat ($r^2$ between 0.56 and 0.96) have been published (Kvist et al., 1988; Ross et al., 1992; Despres et al., 1991; Seidell et al., 1987; Ferland et al., 1989; Weits et al., 1988; Koester et al., 1992; Goran et al., 1998). The different sets of anthropometric measures were usually a combination of circumferences, skinfolds, diameters and their ratios. The accuracy of predicting visceral fat from anthropometry is limited especially when it comes to predicting changes in visceral fat from changes in anthropometric variables (van der Kooy et al., 1993; Ross et al., 1991; Stallone et al., 1991; van der Kooy et al., 1993). In addition, the reported prediction formulae are not generally applicable across populations (Koester et al., 1992) and coefficients of variation of predicted visceral fat areas are on average 25% (Despres et al., 1991; van der Kooy et al., 1993). The advantages and disadvantages of the separate anthropometric measures are discussed below.

**Skinfolds**

Skinfold measurements were originally used to estimate body fat percentage (Durnin & Womersley, 1974; Lohman, 1981) but they have also been used for many decades for the description of subcutaneous fat patterning (Garn, 1955; Edwards, 1950; Mueller & Stallones, 1981; Mueller & Wohlieb, 1981). Seidell et al. (1990, 1992) found that waist circumference
or waist circumference ratios were more strongly related to metabolic disorders (associated with an abdominal fat distribution) than the ratios of extremity to trunk skinfolds. It has been proposed, however, that subcutaneous fat patterning and the waist-to-hip ratio are measuring different aspects of fat distribution and are independently related to several risk factors (Haffner et al., 1987).

The most commonly used skinfold thicknesses on trunk and extremities for assessment of subcutaneous fat patterning are the biceps and triceps skinfolds on the arm and the suprailiac, subscapular, abdominal, mid-thigh, medial calf skinfolds. The coefficient of variation of repeated skinfold measurements by the same trained observer is approximately 5% (Durnin & Womersley, 1974; Lohman, 1981) and between observers it is 10-20% (Bennett & Osborne, 1986; Fuller et al., 1991). Factors affecting the reproducibility are the determination of the anatomical site for a specific skinfold, the method of picking up a skinfold, the alignment of the skinfold crest, time or scale reading and, the experience of the observer (Durnin & Womersley, 1974; Lohman, 1981; Bennett & Osborne, 1986; Fuller et al., 1991). In obese subjects, skinfold measurements are often difficult or impossible to assess and have a poor reproducibility. The measurement sites are usually difficult to determine and the width of the caliper is often not enough for the thickness of the skinfolds (Bray et al., 1978; Gray et al., 1990). The measurement of skinfold thicknesses are, therefore, mainly useful for assessing the subcutaneous fat patterning in lean and moderately obese subjects and not for the indirect assessment of (intra-) abdominal fat.

**Abdominal diameters**

Transverse and sagittal abdominal diameters have been used to predict VAT
accumulation (Kvist et al., 1988a; Sjostrom, 1988). Indeed, Sjostrom (1991) suggested that a large accumulation of VAT would maintain the sagittal (anteroposterior) diameter of the abdomen in supine subjects, while abdominal subcutaneous AT would decrease the sagittal diameter due to gravity. Furthermore, it has been reported that the abdominal sagittal diameter has correlations with the VAT area that are similar in magnitude to those for waist circumference (Despres et al., 1991; Kvist et al., 1988a; Pouliot et al., 1994; Ross et al., 1994). In most of these studies, the sagittal diameters were measured directly on CT or MRI images. In those studies that have compared anthropometrically assessed sagittal diameters to those obtained on CT or MRI images, the correlation coefficients were greater than 0.8 (Koester et al., 1992; van der Kooy et al., 1993b), but these studies were of small samples; thus further study of these relationships is needed.

Kvist et al. (1988) and Sjostrom, (1991) suggested that in subjects in supine position; increasing accumulation of visceral fat would maintain the depth of the abdomen in a sagittal direction while subcutaneous abdominal fat would reduce the abdominal depth due to force of gravity. The sagittal abdominal diameter and transverse abdominal diameter have therefore been studied as indicators for visceral fat (Kvist et al., 1988; Despres et al., 1991; van der Kooy et al, 1993; Seidell et al., 1989). Sagittal diameters are better than WHR and waist circumference to estimate VAT (van der Kooy et al., 1993). Zamboni et al. (1998) found that sagittal diameter measured by CT and anthropometry was a good predictor of VAT with $r = 0.83$ and $0.82$ respectively.

According to the above-mentioned principle, (Kvist et al., 1988; Sjostrom, 1991) these diameters should be measured in subjects in the supine position. For the measurement of the sagittal diameter, a lever or stadiometer can be used to assess the distance between
abdomen and back. Until now, in most studies, the diameters were not measured anthropometrically but derived from CT or MRI images (Kvist et al., 1988; Despres et al., 1991; van der Kooy et al., 1993; Seidell et al., 1989). Measurements can also be made in standing subjects with a whole body caliper. Anthropometrically-assessed abdominal diameters in both supine and standing position have been shown to be strongly correlated with abdominal diameters obtained from images (r coefficients > 0.8) (van der Kooy et al., 1993; Koester et al., 1992). Correlation coefficients between VAT areas or volumes and the sagittal diameter derived from the same scan ranged between 0.46 and 0.96 with the lowest coefficients in the most obese subjects (Kvist et al., 1988; Despres et al., 1991; van der Kooy et al., 1993; Koester et al., 1992).

Information about the reproducibility of the anthropometrically-assessed diameters is not available, but it will probably be comparable to the variability reported for circumference measurements (CV% of about 2%) (Bray et al., 1978). The prediction of the amount of visceral fat from abdominal diameters improved after adjusting the diameters for the thickness of the subcutaneous abdominal fat layer (Kvist et al., 1988; van der Kooy et al., 1993). However, accurate assessment of the thickness of the subcutaneous fat layer is problematic with conventional anthropometric measurements. For instance, measuring abdominal skinfold thicknesses for this purpose is, particularly in obese subjects, not accurate or even not possible (van der Kooy et al., 1993; Bray et al., 1978; Gray et al., 1990). Combinations of ultrasound measurements of subcutaneous fat with diameters or circumferences may yield more satisfactory results.


Circumferences

The most commonly used measure of fat distribution is the waist-to-hip circumference ratio. Ashwell et al. (1985) and, several years later, Seidell et al. (1988) were the first to show that circumference ratios are correlated with visceral fat. After adjustment for degree of total body fatness and age, however, independent associations of waist-to-hip ratio (WHR) to visceral fat were not always significant and sometimes the waist girth alone or the waist-to-thigh ratio were stronger correlates of visceral fat (Weits et al., 1988; Seidell et al., 1988).

Waist and hip circumferences and the waist-to-hip ratio (WHR) have also been studied as potential predictors of VAT accumulation. The WHR has been used widely to investigate the relationship between regional adipose tissue distribution and metabolic profiles (Bjorntorp, 1988; Kissebah & Peiris, 1989; Lapidus et al., 1984; Larsson et al., 1984). Indeed, it has been considered that the relationship between WHR and health risk was due to its ability to predict the accumulation of VAT, but the WHR is only moderately related to the amount of abdominal VAT (Ferland et al., 1989; Ross et al. 1992; Seidell et al., 1988; Sjostrom, 1988). Some have reported that the waist circumference alone is more closely associated to the amount of abdominal VAT and related metabolic disturbances than the WHR (Borkan et al., 1983; Despres et al., 1991; Ferland et al., 1989; Pouliot et al., 1994; Ross et al., 1994; Seidell et al., 1988). Therefore, it has been suggested that waist circumference may be particularly useful in the assessment of the health hazard of obesity, since this measure is a good correlate of both VAT accumulation and total adiposity.

Several studies have shown that abdominal distribution of AT, as indicated by the WHR, is an independent predictor of metabolic aberrations including insulin resistance.
(Kissebah et al., 1982), hyperlipidemia (Anderson et al., 1988; Despres et al., 1985), hypertension (Weinser et al., 1985), and atherosclerosis (Larsson et al., 1984). It is generally believed that the correlative power of WHR is its ability to predict absolute or relative amounts of visceral AT. Because the liberated free fatty acids from much of the visceral AT have direct access to the liver via the portal vein, it has been proposed that visceral AT is a likely mediator for some of the apparent effects of abdominal adiposity on glucose and lipid metabolism (Leibel et al., 1989). What has yet to be firmly established however, is the ability of WHR to predict absolute or relative visceral AT. Three previous studies reported significant relationships between WHR and CT-measured visceral-to-subcutaneous AT ratio (Ashwell et al., 1985; Fujioka et al., 1987; Peiris et al., 1987). Baumgartner et al. (1988), however, reported that WHR was significantly associated with absolute and relative visceral AT in women only. Seidell et al. (1987) reported that WHR did not correlate significantly with visceral-to-subcutaneous ratio measured at the L4 level after adjusting for age and body mass index. Kvist et al. (1986) reported significant relationships between WHR and visceral AT; the $r^2$ values were 69 and 35% for absolute and relative visceral AT, respectively, although did not investigate the effects of age or adiposity.

The results of Ross et al. (1992) show that for absolute visceral AT volume, after controlling for both age and adiposity, WHR explained only 12% of the variation in VAT volume. When abdominal obesity was considered from VAT area measurements obtained at the L4-L5 level, WHR explained 7% of the variation in VAT volume. Furthermore, after controlling for age and adiposity, the observed relationship between WHR and relative VAT volume was nonexistent. These results suggest that, although WHR is an independent predictor of numerous metabolic aberrations, its correlative power may not be explained by
the ability to predict either absolute or relative VAT. Ross et al. (1992) suggested that age is a better predictor of relative VAT mass than either total adiposity or WHR.

An excess of visceral adipose tissue is widely believed to be identified by an increase in waist-to-hip circumference ratio. However, waist-to-hip ratios provide only a rough indication of the amount of adipose tissue in and around the abdomen (van der Kooy et al., 1993; Busetto et al., 1992; Ross et al., 1993; Koester et al., 1992; Depres et al., 1991), and gives no indication of the absolute amount of adipose tissue within the abdomen.

The great advantages of circumference measurements are of course that they are relatively easy and cheap to perform. In addition, circumference measures were found to be reproducible (CV% about 2%) was found to be good (Bray et al., 1978). The disadvantage of the waist circumference as well as waist-to-hip and waist-to-thigh ratio is that they do not allow accurate quantitative distinction between visceral and subcutaneous abdominal fat. Nevertheless, the use of waist-to-hip ratio as a diagnostic tool for types of fat distribution is extremely useful because of the appeal it has to public health policy makers, as well as to the public itself.

Waist circumference has also been used in combination with anthropometric measures such as weight and height. The waist-to-height and waist-to-hip ratios were shown to be similarly associated with risk factors for diabetes and cardiovascular diseases (Kannel et al., 1991; Sasaki et al., 1990). Kannel et al. (1991) reported that the two ratios were reasonably correlated (r = 0.75). A 'conicity' index, developed by Valdez, (1991) is based on the idea that the shape of a body changes from a cylinder to a 'double cone' when subjects accumulate fat in and around their abdomen. This index includes weight, height and waist
circumference and is therefore almost independent of body fatness in contrast to most waist ratios.

Another advantage of circumference measurements is the possibility of instructing subjects to measure the circumferences themselves. The validity of self-reported circumferences appeared reasonable (Kushi et al., 1988; Rimm et al., 1990).

**Use of anthropometry to estimate whole body AT**

Kvist et al. (1988a) measured whole-body (total) AT volume by CT in a group of men and women differing in adiposity and reported a SEE of less than 11% when predicting total AT volume from anthropometry. Among the anthropometric variables tested, the best predictors were weight, hip circumference, and weight/stature. These observations are similar to those reported by Ross et al. (1994) for obese men and women. These authors observed that weight, hip circumference, and body mass index (BMI) were the best single predictors. In a stepwise regression equation, the combination of waist and hip circumferences in women, and these variables with the addition of thigh circumference for men, predicted total AT volume from MRI with an accuracy of about 8%. Taken together, these reports suggest that the maximum precision for anthropometrical predictions of total AT volume is about 8 to 10%.

**Use of anthropometry to estimate BF measured by DXA**

Lopez et al. (1997) found that anthropometry (log 10 of sum of triceps abdominal, suprailiac, front thigh and calf skinfolds and the abdominal/thoracic girth ratio) were best used to determine BF measured by DXA (r = 0.92, SEE = 1.8%; p < 0.001).
Wattanapenpaiboon et al. (1998) found a good agreement between body fat (BF) measured by DXA and BF estimated by four skinfold thicknesses and the prediction equations of Segal et al. (1998).

Ravoglia et al. (1999) found a good correlation between DXA and anthropometry and suggested that because of practical constraints, that often anthropometry and bioelectrical impedance (BIA) are often the only available options for body composition assessment in clinical routine; therefore, further research on the validity and improvement of these methods in older people is indicated. The study by Ravoglia et al. (1999): “Measurement of body fat in healthy elderly men: a comparison of methods” – found DXA and anthropometry were very close and “Because of practical constraints, anthropometry and BIA are often the only available options for body composition assessment in clinical routine, therefore further research on the validity and improvement of these methods in older people is indicated”.

**Predictive Equations to Estimate VAT**

Many researchers have attempted to develop equations that would predict the amount of VAT using anthropometry (Seidell et al., 1987, 1988; Weits et al., 1988; Ferland et al., 1989; Despres et al., 1991; Ross et al., 1994). Kvist and associates (1988a) developed several predictive equations using diameters as independent variables, but were unable to explain more than 80% of the variance in VAT accumulation. It has been shown that in premenopausal women weight, abdominal and subscapular skinfold thickness, WHR, and age in combination can explain up to 74% of the variance in abdominal VAT area (Ferland et al., 1989). It has also been reported that 74% of the variance in abdominal VAT accumulation can be explained by a combination of waist circumference and age in 110 men.
Other equations developed with skinfold thicknesses and circumferences yield similar results with an explained variance of about 70 to 80% (Despres et al., 1991).

Few studies have cross-validated equations developed to predict VAT. Kvist et al. (1988a) tested their predictive equations on small cross-validation groups (7 men, 9 women). The mean difference between VAT areas from CT and predicted values ranged from 8.4 to 11.5% in males and from 18.4 to 27.3% in females depending upon the equation used. Koester et al. (1992) also cross-validated equations developed by their group and others with a cross-validation sample of 21 men. They found that only studies from validation groups with sample characteristics similar to those in the cross-validation group reported satisfactory results, indicating the importance of covariates such as age, sex, adiposity, and health status in the estimation of VAT accumulation.

Depres et al. (1996) cross-validated a predictive equation developed in a sample of 110 men, with a mean age of 31 years, on two samples. One sample was of middle-aged men (n= 34, mean age 55 years) and the other was of young men (n= 41, mean age 25 years). In the middle-aged men, a correlation coefficient of 0.86 was found between the measured and the predicted values with a standard error of estimate of 33.3 cm$^2$ (22.5%). In the young men, the correlation was 0.66 with a standard error of estimate of 29.3 cm$^2$ (42.6%). These results provide further evidence that accurate predictions of VAT accumulation from anthropometry alone are not possible. It was also recommended that attempts to predict VAT should take age and gender into account. The obvious gender differences in AT distribution may lead to gender differences in the relation of total body fat to VAT accumulation. In this regard, (Lemieux et al., 1993) examined the relation of total body fat to VAT accumulation in young men and women. Although positive correlations between body
fat mass and VAT area were noted in each gender, the regression slope was steeper in men than in women, indicating that obesity in men was associated with a greater VAT accumulation than in women. Furthermore, there is a larger accumulation of VAT in the elderly than in young adults, even after control for concomitant variation in total fatness (Enzi et al., 1986; Kotani et al., 1994; Seidell et al., 1988). This phenomenon is observed in men and women. Furthermore, menopause is associated with an increase in VAT accumulation, particularly in the absence of estrogen replacement therapy (Haarbo, Marslew, Gotfredson, & Christiansen, 1991; Ley, Lees, & Stevenson, 1992). Thus, it is important to consider gender and age in the prediction of VAT from anthropometry.

In summary, anthropometric measurements alone cannot provide accurate predictions of VAT. This is further supported by weight gain (Bouchard et al., 1990) and weight loss studies (van der Kooy et al., 1993a) where the change in AT had a poor correlation to the changes in VAT. Thus, only CT and MRI can accurately measure changes in cross-sectional VAT areas in intervention studies. When the use of imaging techniques is not practical, however, anthropometric variables such as the waist circumference may help identify individuals with a preferential accumulation of VAT and thus at increased risk of metabolic complications.

**DXA Combined with anthropometry to estimate VAT**

One of the classic studies in the area of estimating abdominal adipose tissue was performed by Svendsen et al. (1993). These researchers developed an equation for the prediction of VAT by using measurements from anthropometry and DXA combined. Their subject population was 25 postmenopausal women with a wide range of BMI (19.9-53.1
kg/m²). The subjects in this study were very obese with a mean body fat percentage of 40.2 ± 7.4%. Svendsen et al. (1993) estimated the VAT and SAAT from the 1st to the 4th lumbar (L₁-L₄) intervertebral disk region of the abdomen using CT. The fat in this same region was then measured by DXA. Height and weight were measured along with the waist, hip, and umbilicus circumferences. The right subscapular, supra-iliac, and abdominal skinfolds were also measured. An anthropometer was used to measure the abdominal sagittal diameter at the level of the umbilicus while the subjects were in the supine position. Svendsen et al. (1993) also used the log of the sum of skinfolds (right subscapular, supra-iliac, and abdominal) as a variable in their regression analysis.

Svendsen et al. (1993) found that abdominal fat mass from L₁ to L₄ measured by DXA and CT were valid and had a correlation of \( r = 0.90 \) with a SEE of 7% and that DXA explained about 80% of the variation in the VAT measured by CT. These authors found that abdominal fat mass measured (from L₁ to L₄) by DXA, the waist-to-hip ratio, and the log of the sum of skinfold thicknesses could be combined into a prediction equation to accurately predict the mean VAT area (from the L₁-L₄ vertebrae) measured by CT \( (r^2 = 0.91, \text{SEE} = 15\%) \).

Svendsen et al. (1993) also found that the WHR was moderately correlated with VAT \( (r^2 = 0.66) \) and that WHR was the only variable that correlated significantly with the VAT/SAAT ratio \( (r^2=0.40, \ p < 0.001) \). The sagittal diameter was moderately correlated with VAT \( (r^2 = 0.61) \) but not a significant predictor of VAT. These authors concluded that VAT can be predicted in postmenopausal women by DXA combined with anthropometry.

Jensen et al. (1995) also used anthropometry and DXA combined to estimate abdominal adipose tissue. This study had 6 men and 15 women with ages 19-60 y (mean 38
± 12 y) with a BMI range of 18.8 – 35.8 kg/m². The length of the trunk (area of interest) for this study was from the top of the diaphragm to the top of the femur was 38 ± 2 cm. The 6mm thick transverse CT scans were not adjacent and they were interpolated to 10 mm (the slice interval) to cover this area and estimate a VAT volume. Jensen et al. (1995) found that combination of DXA and anthropometry were not significant predictors of CT-measured VAT volume (r = 0.61, p < 0.05). However, the combination of the VAT area from a single CT scan and DXA-measured abdominal fat was an excellent predictor of CT-measured VAT (r = 0.98, p < 0.001). These authors concluded that the VAT area from a single CT scan (or other imaging technique) combined with or without DXA is required for accurate predictions of VAT volume.

Recently Goran et al. (1998) used a anthropometry combined with DXA to derive estimation equations to predict VAT and SAAT (areas from one CT scan at the level of the umbilicus) in healthy pre-pubertal children. The subjects for this study were 113 healthy Caucasian and African-American pre-pubertal children aged 4-10y. Variables used to develop the prediction equation included total body fat and trunk fat measured by DXA, height, weight and various anthropomteric measurements such as: skinfolds (axilla, chest, subscapular, suprailliac, abdomen, triceps, thigh and calf), and circumferences (waist and hip).

Goran et al. (1998) found that VAT was most strongly correlated with the abdominal skinfold (r = 0.88) and trunk fat by DXA (r = 0.93). These authors also found that the VAT area (from one CT scan at the umbilicus) was best predicted by trunk fat and total body fat by DXA and the abdominal skinfold (r² = 0.85, SEE 28.71%). In the absence of DXA the VAT was best predicted by abdominal skinfold, ethnicity and subscapular skinfold (r² = 0.82).
These authors also found that the SAAT was strongly correlated with trunk fat by DXA \((r = 0.96)\), total fat by DXA \((r = 0.93)\) and waist circumference \((r = 0.93)\). SAAT was best predicted by trunk fat from DXA, body weight, waist circumference and abdominal skinfold \((r^2 = 0.96)\). Anthropometry alone could predict SAAT with waist circumference, scapular skinfold, height and abdominal skinfold \((r^2 = 0.92)\). Goran et al. (1998) concluded that VAT and SAAT can be accurately estimated in Caucasian and African-American pre-pubertal children from anthropometry with and without the availability of DXA data.

**Are CT and MRI Reference Techniques?**

Although MRI and CT are not widely available, these techniques might serve as reference techniques for the validation of other methods of body composition assessment. Of principal interest is whether MRI data can be used to calibrate field methods of body fat assessment. Two key issues must be resolved in order to measure body fat by MRI or CT. The principal issue relates to the conversion of AT volume to lipid mass (body fat). This requires knowledge of AT density and its lipid fraction: 
\[
\text{AT lipid mass} = \text{AT volume} \times \text{AT density} \times \text{adipose lipid fraction}
\]

The normal physiological range for AT density and AT lipid fraction is 0.91 to 0.98 g/ml and 0.5 to 0.9, respectively (Martin et al., 1994). Thus the conversion of AT volume to AT lipid mass requires a factor that varies over the range of their product, approximately 0.5 to 0.8 g/ml. This considerable variation cannot be ignored; successful conversion requires a more accurate estimate of the value of this factor. Besides the study by Martin et al. (1994), there is a lack of studies and in vivo methods for estimating either AT density or its lipid fraction in the literature. It has been shown, however, that these values are closely related to the degree of adiposity, with the highest AT densities and lowest
lipid fractions occurring in the leanest individuals, and the lowest AT densities and highest lipid fractions occurring in the fattest individuals (Martin et al., 1994). The second issue when using MRI or CT to estimate total body fat is that after AT lipid has been estimated, non-AT lipid must be added to this value to obtain total body lipid. The amount of non-AT lipid is variable and its estimation difficult.

These observations suggest that the error in the conversion of AT volumes from CT or MRI to lipid mass precludes its use as a reference measure of body fat. It is important to note, however, that the health implications of non-AT lipid are minimal. Therefore, the variable of interest with respect to health risk is AT lipid. As noted earlier, there are uncertainties in the calculation of total body lipid from CT or MRI data, but it may not be necessary to estimate total body lipid. Although, determination of AT lipid mass requires knowledge of the density and lipid fraction of AT, and these vary by site and among individuals, it may be possible to estimate them from measures of total adiposity. Future studies related to the composition of human AT could help the application of this approach.

Conclusions

CT and MRI are exciting new tools that may become reference methods in the development of improved equations for assessing human body composition. At this time, however, there is a need for further validation of both methods. Preliminary validation data demonstrate that CT and MRI are the methods of choice for the precise measurement of SAAT and VAT. The assessment of VAT accumulation is critical in the assessment of the health risk since visceral obesity is closely associated with numerous metabolic alterations predictive of an increased risk of diabetes mellitus and cardiovascular diseases. Although the
accurate prediction of VAT by anthropometry remains a problem, preliminary results suggest that waist circumference provides useful information in the assessment of the health risk associated with visceral obesity in each gender.
CHAPTER 3: METHODS

Study Design

This study was a cross sectional correlational analysis (i.e. descriptive study with no control group). All anthropometric, DXA and MRI measurements (for each subject) were performed on the same day.

Subjects

Middle aged men were selected for this study because they are at a high risk for cardiovascular problems (Larson et al., 1984; Ducimetiere et al., 1986; Donahue et al., 1987). There were 12 male Caucasian volunteers 50-69 years of age who underwent the anthropometry, whole body DXA and abdominal MRI scanning. Subjects were recruited mostly by newspapers, newsletters, and flyers distributed at community centres, fitness centres, hospitals and university. Subjects were also free of any serious disease, metal implants and were apparently healthy. A broad range of BMI was desired to give a better range for the analysis of estimating VAT mass at different levels of adiposity (i.e. the more heterogeneous and larger the sample size would result in a better range for the correlational analysis).

A similar study using CT as the criterion measure found that abdominal adipose area estimated by DXA and anthropometry combined could accurately predict the mean VAT area measured by CT with an $r^2 = 0.91$ and SEE = 15% (Svendsen et al., 1993). From the values obtained in this study by Svendsen et al. (1993) it was calculated that this study would have a power of 0.90 with 11 subjects (using Cohen's Power Tables – two tailed test, with the level
Recently Thomas et al. (1998) stated that:

"Conventional indirect techniques, including underwater weighing, body water dilution, impedance, and anthropometry, measure body fat content by empirically determined relationships on the basis of population averaging. A critical difference between MRI and the other techniques is that MRI area measurement is amenable to absolute calibration. This leaves only tissue distribution and tissue content as possible sources of measurement errors in different individuals. MRI based studies are likely to be less affected by individual variability and may therefore achieve higher statistical power for a given sample size."

The methods and procedures used in this investigation were approved by the clinical research ethics board of the University of British Columbia, Canada. All participants were requested to sign an informed consent before being tested (see Appendix A for copy of the Subject Consent Form).

Measurement of Height, Weight and Anthropometry

Body weight was measured on a scale calibrated to 0.1 kg. Barefoot standing height was measured to the nearest 0.1 cm. Skinfold and circumference measurements were obtained using the procedures described by Lohman et al. (1988). Skinfold thicknesses (mm) were obtained using a Harpenden skinfold caliper at the following sites: triceps, biceps, chest, subscapular, suprailliac, abdominal, mid-thigh, and medial calf. Circumference measurements were taken at the following sites: relaxed arm, forearm, waist, hip, umbilicus, mid-thigh, calf. Circumferences were obtained with the subject in the standing position. An anthropometer was used to measure the abdominal sagittal diameter (cm) at the level of the umbilicus while the subjects were in the standing position.

Body fat distribution was estimated by anthropometry using variables utilized by
other researchers in similar studies such as: waist-to-hip ratio (WHR), sum of skinfold measures and log of the sum of skinfold measures. This present study utilized the same sum of skinfolds as Despres et al. (1984, 1991), who used: 1) the sum of three skinfolds (subscapular, suprailiac, abdominal; mm) to get an estimate of subcutaneous adipose tissue in the trunk; 2) sum of four skinfolds (triceps, biceps, thigh, calf; mm) to get an estimate of subcutaneous adipose tissue in the extremities; 3) the sum of seven skinfolds (triceps, biceps, subscapular, suprailiac, abdominal, thigh, calf; mm) as an indicator of total body subcutaneous adipose tissue. This study also used the log of the sum of three skinfolds (abdominal, suprailiac, subscapular; mm) which were found by Svendsen et al. (1993) to be a good predictor of the mean VAT area between the L1-L4 vertebrae (i.e. taking the log of the sum of skinfolds transforms a positively skewed curve to a more normal distribution of data). All anthropometric measurements were at the locations as specified by the Anthropometric Standardization Reference Manual by Lohman et al. (1988) except that the abdominal skinfold was taken vertically instead of horizontally.

**Measurement of Body Composition by DXA**

Total body and regional body composition were measured by DXA using a Hologic QDR-4500W. The radiation exposure from this procedure was negligible and estimated to be 0.06 mR. Subjects were scanned in light clothing, while in the supine position with arms by their side. Each volunteer was positioned with their arms sufficiently separated from their trunk to allow the creation of a region of interest that included only truncal contents. All DXA scans were performed and analyzed by using the Hologic Whole Body V8.20a:5 software program for body composition analysis. The DXA scans provided a three-
compartment model of body composition. The DXA scan analysis provided the following in vivo parameters: 1) fat mass, 2) bone mineral mass, and 3) bone-free lean mass (i.e. body mass minus fat mass and bone mineral mass). These parameters were recorded for the whole body, arms, legs and trunk. The trunk included the thoracic and abdominal areas. The trunk was defined as the whole body less the head, neck, arms and legs. The cutoff landmarks were the neck at the 1st rib, shoulder (humeroscapular) joint and hip (coxal) joint. This region was chosen as the trunk region because it is commonly the default positioning of the software during a whole body DXA analysis and has easily distinguishable landmarks. Other researchers such as Svendsen et al. (1993) used L1-L4 as the landmarks, but this introduces some subjectivity and error into the exact location of the cutoff landmarks. Also, Treuth et al. (1995) found that by dividing the trunk region into three specific regions did not improve the estimation of VAT area at L4-L5. (see Appendix C for example of DXA scan). The total time for the DXA analysis for each subject was about 10 minutes.

Measurement of Abdominal Adipose Tissue Distribution by MRI

MR images were obtained with a SHIMADZU SMT-100x MRI whole body scanner. A spin-echo sequence with a 600-ms repetition time and a 11-ms echo time was used for all MRI acquisitions. These parameters were selected to: 1) acquire 16-20 adjacent transverse scans (no gaps) with a thickness of 20 mm of the abdominal region in a single MRI acquisition sequence, 2) obtain MR images that were T1 weighted (42.6MHZ) which resulted in reasonable AT-lean tissue contrast, and 3) maintain a good signal-to-noise ratio. All images were acquired on a 256 x 179.2 matrix within a 45 x 45 cm field of view, giving a 4.25 mm² pixel area (4.25mm² x 20 mm thickness = 85 mm³ pixel volume). The subjects
laid in the magnet in a supine position, with arms placed straight above the head. All data sets included the region from the L1 to L5 vertebrae. (see Appendix C for example of MR images).

To plan the data acquisition, a sagittal image (i.e. scout scan) of the abdomen region was taken to identify the region of interest (i.e. superior plate of the L1 vertebra to the inferior plate of the L5 vertebra). All image data were transferred onto MRI x-ray films.

**Transfer of MRI images into analysis software**

Borkan et al. (1982) using CT used 7 scans of 10 mm thickness 20 mm apart to look at the region from 6 cm below the umbilicus to 6 cm above the umbilicus. Svendsen et al. (1993) measured mean VAT area using CT from the L1 to L4 lumbar vertebrae. A recent study by Han et al. (1997) looked at the volumes and areas of transverse scans of VAT in the trunk from the L1 to L5 vertebrae using nine scans of 20 mm thickness. For this present study the landmarks for the eleven MRI cross-sectional scans (i.e. region of interest) were from the superior plate of the L1 vertebra to the inferior plate of the L5 vertebra. This area corresponds approximately to the top of the kidneys to the anterior superior iliac crest.

In this study, MRI scans superior to the L1 vertebra had an increase in movement artifacts due to respiratory movements and thus the AT-lean tissue contrast was not as well defined as the MRI scans were below the L1 vertebra. A signal averaging technique could have been used to attempt to reduce these artifacts as described by Ross et al. (1992). But this signal averaging technique would have required using at least four averages and added at least 30 minutes per subject for total MRI data acquisition. This was beyond the scope of this study.
The eleven adjacent MRI scans (transferred onto x-ray films) for each subject were then scanned into an Adobe PhotoShop software file (600 dpi – gray image) using an external-scanner Umax Vista 5.8 especially designed for MRI negatives. To facilitate the processing and storage of the MR images, the MRI data were linearly compressed from 12-bit (0 - 4,095 range) to 8-bit (0 - 255 range) values. Each MRI negative was scanned into the PhotoShop program along with a corresponding scale from the MRI scan. From this scale it was determined that 1 cm$^2$ from the original subject corresponded to 393 ± 6 pixels on the scanned MRI image in the Photoshop software program.

Segmentation of AT

By a pilot study it was determined that by using a cutoff threshold of 170 (on a scale from 0 – 255) on the histogram in the Photoshop software program would result in the best representation and distinction of AT from other tissues. The threshold selected for AT was based on the analysis of a sample of typical images and the respective gray level histograms produces by the Potoshop software program. Measurement errors of AT from individual variation were minimized by using internal markers such as looking at the border between the SAAT and the muscles of the abdomen. The area highlighted containing the SAAT and the muscles of the abdomin resulted in two distinct pixel intensity peaks for lean and adipose respectively (see Appendix C for an example). From these preliminary studies it was determined that the optimal threshold for AT was 170 on a scale histogram scale (0 - 255) of the highlighted area in the Photoshop software program. Therefore, any pixel intensities above 170 on the histogram would be considered as representing AT. The contrast was also increased by 75% on each scan in the Photoshop software program to increase the
differentiation between AT and lean tissue. The pixels that were identified as AT in response to the threshold selected were then counted.

**Calculation of AT Volume and Mass**

Using the Adobe PhotoShop software the various adipose tissue compartments (i.e. VAT, SAAT and TAAT) were traced by encircling the abdominal and thoracic cavities halfway through the muscle wall (i.e. rectus abdominus, obliques, quadratus lumbarum and the long back muscles) on the computer screen using a mouse for each scan. The area of SAAT was obtained as the difference between TAAT and VAT. The software program then counted the number of pixels in each highlighted area (i.e. traced area), which were then converted to areas (cm$^2$) (i.e. number of pixels counted divided by 393 pixels/cm$^2$ resulted in the true tissue area of AT in cm$^2$).

The AT area for each compartment (i.e. VAT, SAAT, TAAT) in each subject was then multiplied by the scan thickness (20 mm) to get the volume of each AT compartment in each scan. The volumes of the 11 transverse adjacent scans were then summed to give the total volume for VAT, SAAT and TAAT for the region from the L1 to L5 vertebrae in each subject. The volumes were then converted to mass of adipose tissue by using 0.925 g/ml as the density of AT. This density was derived assuming that AT contains 80% fat, 2% protein, 18% water with negligible minerals (Garrow, 1974), with corresponding densities at body temperature (37°C) of 0.900, 1.34 and 0.993 kg/l (Siri, 1961), giving an average AT density of 0.925 kg/l. This was also the same density value of AT used by Han et al. (1997).

As explained in the Literature Review the lipid fraction and the density of AT is not the same in all individuals. The content of AT ranges from 80-85% fat (lipid), 2-3% protein,
13-18% water with negligible minerals (Garrow, 1974), with a corresponding densities at body temperature (37°C) of 0.920 - 0.925 kg/l (Siri, 1961; Thomas, 1962; Sohlstrom et al., 1993; Martin et al., 1994; Tothill et al., 1996).

Reliability

All scans were analyzed by the same investigator. The test re-test reliability for multiple repeated estimates of adipose tissue compartments were made to determine reproducibility of the MRI and DXA measurements; the coefficient of variation for repeated measures was determined.

Statistical Analysis:

A simple Pearson correlation analysis was used to assess the simple relationship between variables: age, height, weight, body mass index, skinfold thicknesses (triceps, biceps, chest, subscapular, suprailliac, abdominal, mid-thigh, calf), sum of three skinfolds (subscapular, suprailliac, abdominal), sum of four skinfolds (triceps, biceps, mid-thigh, calf), sum of seven skinfolds (subscapular, suprailliac, abdominal, triceps, biceps, mid-thigh, calf), log of the sum of three skinfold thicknesses (subscapular, abdominal, suprailliac), circumferences or girths (arm, forearm, umbilicus, waist, hip, mid-thigh, calf), sagittal diameter), waist-to-hip ratio and DXA fat measures (body fat mass, trunk fat); and MRI AT masses in different regions (VAT, SAAT, TAAT).

Development of Prediction Equations to Estimate VAT

A forward stepwise multiple regression analysis was performed to develop prediction
equations to estimate adipose tissue mass in different regions as determined by the MRI. VAT mass was the dependent variable and the independent variables were: age, height, weight, body mass index, skinfold thicknesses (subcapular, suprailliac, abdominal), sum of three skinfolds (subcapular, suprailliac, abdominal), sum of four skinfolds (triceps, biceps, thigh, calf), sum of seven skinfolds (subcapular, suprailliac, abdominal, triceps, biceps, mid-thigh, calf), log of the sum of three skinfold thicknesses (subcapular, suprailliac, abdominal), circumferences or girths (umbilicus, waist, hip), waist-to-hip ratio, sagittal diameter, and DXA fat measures (body fat mass, trunk fat mass).

**Cross-Validation with Other Studies in the Literature**

Multiple prediction equations from the literature were also cross-validated with the values measured with this present study. For example, the equation developed by Svendsen et al. (1993) to estimate mean the VAT area from L1-L4 was cross validated with the measurements made by this present study.

**Development of Prediction Equations to Estimate BF and TF**

Multiple regression analyses were applied to identify the best predictors of body fat and trunk fat mass measured by DXA using only anthropometric variables. The multiple regression analysis was performed with the body fat mass and trunk fat mass as determined by DXA being the dependent variables and the independent variables were: age, height, weight, body mass index, skinfold thicknesses (triceps, biceps, chest, subcapular, suprailliac, abdominal, mid-thigh, calf), sum of three skinfolds (subcapular, abdominal, suprailliac), sum of four skinfolds (triceps, biceps, mid-thigh, calf), sum of seven skinfolds (subcapular,
suprailiac, abdominal, triceps, biceps, mid-thigh, calf), log of the sum of three skinfold thicknesses (subscapular, abdominal, suprailiac), circumferences or girths (arm, forearm, umbilicus, waist, hip, mid-thigh, calf), waist-to-hip ratio, and sagittal diameter.

The final prediction equations were the ones with the highest correlation ($r^2$), where all of the independent variables were significant at the 0.05 level. For the stepwise regression the probability of F-to-enter and probability of F-to-remove as entry and removal criteria were 0.05 and 0.10 respectively. All statistical analyses were completed by using SPSS software (SPSS Inc. Chicago). Significance was set at the 0.05 level for all tests.
CHAPTER 4: RESULTS

Subject Characteristics

There were originally 12 male Caucasians who volunteered and gave their full informed consent to take part in the study. The data of one subject had to be omitted because some of the MRI scans for this subject had a poor signal-to-noise ratio and some of the SAAT appeared as a shadow (i.e. "ghosting"), which was the consequence of randomly occurring MRI artifacts (see Appendix C for example). The body of the same subject was also very large and some tissue was outside of the MRI scanning area. Therefore, it was decided to omit the data from this subject and the final number of subjects used for the data analysis for this study was eleven. The physical characteristics of the eleven middle aged men used in the data analysis are summarized in Table 1.

The group of subjects was a mixture of obese and non-obese middle aged men who had a mean age of 58.0 ± 6.0 y (range 50.0 – 69.0 y). The subjects in this study had a wide range of Body Mass Index’s (BMI’s) from 20.1 to 32.6 (kg /m²) with a mean of 26.9 ± 3.4 (kg /m²). The waist-to-hip ratio (WHR) in the group of subjects ranged from 0.815 to 0.950 with a mean of 0.885 ± 0.048. The sum of three skinfolds (subscapular, abdominal, suprailliac) ranged from 34.8 to 130 (mm) with a mean of 72.8 ± 28.9 (mm). The sum of four skinfolds (triceps, biceps, thigh, calf) ranged from 24.8 to 98.7 (mm) with a mean of 53.1 ± 20.7 mm. The sum of seven skinfolds (triceps, biceps, thigh, calf, subscapular, abdominal, suprailliac) ranged from 59.6 to 229 (mm) with a mean of 126 ± 48.0 mm. The log of the sum of three skinfolds (subscapular, abdominal, and suprailliac) ranged from 1.44 to 2.05 with a mean of 1.73 ± 0.19.
Table 1: Physical characteristics, body composition, and anthropometric measurements of the men used for the data analysis in this study (n=11)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range (min. – max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>58 ± 6</td>
<td>50 – 69</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177 ± 7</td>
<td>166 – 192</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.7 ± 14.9</td>
<td>59.4 – 108</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.9 ± 3.4</td>
<td>20.1 – 32.6</td>
</tr>
<tr>
<td><strong>Skinfolds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td>12.2 ± 5.1</td>
<td>5.3 – 23.0</td>
</tr>
<tr>
<td>Biceps (mm)</td>
<td>6.8 ± 2.2</td>
<td>3.2 – 10.2</td>
</tr>
<tr>
<td>Chest (mm)</td>
<td>17.2 ± 7.1</td>
<td>8.7 – 32.0</td>
</tr>
<tr>
<td>Subscapular (mm)</td>
<td>18.8 ± 8.3</td>
<td>7.9 – 32.5</td>
</tr>
<tr>
<td>Suprailiac (mm)</td>
<td>29.1 ± 11.6</td>
<td>14.2 – 50.5</td>
</tr>
<tr>
<td>Abdominal (mm)</td>
<td>24.9 ± 10.6</td>
<td>10.0 – 47.0</td>
</tr>
<tr>
<td>Mid-thigh (mm)</td>
<td>21.1 ± 9.5</td>
<td>10.5 – 44.5</td>
</tr>
<tr>
<td>Medial Calf (mm)</td>
<td>13.1 ± 5.7</td>
<td>5.4 – 21.2</td>
</tr>
<tr>
<td><strong>Girths (Circumferences)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relaxed arm (cm)</td>
<td>31.6 ± 2.9</td>
<td>25.5 – 35.4</td>
</tr>
<tr>
<td>Forearm (cm)</td>
<td>28.4 ± 1.8</td>
<td>25.3 – 31.1</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>93.4 ± 9.8</td>
<td>76.4 – 108.0</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>105 ± 6.3</td>
<td>93.8 – 115.5</td>
</tr>
<tr>
<td>Umbilical (cm)</td>
<td>99.2 ± 11.3</td>
<td>79.0 – 117.7</td>
</tr>
<tr>
<td>Mid-thigh (cm)</td>
<td>52.1 ± 4.3</td>
<td>42.9 – 57.0</td>
</tr>
<tr>
<td>Calf (cm)</td>
<td>37.3 ± 2.6</td>
<td>32.2 – 41.5</td>
</tr>
<tr>
<td>Sagittal diameter (cm)</td>
<td>26.1 ± 3.8</td>
<td>19.0 – 30.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.885 ± 0.048</td>
<td>0.815 - 0.950</td>
</tr>
<tr>
<td>SOTSF (mm)</td>
<td>72.8 ± 28.9</td>
<td>34.8 – 130</td>
</tr>
<tr>
<td>SOFSF (mm)</td>
<td>53.1 ± 20.7</td>
<td>24.8 – 98.7</td>
</tr>
<tr>
<td>SOSSF (mm)</td>
<td>126 ± 48.0</td>
<td>59.6 – 229</td>
</tr>
<tr>
<td>LOGSUMSF</td>
<td>1.73 ± 0.19</td>
<td>1.44 – 2.05</td>
</tr>
</tbody>
</table>

SOTSF = sum of three skinfolds (subscapular, suprailiac and abdominal); SOFSF = sum of four skinfolds (triceps, biceps, thigh and calf); SOSSF = sum of seven skinfolds (SOTSF + SOFSF); LOGSUMSF = log of SOTSF
Fat Measured by DXA

The BF and TF measurements from the DXA analysis are shown in Table 2. The body fat (BF) measured by DXA for the group of subjects ranged from 11.3 to 32.0 (kg). The mean BF and TF mass measured by DXA were 20.0 ± 6.6 (kg) and 10.8 ± 3.76 (kg) respectively. These results suggest that, on average, about half of the BF was located in the trunk region. (see Appendix B for example of DXA analysis.)

Table 2: DXA and MRI measurements of the men (n=11)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range (min. – max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DXA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>20.0 ± 6.6</td>
<td>11.3 – 32.0</td>
</tr>
<tr>
<td>Body fat percentage (%)</td>
<td>22.5 ± 6.2</td>
<td>9.70 – 31.3</td>
</tr>
<tr>
<td>Trunk fat (kg)</td>
<td>10.8 ± 3.76</td>
<td>5.73 – 16.6</td>
</tr>
<tr>
<td>Trunk fat percentage (%)</td>
<td>25.9 ± 5.2</td>
<td>16.50 – 31.80</td>
</tr>
<tr>
<td><strong>Mean area for 11 MRI scans (L1 – L5)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAAT area (cm²)</td>
<td>176 ± 57.8</td>
<td>104 – 280</td>
</tr>
<tr>
<td>SAAT area (cm²)</td>
<td>87.5 ± 36.2</td>
<td>43.7 – 170</td>
</tr>
<tr>
<td>VAT area (cm²)</td>
<td>88.9 ± 39.0</td>
<td>44.7 – 141</td>
</tr>
</tbody>
</table>

The trunk region measured by DXA was defined as the whole body less the head, neck, arms and legs. TAAT = total abdominal adipose tissue; SAAT = subcutaneous abdominal adipose tissue; VAT = visceral adipose tissue. The abdominal region measured by MRI was defined as the region from the L1 to the L5 vertebrae.

AT Measured by MRI

Distinct contrasts between AT and lean tissues were clearly identifiable on the MRI scans obtained. Appendix C illustrates an example of a cross-sectional abdominal image.
obtained at the L4-L5 level. As can be seen in Appendix C, the SAAT and VAT compartments were clearly distinct from lean tissue structures and thus were segmented in a straightforward manner. For this study the landmarks for the eleven MRI scans for each subject (i.e. region of interest) were from the superior plate of the L1 vertebra to the inferior plate of the L5 vertebra. This area corresponds approximately to the top of the kidneys to the anterior superior iliac crest.

The area for the VAT, TAAT and SAAT was determined for each MRI scan. The areas for each AT compartment (of the 11 MRI scans for each subject) were added together to give the total area of VAT, SAAT and TAAT for each subject. The mean areas for TAAT, SAAT and VAT measured by MRI were 176 ± 57.8, 87.5 ± 36.2 and 88.9 ± 39.0 cm$^2$ respectively for the subjects (see Table 2). Table 2 also shows that the subjects had a broad range of abdominal tissue areas where the standard deviation of the SAAT and VAT area was almost 50%. Also, from Table 2 it can be seen that the mean SAAT and VAT areas were very similar. This suggests that, for the subjects in this study, about half of the abdominal adipose tissue was situated subcutaneously and half of the abdominal adipose tissue was situated in the visceral compartment when averaged over the L1-L5 vertebrae region.

Calculations of AT Volume and Mass

Multiplying the AT area (cm$^2$) in each compartment of each MRI scan by the thickness of each MRI scan (2.0 cm) resulted in the volume (cm$^3$) of adipose tissue in each compartment for each scan. The volume of each scan compartment was then added to give a final volume for VAT, SAAT and TAAT for the L1-L5 region for each subject. The adipose
tissue volume (cm³) was then multiplied by an assumed adipose tissue density (0.925 g/cm³) to give the adipose tissue mass (kg) for each compartment (Table 3).

Table 3: Calculated values from MRI measurements of the men (n=11)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range (min. – max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total adipose volume from L1 to L5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAAT volume (litres)</td>
<td>3.88 ± 1.27</td>
<td>2.29 – 6.15</td>
</tr>
<tr>
<td>SAAT volume (litres)</td>
<td>1.93 ± 0.79</td>
<td>0.960 – 3.74</td>
</tr>
<tr>
<td>VAT volume (litres)</td>
<td>1.96 ± 0.86</td>
<td>1.07 – 3.10</td>
</tr>
<tr>
<td><strong>Total adipose mass from L1 to L5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAAT mass (kg)</td>
<td>3.61 ± 1.18</td>
<td>2.13 – 5.72</td>
</tr>
<tr>
<td>SAAT mass (kg)</td>
<td>1.79 ± 0.74</td>
<td>0.894 – 3.48</td>
</tr>
<tr>
<td>VAT mass (kg)</td>
<td>1.82 ± 0.80</td>
<td>0.995 – 2.89</td>
</tr>
</tbody>
</table>

The abdominal region measured by MRI was defined as the region from the 1st to the 5th lumbar vertebrae. TAAT = total abdominal adipose tissue; SAAT = subcutaneous abdominal adipose tissue; VAT = visceral adipose tissue. (Density of adipose tissue calculated at 0.925 kg/l).

Because adipose tissue contains chemical entities other than fat (i.e. water, proteins, etc.), in order to get fat (lipid) mass the adipose tissue mass would have to be multiplied by the fraction of fat in the adipose tissue. As previously mentioned, fat fractions are not constant and varies with whole body adiposity (Martin et al., 1994). It was decided to keep the units as adipose mass because adipose tissue is more biologically significant than fat and also prevents any further assumptions and error of estimation.

Large interindividual differences were observed for all MRI-measured variables. TAAT volume ranged from 2.3 to 6.2 litres, SAAT volume from 0.96 to 3.74 litres, and VAT
volume from 1.07 to 3.10 litres. The mean SAAT volume (1.93 ± 0.79 litres) and mean VAT volume (1.96 ± 0.86 litres) represented 49.6% and 50.4% respectively of the mean TAAT volume (3.88 ± 1.27) for the L1-L5 region.

Reliability

Seven repeated measurements were made of the same MRI scan to determine the area of VAT from an MRI image. The segmentation of AT for the scans was performed using a single threshold, 170 on a (0 - 255) scale, above which pixels were considered AT. The test-retest reliability for VAT was re-analyzed by the same investigator. The standard error of the mean for repeated measurements of transverse MR images obtained at the L4-L5 level was calculated to be 1.12%.

These results indicate that the reliability of the MRI measurements are good and show good reproducibility and confirm the findings of other authors (Staten et al., 1989; Seidell et al., 1990; Ross et al., 1991). Han et al., (1997) found the coefficient of variation using a similar method for repeated calculations to be 0.89%.

The test re-test reliability for body fat and trunk fat by DEXA was found to be 0% and 1.03% respectively, when ten scans were re-analysed by the same investigator.

Adipose Tissue Distribution and Single Scan Analysis

Figure 1 shows the mean distribution of adipose tissue from the superior plate of the L1 vertebra (scan #1) to the inferior plate of vertebra L5 (scan #11) for the subjects.

Data for each of the 11 MRI scans can be seen in Table 4. Inspection of the AT area measurements level (Table 4) shows that the largest mean value for TAAT area (217 cm²)
was obtained at the level of L3-L4 (scan #6). Superior to L2-L3 (scan #4) the mean VAT area is greater than the mean SAAT area. Inferior to L2-L3 the SAAT mean area is greater than the mean area for the VAT.

**Figure 1:** Mean distribution of adipose tissue areas from the 11 men. VAT = visceral adipose tissue, SAAT = subcutaneous abdominal adipose tissue, TAAT = total abdominal adipose tissue. Scan number 1 is near the superior plate of the L1 vertebra, scan 7 is near the umbilicus, and scan 11 is near the inferior plate of the L5 vertebra. Each adjacent MRI scan was 20 mm in thickness.

The largest mean SAAT area (120 cm$^2$) was obtained at the L3-L4 intervertebral disc region (scan #6). For VAT, the largest mean VAT area (131 cm$^2$) was measured at the L1 region (scan #1).
### Table 4: Mean adipose areas (cm$^2$) for different compartments from the lumbar vertebrae levels L1 to L5 for the men (n=11).

<table>
<thead>
<tr>
<th>Scan Number</th>
<th>Mean VAT (cm$^2$)</th>
<th>Mean SAAT (cm$^2$)</th>
<th>Mean TAAT (cm$^2$)</th>
<th>Correlation between VAT area for each scan and total VAT volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>131</td>
<td>51.0</td>
<td>182</td>
<td>0.74 (p &lt; 0.010)</td>
</tr>
<tr>
<td>2</td>
<td>121</td>
<td>58.7</td>
<td>180</td>
<td>0.66 (p &lt; 0.028)</td>
</tr>
<tr>
<td>3</td>
<td>117</td>
<td>69.7</td>
<td>187</td>
<td>0.89 (p &lt; 0.001)</td>
</tr>
<tr>
<td>4</td>
<td>108</td>
<td>87.2</td>
<td>195</td>
<td>0.95 (p &lt; 0.001)</td>
</tr>
<tr>
<td>5</td>
<td>104</td>
<td>110</td>
<td>214</td>
<td>0.95 (p &lt; 0.001)</td>
</tr>
<tr>
<td>6</td>
<td>97.4</td>
<td>120</td>
<td>217</td>
<td>0.91 (p &lt; 0.001)</td>
</tr>
<tr>
<td>7</td>
<td>79.1</td>
<td>112</td>
<td>191</td>
<td>0.78 (p &lt; 0.005)</td>
</tr>
<tr>
<td>8</td>
<td>67.7</td>
<td>108</td>
<td>175</td>
<td>0.46 (p &lt; 0.155)</td>
</tr>
<tr>
<td>9</td>
<td>54.7</td>
<td>91.2</td>
<td>146</td>
<td>0.30 (p &lt; 0.383)</td>
</tr>
<tr>
<td>10</td>
<td>47.7</td>
<td>80.4</td>
<td>128</td>
<td>0.50 (p &lt; 0.115)</td>
</tr>
<tr>
<td>11</td>
<td>50.0</td>
<td>75.2</td>
<td>125</td>
<td>0.17 (p &lt; 0.620)</td>
</tr>
<tr>
<td>Mean area</td>
<td>88.9</td>
<td>87.5</td>
<td>176</td>
<td></td>
</tr>
</tbody>
</table>

Scan #1 is near the superior plate of the L1 vertebra, scan #7 is near the umbilicus, and scan #11 is near the inferior plate of the L5 vertebra. Each MRI scan was 20 mm in thickness.

### Relationship between MRI Measured AT Area and Total VAT Volume

A correlation analysis for the mean VAT area measurements obtained on the eleven abdominal MRI scans and total VAT volume for L1-L5 was performed and can be seen in the last column of Table 4. For the eleven abdominal images acquired, the correlation with total VAT volume ranged from 0.17 to 0.95. The predictive value of the images below the L4 vertebra (scan #8) were relatively low and thus were not as good predictors as the scans obtained near the L2-L3 vertebrae (scans #4 and #5).

The scan with the highest correlation ($r = 0.95, P < 0.001$) and therefore the best predictor of total VAT mass was the VAT area on scan number 4 which corresponds to the L2-L3 intervertebral disc region.

A linear regression analysis was performed to assess the relationship between the
total VAT mass (dependent variable) and the VAT area measured at L2-L3 (scan #4) by MRI (independent variable). The following prediction equation to estimate VAT mass from one scan at the L2-L3 region had an $r^2 = 0.90$ with a SEE of 9.72%.

Equation #1:

$$\text{VAT mass}_{L1-L5} (\text{kg}) = (0.00999 \times \text{area of VAT in one MRI scan at L2-L3}) + 0.742$$

These results therefore suggest that a single scan at the L2-L3 intervertebral disc region can be used to accurately estimate VAT mass in middle aged men. Although the correlation observed are biased by the inclusion of the abdominal image of interest, the VAT areas of single abdominal scans acquired in the L2-L3 region are, nevertheless, strongly predictive of the total volume of VAT obtained by multiple images.

Relationship between Anthropometry and VAT Measured by MRI

The simple Pearson correlation coefficients ($r$) between abdominal adipose tissues measured by MRI and selected anthropometric variables are shown in Table 5. The suprailiac skinfold was the skinfold with the highest correlation with VAT mass ($r = 0.73$). The WHR had a correlation of 0.83 with VAT mass and the umbilical and waist girth had a correlation of 0.78 and 0.77 respectively to VAT mass.

Relationship between DXA and AT measured by MRI

The simple Pearson correlation coefficients ($r$) between abdominal adipose tissue masses measured by MRI, and DXA variables are also shown in Table 5.
Table 5: Correlation coefficients (r) between adipose tissue mass determined by MRI and body composition measured by DXA and simple anthropometric measurements. (n = 11)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>VAT</th>
<th>SAAT</th>
<th>TAAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>(y)</td>
<td>0.52</td>
<td>0.03</td>
<td>-0.26</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>(cm)</td>
<td>0.15</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>(kg)</td>
<td>0.68</td>
<td>0.73</td>
<td>0.77</td>
</tr>
<tr>
<td>SKINFOLDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUBSCASF</td>
<td>(mm)</td>
<td>0.48</td>
<td>0.82</td>
<td>0.73</td>
</tr>
<tr>
<td>SUPRASF</td>
<td>(mm)</td>
<td>0.73</td>
<td>0.97</td>
<td>0.93</td>
</tr>
<tr>
<td>ABDOMSF</td>
<td>(mm)</td>
<td>0.62</td>
<td>0.89</td>
<td>0.84</td>
</tr>
<tr>
<td>GIRTHS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAISTGR</td>
<td>(cm)</td>
<td>0.77</td>
<td>0.78</td>
<td>0.85</td>
</tr>
<tr>
<td>UMBILGR</td>
<td>(cm)</td>
<td>0.78</td>
<td>0.81</td>
<td>0.87</td>
</tr>
<tr>
<td>HIPGR</td>
<td>(cm)</td>
<td>0.57</td>
<td>0.68</td>
<td>0.69</td>
</tr>
<tr>
<td>SAGDIA</td>
<td>(cm)</td>
<td>0.69</td>
<td>0.77</td>
<td>0.80</td>
</tr>
<tr>
<td>BMI</td>
<td>kg/m²</td>
<td>0.66</td>
<td>0.36</td>
<td>0.53</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td>0.83</td>
<td>0.68</td>
<td>0.81</td>
</tr>
<tr>
<td>LOGSUMSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOTSF</td>
<td>(mm)</td>
<td>0.65</td>
<td>0.94</td>
<td>0.89</td>
</tr>
<tr>
<td>SOFSF</td>
<td>(mm)</td>
<td>0.66</td>
<td>0.88</td>
<td>0.86</td>
</tr>
<tr>
<td>SOSSF</td>
<td>(mm)</td>
<td>0.68</td>
<td>0.94</td>
<td>0.91</td>
</tr>
<tr>
<td>DEXA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFDXA</td>
<td>(kg)</td>
<td>0.84</td>
<td>0.91</td>
<td>0.96</td>
</tr>
<tr>
<td>BFDXA</td>
<td>(kg)</td>
<td>0.76</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAAT</td>
<td>(kg)</td>
<td>0.89</td>
<td>0.94</td>
<td>1.00</td>
</tr>
<tr>
<td>SAAT</td>
<td>(kg)</td>
<td>0.68</td>
<td>1.00</td>
<td>0.94</td>
</tr>
<tr>
<td>VAT</td>
<td>(kg)</td>
<td>1.00</td>
<td>0.68</td>
<td>0.89</td>
</tr>
</tbody>
</table>

TFDXA = Trunk fat measured by DXA, BFDXA = body fat measured by DXA, The trunk region measured by DXA was defined as the whole body less the head, arms and legs. TAAT = total abdominal adipose tissue; SAAT = subcutaneous abdominal adipose tissue; VAT = visceral adipose tissue. The abdominal region measured by MRI was defined as the region from the L1 to the L5 vertebrae. SOTSF = sum of three skinfolds; SOFSF = sum of four skinfolds; SOSSF = sum of seven skinfolds. LOGSUMSF = log of SOTSF

1 = p < 0.05
2 = p < 0.001
The linear regression relationship between the TAAT mass measured from L1-L5 by MRI (dependent variable) and the trunk fat mass measured by DXA (independent variable) is shown in Figure 2 ($r^2 = 0.92$, SEE = 0.360 (kg) or 20 %). This means that 92% of the variability in TAAT mass from the L1 to L5 vertebrae could be explained by the trunk fat mass (kg) measured by DXA. Clearly the trunk fat measured by DXA is an excellent predictor of abdominal obesity.

**Equation #2:** \[ \text{TAAT mass}_{L1-L5} \text{ (kg)} = 0.298(\text{TFDXA}) + 0.354 \]

**Figure 2:** Correlation between total abdominal adipose tissue (TAAT) mass (kg) determined by MRI (L1-L5) plotted as a function of trunk fat mass (kg) measured by DXA. Line shown is the regression line.

The linear regression relationship between the VAT mass (L1-L5) measured by MRI (dependent variable) and trunk fat mass (kg) measured by DXA (independent variable) is shown in Figure 3 ($r^2 = 0.70$, SEE = 0.311 (kg) or 17.3 %). Therefore, only 70% of the
variability in VAT mass from the L1 to L5 vertebrae could be explained by the trunk fat measured by DXA.

**Equation #3:** \( \text{VAT mass}_{L1-L5} (\text{kg}) = 0.120(\text{TFDXA}) + 0.503 \)

![Graph showing correlation between VAT mass and TFDXA](image)

\( y = 0.120x + 0.503 \)
\( R^2 = 0.70 \)

**Figure 3:** Correlation between visceral adipose tissue (VAT) mass (kg) determined by MRI (L1-L5) plotted as a function of trunk fat mass (kg) measured by DXA. Line shown is the regression line.

Also, notice that there is a decreased correlation at higher levels of obesity. These plots show that the accuracy of the prediction equations is randomly scattered, and there is a loss in prediction accuracy at increased levels of VAT mass.

**Development of Prediction Equations to Estimate VAT Mass**

The TAAT, SAAT and VAT masses were all positively correlated with each other (\( r > 0.68, P < 0.02 \)). The SAAT and TAAT masses were more closely correlated to each other
than the VAT mass (Table 5). The lowest correlation was between SAAT and VAT mass. The trunk fat mass measured with DXA had the highest correlation of all non-MRI variables with VAT mass \((r = 0.84; p < 0.001)\) and the frequently used WHR had the second highest correlation with VAT mass \((r = 0.83; p < 0.001)\). Therefore, trunk fat measured by DXA explained 70% of the variation in VAT mass and WHR could explain 69% of the variation in VAT mass. TFDXA and WHR were the only non-MRI variables which could explain the variation in VAT mass with a probability less than \(p < 0.001\).

A forward stepwise multiple regression analysis was performed to develop prediction equations to estimate VAT mass in the L1-L5 region as determined by MRI. VAT mass was the dependent variable and the independent variables were: age, height, weight, body mass index, skinfold thicknesses (subscapular, suprailiac, abdominal), sum of three skinfolds (subscapular, suprailiac, abdominal), sum of four skinfolds (triceps, biceps, thigh, calf), sum of seven skinfolds (subscapular, suprailiac, abdominal, triceps, biceps, thigh, calf), log of the sum of three skinfold thicknesses (subscapular, suprailiac, abdominal), circumferences or girths (umbilicus, waist, hip), waist-to-hip ratio, sagittal diameter, and DXA fat measures (body fat mass, trunk fat mass). When offered in a stepwise regression equation the first variable to enter the equation was the trunk fat measured by DXA \((r^2 = 0.70)\), followed by the subscapular skinfold, and then the sum of seven skinfolds (Table 6). None of the other variables offered to the equation by stepwise regression were significant predictors. The final equation (Equation #6) to estimate VAT mass with the highest \(r^2\) value (0.95) and lowest SEE (8.00%) contained only three terms.
Equation #6:

\[ \text{VAT}_{L1-L5} \text{ (kg)} = 0.304 \text{ (TFDXA)} - 0.0526 \text{ (SUBSCASF)} - 0.00707 \text{ (SOSSF)} + 0.414 \]

Where \( \text{VAT} \) is visceral adipose tissue mass determined by MRI (kg), TFDXA is trunk fat mass determined by DXA (kg), SUBSCASF is the subscapular skinfold (mm) and SOSSF is sum of seven skinfolds (mm).

Table 6: Multiple regression equations for estimation of \( \text{VAT}_{L1-L5} \) mass (kg) \( (n = 11) \)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient estimate (SEE)</th>
<th>Partial ( r^2 )</th>
<th>Equation ( r^2 )</th>
<th>SEE %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equation #4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.509 (0.301)</td>
<td>0.70</td>
<td>0.70</td>
<td>17.3</td>
</tr>
<tr>
<td>Trunk fat mass by DXA</td>
<td>0.121 (0.027)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Equation #5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.429 (0.186)</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk fat mass by DXA</td>
<td>0.226 (0.031)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>- 0.0558 (0.1396)</td>
<td>0.20</td>
<td>0.90</td>
<td>10.6</td>
</tr>
<tr>
<td><strong>Equation #6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.414 (0.140)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk fat mass by DXA</td>
<td>0.304 (0.038)</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>- 0.0526 (0.0106)</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of seven skinfolds</td>
<td>- 0.00707 (0.00267)</td>
<td>0.05</td>
<td>0.95</td>
<td>8.00</td>
</tr>
<tr>
<td><strong>Equation #7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.557 (0.149)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk fat mass by DXA</td>
<td>0.359 (0.056)</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>- 0.0577 (0.0106)</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat mass by DXA</td>
<td>- 0.0765 (0.0289)</td>
<td>0.05</td>
<td>0.95</td>
<td>8.01</td>
</tr>
<tr>
<td><strong>Equation #8 (anthropometry only)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-6.52 (1.85)</td>
<td>0.69</td>
<td>0.69</td>
<td>17.4</td>
</tr>
<tr>
<td>WHR</td>
<td>9.42 (2.09)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional Estimation Equations

It was then decided to remove the sum of seven skinfolds from the list of available independent variables because it may not always be practical to do seven skinfolds on each
subject; especially for large populations. A new forward stepwise multiple regression analysis was performed to estimate VAT mass from the L1-L5 vertebrae.

The resulting equation to estimate VAT mass with an $r^2$ value (0.95) and SEE (8.01%) contained only three terms; two DXA measurements and one skinfold measurement.

**Equation #7:**

$$VAT_{L1-L5} (kg) = 0.359 \text{ (TFDXA)} - 0.0577 \text{ (SUBSCASF)} - 0.0765 \text{ (BFDXA)} + 0.557$$

Where VAT is visceral adipose tissue mass determined by MRI (kg), TFDXA is trunk fat mass determined by DXA (kg), SUBSCASF is the subscapular skinfold (mm) and BFDXA is body fat mass determined by DXA (kg).

**Relationship between Circumferences and VAT Measured by MRI**

The umbilical and waist girth were also highly correlated with VAT mass ($r = 0.78$ and 0.77 respectively). The umbilical and waist girth, measured by anthropometry could explain about 60% of the variation in VAT mass from the L1-L5 vertebrae.

The WHR had the highest correlation with VAT mass for all of the anthropometric variables. When the anthropometric variables were used alone to derive an estimation equation to predict VAT mass, the only variable that entered the stepwise regression was the WHR ($r^2 = 0.69$, SEE = 17.4%). (See Equation #8 in Table 6).

**Cross Validation with Other Studies from the Literature**

On account of the small sample size in the present study the correlation coefficients between the variables measured and VAT mass from this study ($n = 11$) were compared to those found by Treuth et al. (1995), who had a very large sample size ($n = 151$). Even though Treuth et al. (1995) used a population of obese women the correlation coefficients
between the different variables and VAT were very similar to those found in this present study (see Table 7).

**Table 7**: Correlation coefficients of the variables with VAT mass from this study (n = 11) compared to those found by Treuth et al. (1995) (n = 151).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Correlation coefficients (r) from Treuth et al. (1995)</th>
<th>Correlation coefficients (r) from this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>(y)</td>
<td>0.51*</td>
<td>0.52</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>(kg)</td>
<td>0.69*</td>
<td>0.68*</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>(cm)</td>
<td>-0.01*</td>
<td>0.15</td>
</tr>
<tr>
<td>BMI</td>
<td>(kg/m²)</td>
<td>0.69*</td>
<td>0.66*</td>
</tr>
<tr>
<td>LOGSUMSF</td>
<td>(mm)</td>
<td>0.71*</td>
<td>0.68*</td>
</tr>
<tr>
<td>SAGDIA</td>
<td>(cm)</td>
<td>0.85*</td>
<td>0.69*</td>
</tr>
<tr>
<td>WAISTGR</td>
<td>(cm)</td>
<td>0.84*</td>
<td>0.77*</td>
</tr>
<tr>
<td>HIPGR</td>
<td>(cm)</td>
<td>0.75*</td>
<td>0.57</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td>0.61*</td>
<td>0.83*</td>
</tr>
<tr>
<td>UMBILGR</td>
<td>(cm)</td>
<td>0.71*</td>
<td>0.78*</td>
</tr>
<tr>
<td>BFDXA</td>
<td>(%)</td>
<td>0.75*</td>
<td>0.76*</td>
</tr>
<tr>
<td>TFDXA</td>
<td>(%)</td>
<td>0.77*</td>
<td>0.84*</td>
</tr>
</tbody>
</table>

TFDXA = Trunk fat determined by DXA, BFDXA = body fat determined by DXA, VAT = Visceral adipose tissue (L1-L5). LOGSUMSF = log of sum of three skinfolds.  

Also, because of the limited number of subjects in this study, rather than cross validate the derived prediction equations, this study opted to cross validate some of the VAT prediction equations from the literature. Table 8 shows the linear correlation between VAT measured in the eleven men by MRI in this study and that predicted by equations reported by other authors in the literature. All formulas from the literature were generated by measuring abdominal tissue areas or volumes by CT or MRI and choosing a set of anthropometric variables which resulted in the highest explained variance.
<table>
<thead>
<tr>
<th>Author</th>
<th>Equation</th>
<th>S.E.E%</th>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>Independent Variables</th>
<th>Dependent Variable</th>
<th>S.E.E%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hahn et al. (1997)</td>
<td>( \text{MR (VAT area at L2-L3)} )</td>
<td>0.86</td>
<td>0.09</td>
<td>96 ± 6</td>
<td>16</td>
<td>( M : 16 )</td>
<td></td>
</tr>
<tr>
<td>Teuch et al. (1995)</td>
<td>( \text{CT (VAT area at L4-L5)} )</td>
<td>35.5</td>
<td>0.14</td>
<td>54 ± 2</td>
<td>15</td>
<td>( P : 15 )</td>
<td></td>
</tr>
<tr>
<td>Svensen et al. (1993)</td>
<td>( \text{MR (Average area of VAT L1-L4)} )</td>
<td>17.9</td>
<td>8.8</td>
<td>41 ± 15</td>
<td>27</td>
<td>( M : 27 )</td>
<td></td>
</tr>
<tr>
<td>Ross et al. (1992)</td>
<td>( \text{CT (VAT area at L4-L5)} )</td>
<td>2.93</td>
<td>0.15</td>
<td>31 ± 8</td>
<td>10</td>
<td>( M : 10 )</td>
<td></td>
</tr>
<tr>
<td>Depes et al. (1991)</td>
<td>( \text{CT (VAT area at L4-L5)} )</td>
<td>36.4</td>
<td>0.06</td>
<td>39 ± 6</td>
<td>10</td>
<td>( M : 10 )</td>
<td></td>
</tr>
<tr>
<td>Fortland et al. (1989)</td>
<td>( \text{VAT volume at L3-L5)} )</td>
<td>36.4</td>
<td>0.06</td>
<td>39 ± 6</td>
<td>10</td>
<td>( M : 10 )</td>
<td></td>
</tr>
<tr>
<td>Kriisel et al. (1989)</td>
<td>( \text{CT (VAT area at L4-L5)} )</td>
<td>36.4</td>
<td>0.06</td>
<td>39 ± 6</td>
<td>10</td>
<td>( M : 10 )</td>
<td></td>
</tr>
<tr>
<td>Seidel et al. (1987)</td>
<td>( \text{CT (VAT area at L4-L5)} )</td>
<td>36.4</td>
<td>0.06</td>
<td>39 ± 6</td>
<td>10</td>
<td>( M : 10 )</td>
<td></td>
</tr>
</tbody>
</table>

**Note:**
- VAT: Visceral Adipose Tissue
- CT: Computed Tomography
- MRI: Magnetic Resonance Imaging

**Table 8:** Linear correlation between VAT measured in the 11 men by MRI in this study and that predicted by equations from the literature.
For example cross-validation with Svendsen et al. (1993). The mean VAT area for the present study obtained for L1-L4 was \( 79.1 \pm 28.6 \text{ cm}^2 \), whereas the predicted mean area using the equation of Svendsen et al. (1993) was \( 80.9 \pm 66.2 \text{ cm}^2 \). The linear correlation \( (r^2) \) value obtained when the predicted estimate of mean VAT area from L1-L4 was regressed against the MRI-measured values in the present study was moderate \( (r^2 = 0.68, \text{ SEE} = 17.9\%) \).

Only the Han et al. (1997) equation was generated for a population and region of interest (L1-L5 vertebrae) similar to this study. This may also explain why the predicted amount of VAT mass by using the equation derived by Han et al. (1997) had the highest correlation \( (r^2 = 0.90) \) with the measured amount of VAT mass from this study.

**Correlation Analysis of BF Measured by DXA**

The simple Pearson correlation coefficients \( (r) \) between BFDXA and TFDXA and other measures of anthropometry and MRI are shown in Table 9.
Table 9: Correlation coefficients of variables with body fat (BF) mass measured by DXA (n = 11)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>BFDXA</th>
<th>TFDXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>(y)</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>(cm)</td>
<td>0.30</td>
<td>0.17</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>(kg)</td>
<td>0.88₂</td>
<td>0.84₂</td>
</tr>
<tr>
<td>SKINFOLDS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRICEPSF</td>
<td>(mm)</td>
<td>0.88₂</td>
<td>0.88₂</td>
</tr>
<tr>
<td>BICEPSF</td>
<td>(mm)</td>
<td>0.87₂</td>
<td>0.74₁</td>
</tr>
<tr>
<td>CHESTSF</td>
<td>(mm)</td>
<td>0.92₂</td>
<td>0.88₂</td>
</tr>
<tr>
<td>SUBSCASF</td>
<td>(mm)</td>
<td>0.82</td>
<td>0.85₁</td>
</tr>
<tr>
<td>SUPRASF</td>
<td>(mm)</td>
<td>0.96₂</td>
<td>0.96₁</td>
</tr>
<tr>
<td>ABDOMSF</td>
<td>(mm)</td>
<td>0.89₂</td>
<td>0.85₁</td>
</tr>
<tr>
<td>THIGHSF</td>
<td>(mm)</td>
<td>0.87₂</td>
<td>0.80₁</td>
</tr>
<tr>
<td>CALFSF</td>
<td>(mm)</td>
<td>0.89₂</td>
<td>0.81₁</td>
</tr>
<tr>
<td>GIRTHS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARMGR</td>
<td>(cm)</td>
<td>0.78₁</td>
<td>0.69₁</td>
</tr>
<tr>
<td>FARMGR</td>
<td>(cm)</td>
<td>0.64₁</td>
<td>0.54</td>
</tr>
<tr>
<td>WAISTGR</td>
<td>(cm)</td>
<td>0.94₁</td>
<td>0.93₁</td>
</tr>
<tr>
<td>UMBILGR</td>
<td>(cm)</td>
<td>0.92₁</td>
<td>0.90₁</td>
</tr>
<tr>
<td>HIPGR</td>
<td>(cm)</td>
<td>0.83₁</td>
<td>0.79₁</td>
</tr>
<tr>
<td>THIGHGR</td>
<td>(cm)</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>CALFGR</td>
<td>(cm)</td>
<td>0.61₁</td>
<td>0.52</td>
</tr>
<tr>
<td>SAGDIA</td>
<td>(cm)</td>
<td>0.85₁</td>
<td>0.87₁</td>
</tr>
<tr>
<td>BMI</td>
<td>(kg/m²)</td>
<td>0.60₁</td>
<td>0.68₁</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td>0.85₁</td>
<td>0.88₁</td>
</tr>
<tr>
<td>SOTSF</td>
<td>(mm)</td>
<td>0.95₁</td>
<td>0.94₁</td>
</tr>
<tr>
<td>SOFSF</td>
<td>(mm)</td>
<td>0.94₁</td>
<td>0.85₁</td>
</tr>
<tr>
<td>SOSSF</td>
<td>(mm)</td>
<td>0.98₁</td>
<td>0.93₁</td>
</tr>
<tr>
<td>LOGSUMSF</td>
<td></td>
<td>0.94₁</td>
<td>0.95₁</td>
</tr>
<tr>
<td>DEXA DATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFDXA</td>
<td>(kg)</td>
<td>0.97₁</td>
<td>1.00₁</td>
</tr>
<tr>
<td>BFDXA</td>
<td>(kg)</td>
<td>1.00₁</td>
<td>0.97₁</td>
</tr>
<tr>
<td>MRI DATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAAT</td>
<td>(kg)</td>
<td>0.93₁</td>
<td>0.96₁</td>
</tr>
<tr>
<td>SAAT</td>
<td>(kg)</td>
<td>0.92₁</td>
<td>0.91₁</td>
</tr>
<tr>
<td>VAT</td>
<td>(kg)</td>
<td>0.76₁</td>
<td>0.84₁</td>
</tr>
</tbody>
</table>

TFDXA = Trunk fat determined by DXA, BFDXA = body fat determined by DXA, TAAT = Total abdominal adipose tissue, SAAT = Subcutaneous abdominal adipose tissue, VAT = Visceral adipose tissue, SOTS = sum of three skinfolds (subscapular, suprailiac and abdominal); SOFS = sum of four skinfolds (triceps, biceps, thigh and calf), SOSSF = sum of seven skinfolds (SOTSF + SOSSF)

₁ = p < 0.05
₂ = p < 0.001
The sum of seven skinfolds (SOSSF) had the highest correlation coefficient with BFDXA \((r = 0.98; p < 0.001)\) and the second highest was the TFDXA \((r = 0.97)\). The suprailiac skinfold was also very highly correlated with BFDXA \((r = 0.96; p < 0.001)\).

Therefore, the sum of seven skinfolds could explain about 96% of the variation in the body fat and the suprailiac skinfold could explain about 92% of the variation in the body fat measured by DXA in this study.

Development of Prediction Equations to Estimate BF by Anthropometry

The stepwise linear regression for estimation of BFDXA with all of the measured anthropometric variables used in shown in Table 10. The first variable to enter the stepwise linear regression was the sum of seven skinfolds, followed by the abdominal skinfold. The partial correlation \(r^2\) of the abdominal skinfold was 0.03 and therefore it was decided to stop the regression analysis at two variables. The final equation to estimate BFDXA mass (kg) with the highest \(r^2\) value (0.98) and lowest SEE (5.22%) contained only two terms:

Equation #10:

\[
\text{BODY FAT (kg)} = 0.212 \times \text{(SOSSF)} - 0.369 \times \text{(ABDOMSF)} + 2.50
\]

Where SOSSF is sum of seven skinfolds (mm) and ABDOMSF is the abdominal skinfold (mm).

Because the measurement for the sum of seven skinfolds may be time consuming to perform and may not always be practical it was decided to derive another prediction equation without the sum of seven skinfolds as one of the independent variables offered to the regression.
Table 10: Multiple regression equations for estimation of BF mass (kg) determined by DXA using anthropometric variables only (n = 11)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient estimate (SEE)</th>
<th>Partial $r^2$</th>
<th>Equation $r^2$</th>
<th>SEE %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equation #9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>3.22 (1.34)</td>
<td></td>
<td>0.95</td>
<td>7.59</td>
</tr>
<tr>
<td>Sum of seven skinfolds (mm)</td>
<td>0.133 (0.010)</td>
<td>0.95</td>
<td>0.95</td>
<td>7.59</td>
</tr>
<tr>
<td><strong>Equation #10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.50 (0.947)</td>
<td></td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Sum of seven skinfolds (mm)</td>
<td>0.212 (0.024)</td>
<td>0.95</td>
<td>0.98</td>
<td>5.22</td>
</tr>
<tr>
<td>Abdominal skinfold (mm)</td>
<td>-0.369 (0.111)</td>
<td>0.03</td>
<td>0.98</td>
<td>5.22</td>
</tr>
<tr>
<td><strong>Equation #11</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>4.151 (1.630)</td>
<td></td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Suprailiac skinfold (mm)</td>
<td>0.544 (0.052)</td>
<td>0.92</td>
<td>0.92</td>
<td>9.58</td>
</tr>
<tr>
<td><strong>Equation #12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-15.521 (7.055)</td>
<td></td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Suprailiac skinfold (mm)</td>
<td>0.341 (0.082)</td>
<td>0.92</td>
<td>0.96</td>
<td>7.18</td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>0.274 (0.097)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The resulting equation had only one skinfold and one girth ($r^2 = 0.96$; SEE = 7.18%).

**Equation #12:**

\[ \text{BODY FAT (kg)} = 0.274 (\text{WAISTGR}) + 0.341 (\text{SUPRASF}) - 15.5 \]

Where SUPRASF is the suprailiac skinfold (mm) and WAISTGR is the waist girth (cm).
Cross Validation with Other Equations to Estimate BF

Because of the limited number of subjects in this study, rather than cross validate the derived prediction equations, this study opted to cross validate with some of the BF prediction equations from the literature. In Table 11 comparisons were made between measured BF and predicted amount of BF in this study population, by using prediction equations reported in the literature. This study found that body fat measured by DXA had a correlation of $r^2 = 0.82$ with body fat predicted by the Durnin & Womersely (1974) and $r^2 = 0.77$ with the Yuhaz equation (Carter, 1982).

**Table 11:** Linear regression between body fat % measured in the 11 men by MRI in this study and that predicted by equations adapted from the literature.

<table>
<thead>
<tr>
<th>Author</th>
<th>Dependent Variable</th>
<th>$r^2$</th>
<th>SEE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sloan &amp; Weir (1967)</td>
<td>% fat</td>
<td>0.74</td>
<td>14.8</td>
</tr>
<tr>
<td>Durnin &amp; Womersely (1974)</td>
<td>% fat</td>
<td>0.82</td>
<td>12.4</td>
</tr>
<tr>
<td>Jackson &amp; Pollock (1978)</td>
<td>% fat</td>
<td>0.64</td>
<td>17.5</td>
</tr>
<tr>
<td>Yuhaz (1982)</td>
<td>% fat</td>
<td>0.77</td>
<td>13.9</td>
</tr>
</tbody>
</table>

An interesting finding was the similarity between Equation #9 in this study (using the sum of seven skinfolds) and the Yuhaz equation, which uses a sum of six skinfolds.

**Yuhaz equation (Carter 1982):**

\[
\text{% fat} = 0.1051 \times \text{[sum of six skinfolds (triceps, subscapular, suprailiac, abdominal, front thigh and medial calf skinfolds)]} + 2.585
\]

**Development of Prediction Equations to Estimate TF by Anthropometry**

The stepwise linear regression for estimation of TFDXA mass (kg) with all of the measured anthropometric variables used in shown in Table 12.
Table 12: Multiple regression equations for estimation of trunk fat (TF) mass (kg) determined by DXA using anthropometric variables only (n = 11 men)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient estimate (SEE)</th>
<th>Partial ( r^2 )</th>
<th>Equation ( r^2 )</th>
<th>SEE %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equation #13</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.77 (0.99)</td>
<td>0.91</td>
<td>0.91</td>
<td>10.78</td>
</tr>
<tr>
<td>Suprailiac skinfold (mm)</td>
<td>0.310 (0.032)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Equation #14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-9.01 (4.67)</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suprailiac skinfold (mm)</td>
<td>0.199 (0.054)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td>0.150 (0.064)</td>
<td>0.04</td>
<td>0.95</td>
<td>8.80</td>
</tr>
</tbody>
</table>

The first variable to enter the equation was the suprailiac skinfold (\( r^2 = 0.91 \)), followed by the waist girth. The resulting equation to estimate trunk fat mass (kg) had an \( r^2 \) value of 0.95 and SEE of 8.80%.

**Equation #14:**

\[
\text{TRUNK FAT (kg)} = 0.199 \text{(SUPRASF)} + 0.150 \text{(WAISTGR)} - 9.01
\]

Where SUPRASF is the suprailiac skinfold (mm) and WAISTGR is the waist girth (cm).

It is interesting to note that the best prediction equation to estimate TFDXA (Equation #14) had the same two variables that best predicted the BFDXA (Equation #12). Thus the suprailiac skinfold and waist girth are strong predictors and good variables to use when estimating TF or BF measured by DXA.
CHAPTER 5: DISCUSSION

The aim of this study was to derive a prediction equation for estimating VAT mass by using DXA combined with anthropometric measurements (using AT measured by MRI as the criterion). Eleven men over 50 years of age with a large range of adiposity were utilized for the data analysis for this study in order to improve the estimation of VAT mass through more readily available and accurate means. Middle aged men are at high risk for cardiovascular disease and the ability to estimate VAT mass would be very beneficial in order to investigate the association of VAT with metabolic complications and the risk factors for CVD.

Study Design & Significance of Study

This study is the first attempt to use DXA combined with anthropometry to predict VAT mass measured by a series of adjacent MRI scans from the L1 to L5 lumbar vertebrae region in middle aged men. This study was a descriptive study with no control group. This study used eleven men over the age of 50 y to examine the relationship of anthropometry and DXA to abdominal adipose tissue distribution measured by MRI.

Subject Characteristics

The strength of this study is that subjects with wide a range of adiposity were used. The BMI’s ranged from 20.1 to 32.6 kg/m$^2$ and the sum of seven skinfolds ranged from 59.6-229 mm (Table 1) for the eleven subjects that underwent the data analysis in this study. Other researchers have used a low number of subjects also. Han et al. (1997) looked at 16 NIDDM men (ages 44-66 y) and Kvist et al., (1988) looked at 17 men (ages 24-56 y).
Fat Measured by DXA

Another strength from this study is the use of DXA which is an indirect method to measure BF and TF. Conventional indirect techniques, including underwater weighting, body water dilution, impedance, and anthropometry, measure body fat content by empirically determined relationships on the basis of population averaging. A critical difference between DXA and the other techniques is that DXA measurement is amenable to absolute calibration. This leaves tissue distribution and tissue content as possible sources of measurement errors in different individuals. Thus DXA measurements are less likely to be affected by individual variability and may therefore achieve higher statistical power for a given sample size. But it is also important to note that DXA software does make assumptions which include that the head assumes 17.0% brain fat and that the lean body mass is 73.2% water. Other possible sources of error may be the type of DXA machine and software used and different observer's interpretation of the cutoff landmarks for the trunk.

This study demonstrates that measurement by DXA gives an accurate measurement of regional fat content and can be used to improve the estimation of abdominal adipose tissue compartments. The TFDXA was found to be about 54% of the BFDXA for the subjects in this study. It is important to note that the TFDXA in this study included both the thoracic and abdominal areas.

AT Measured by MRI

The adipose areas measured in this study were similar to those found in a few other studies. Svendsen et al. (1993) who used 25 postmenopausal women aged 52 ± 2 y found the
mean area of VAT from L1 to L4 to be 118 cm$^2$. In this study the mean area of VAT from levels L1 to L4 were found to be 108 cm$^2$. Men have been found to have a greater accumulation of VAT than the amount of VAT found in premenopausal women (Lemieux et al. 1993). However, at menopause, in the absence of hormonal replacement therapy, an acceleration of abdominal fat accumulation in noted (Haarbo et al., 1991). Therefore more research is warranted to look at the amount of VAT found in postmenopausal women compared to middle aged men.

The adipose areas measured in this study were somewhat lower than measured by other studies. Ross et al. (1992) found at the level of the umbilicus (L4-L5) that the mean VAT area was 118 cm$^2$ (i.e. measured in men with mean age of 40.8 y and BMI of 28.5 kg/m$^2$). Van der Kooy et al. (1993) found at the level of the umbilicus (L4-L5) that the mean VAT area measured in their studies was 156 cm$^2$ with 40 men with mean age of 40 y and mean BMI of 30.7 kg/m$^2$. Han et al. (1997) found that at the level of the umbilicus (L4-L5) that the mean VAT area measured in their study was 175.5 cm$^2$. The 16 men in the study by Han et al. (1997) had a mean age of 56.2 y and a slightly greater mean BMI (27.9 kg/m$^2$) than the subjects in this study. In this study the mean VAT area at the level of the umbilicus (L4-L5) was measured to be 79.1 cm$^2$ (Table 4) with subjects with a mean BMI of 26.9 kg/m$^2$.

The variance between studies may be partially explained by differences in adiposity and age of subjects. There may have also been different threshold levels or methods to distinguish adipose tissue from other tissues.

For this present study a single threshold was set at 170 on the histogram for all subjects to distinguish adipose tissue from other tissues. The most appropriate single threshold value for lipid is scanner and sequence dependent (Han et. al., 1997). Thus, the
threshold used in this study may not apply precisely to other studies. Measurement errors of adipose tissue from individual variation could be minimized if internal markers are used. For example a highlighted area at the border between SAAT and the muscles of the abdomen can be used to establish the adipose threshold on the histogram. The resulting histogram with only SAAT and muscles of the abdomen should result in two separate and distinguishable peaks on the histogram. One peak for lean tissue (muscle) and one peak for the adipose tissue (see Appendix C).

**Calculation of AT Volume and Mass**

This study estimated the VAT, SAAT volume from a series of cross-sectional MRI scans of the abdominal region from the L1 vertebra (about the top of the kidneys) to the inferior plate of the L5 vertebra. The mean VAT volume estimated in this study (1.95 ± 0.86) was very close to the VAT volume measured by Jensen et al. (1995) (1.96 ± 2.13) who measured VAT mass from the dome of the diaphragm to the top of the femur. The population used by Jensen et al. (1995) was six men and fifteen women aged 38 ± 12y (range 19-60y) with BMI values of 26.6 ± 5.1 kg/m². As mentioned previously, subjects of different ages and sex have been found to have different adipose tissue distribution.

The results from the MRI measurements in this study found that the VAT and SAAT had similar mean volumes (1.96 ± 0.86 and 1.92 ± 0.80 litres respectively) for the abdominal region from the L1 to L5 lumbar region. Han et al. (1997) found that in the L1-L5 region that the SAAT had a smaller mass than the VAT which were 1.56 ± 0.09 and 2.34 ± 0.47 kg respectively.

Other researchers have reported that the SAAT is usually 2-3 times the area of the
VAT (Ross et al. 1994; Treuth et al., 1995; Goran et al., 1998). But these studies also only used a single CT scan at the level of the umbilicus or L4-L5 and not the whole L1-L5 region as this study does.

Researchers such as Ross et al. (1994), who reported larger SAAT than VAT areas, had subjects with greater BMIs (32 ± 3.6) than this study. Other studies with greater SAAT than VAT volumes also usually included the region from the L1 or diaphragm to the trochanter (top of femur) or symphysis pubis and thus included a lot of SAAT from the buttocks (Jensen et al., 1995; Abate et al., 1997; Thomas et al., 1998). Perhaps the ratio of VAT to SAAT changes at different levels of the abdomen and when the VAT and SAAT for each scan are summed for the L1 to L5 region the total volumes of VAT and SAAT are very similar.

This present study found the mean VAT mass from L1 to L5 to be 1.82 kg for subjects with a mean BMI of 26.9 kg/m$^2$. Abate et al. (1994) using dissection of two male cadavers aged 27 and 31 y with BMI's slightly higher (BMI 27-31 kg/m$^2$) than the mean for the subjects in the present study found the VAT mass from the L1 to L4 lumbar vertebrae to be 2.61 and 3.61 kg respectively. Han et al. (1997) measuring the same L1 to L5 region with MRI stated that the mass of VAT was 2.34 kg.

An interesting observation from this investigation was the narrow range of VAT mass despite the wide range of BMIs. The VAT mass in the most obese subject was only 1.87 kg more than that of the leanest subject (Table 3). This raises the question of whether such small differences in absolute VAT mass could account for the major differences in metabolic alterations. The answer to this question, of course awaits more extensive investigation.

It is also important to note that when comparing other data with the data from this
study that there are sex differences in the deposition of adipose tissue. In the study by Thomas et al. (1998) similar volumes of VAT were found in the 11 women aged 32.58 ± 2.74 y that had similar BMIs to the middle aged men in this study. According to Ross et al. (1994) women have less VAT and more SAAT in the abdominal region. Ross et al. (1994) also stated that for women the distribution of VAT is skewed towards the lower abdomen (largest mean value for VAT was 5-10 cm above L4-L5) and that for the men VAT was normally distributed (largest mean value for VAT was 10-15 cm above L4-L5). The women on the other hand had a consistent amount of VAT from the L1 to L5 level Thomas et al. (1998). Han et al. (1997) found that the VAT areas were similar for the women at all levels between L1-L5, and as with this study, that the VAT area for the men was greatest at the L1 level and decreased towards the L5 level.

**Adipose Distribution and Single Scan Analysis**

For this study the landmarks for the area of interest were from the superior plate of the L1 lumbar vertebra to the inferior plate of the L5 lumbar vertebra. This area corresponds approximately to the levels of the top of kidneys to the anterior superior iliac crest.

The distribution of adipose tissue measured by MRI for this region were very similar to the findings of Ross et al. (1992). The findings from this study confirm Ross et al. (1994) who stated that “the largest mean values for VAT were obtained on the images 10 and 15 cm above the L4-L5 level.” The mean VAT area was found by Ross et al. (1992) and this study to decrease as one moves from the level of the L1 vertebra to the L5 vertebra (see Figure 1). Above L2-L3 level the mean VAT area is greater than the mean SAAT area, and inferior to L2-L3 level the SAAT mean area is greater than the mean area for VAT (see Figure 1).
Similar results were found by Borkan et al. (1982). Borkan et al. (1982) found that VAT has its largest area in the upper abdomen, with a gradual decrease in the lower abdomen levels. Borkan et al. (1982) also found that the SAAT area shows considerable variation between subjects but tends to increase in the lower abdomen. This study supports these findings in that the SAAT area also tends to increase in the lower abdomen.

Correlation analysis was performed to evaluate the extent to which a single scan was related to the total volume of adipose from the L1 to L5 vertebrae. Researchers in the past (Kvist et al., 1988; Ross et al., 1992) have stated that a single scan at the L4-L5 region (approximately the umbilicus level) were highly correlated with VAT volume ($r > 0.95$).

Kvist et al. (1988) and Shoen et al. (1998) have found that AT area measurements obtained at the L4-L5 level were highly predictive of the corresponding VAT volume with both male and female subjects. Borkan et al. (1982) reported that the AT area measurements from multiple slices taken in the umbilicus region were highly correlated with one another. These studies suggest that, in cross-sectional studies, TAAT and VAT determined from a single scan or near at the level of the umbilicus are highly predictive of visceral volume measurements acquired by multiple scans. What has not been demonstrated, however, is whether diet or exercise intervention would result in selective effects on subcutaneous or visceral AT depots in the abdomen region that would be identified only through multiple scan acquisitions. MRI is particularly suited to intervention studies, because numerous slices (20-30) of the abdomen could be acquired sequentially without hazard to the subject.

Thomas et al. (1998) found that there is an increase in measurement uncertainty as slices are removed from a contiguous data set. This uncertainty highlights the possible inaccuracies in using single slice data sets. Thomas et al. (1998) also stated that one scan is
not a good predictor of AT volume due to large variances between individuals.

More recent authors (Han et. al., 1997; Abate et al., 1997) have found that a single axial MRI scan at the L2-L3 intervertebral region has a good correlation ($r = 0.96$ and 0.93 respectively) to estimate VAT volume. Han et al. (1997) and this present study found that the frequently used L4-L5 vertebrae have a lower correlation with VAT mass. For the present study an MRI scan at the L2-L3 intervertebral disk region was found to have the best and most consistent predictive value for the VAT volume ($r = 0.95$, $p < 0.001$).

This study concludes that measuring the VAT area at the L2-L3 level by MRI is an acceptably reliable and accurate method for estimating the total VAT volume in the L1-L5 region in middle aged men.

**Relationship between DXA and AT Measured by MRI**

Figure 2 shows a good correlation ($r^2 = 0.92$) between TAAT mass and trunk fat measured by DXA. But Figure 3 only shows a moderate correlation ($r^2 = 0.70$) between VAT mass measured by MRI and trunk fat measured by DXA. Therefore as expected TFDXA is more closely related to TAAT than to VAT due to interindividual variations in the VAT to SAAT ratio.

Further the correlation between SAAT and VAT was only moderate ($r^2 = 0.68$). Table 5 shows an even lower correlation ($r^2 = 0.63$) was found between total body fat measured by DXA and VAT. Thus measurements of BF are only a moderate predictor of VAT mass. Even lower was the correlation between BMI and VAT ($r^2 = 0.66$). These results confirm the results found by Thomas et al. (1998) that there is significant variation in the percentage of VAT, SAAT and TAAT, across the BMI range, which cannot be easily
predicted from total body fat and/or subcutaneous fat.

In view of the small sample size the high correlation coefficient (r = 0.84) between the MRI measured VAT mass and the trunk fat measured by DXA and anthropometric variables was encouraging and also very close to the correlation coefficient (r = 0.80) found by Svendsen et al. (1993) between mean VAT area and TFDXA. VAT mass was best predicted by trunk fat determined by DXA (Table 6). The trunk fat mass measured by DXA could, in particular, explain 70% of the variation in VAT mass.

The use of MRI scanning as a criterion measure "gold standard" showed an excellent correlation between DXA measured trunk fat and TAAT mass measured by MRI (Figure 2). Some variation in the MRI measurement may be due to increased signal-to-noise ratio (motion artifacts), in particular bowel and respiratory movements. Figures 2 and 3 show that there is a loss in accuracy of estimating VAT from DXA measurements at increased levels of VAT mass. These findings are very similar to those found by Goran et al. (1998) who also found "the estimate error is inflated by the lack of accuracy of the prediction equations at extremely high levels of VAT and SAAT". This bias might be due to the inability to accurately measure skinfolds and circumferences in very obese subjects or that the lipid fraction may change at increased levels of obesity. Another explanation for the SEE of 17.3% could be that DXA only measures the soft tissue composition directly in non-osseous pixels and assumes a similar composition in pixels containing bone. Many of the assumptions with DXA are software specific and may vary with type of DXA machine and software used for the analysis.

Thus further cross-validation studies are needed, particularly in obese subjects and in other ethnic groups. Also, other anthropometric measures not included in this study should
be explored for their potential in improving the accuracy of predicting VAT mass (for example, ultrasound measures of tissue thickness).

**Relationship between Anthropometry and AT Measured by MRI**

The simple Pearson correlation coefficients (r) between abdominal adipose tissues measured by MRI and other measures of MRI, and selected anthropometric variables are shown in Table 5. The suprailiac skinfold was the skinfold with the highest correlation with VAT (r = 0.73). Height had the lowest correlation with VAT mass (r = 0.15).

**Relationship between Circumferences and AT Measured by MRI**

Pouliot et al. (1994) found that the waist girth was the best anthropometric correlate of VAT. By using waist girth and age Pouliot et al. (1994) was able to predict up to 75% of the variance in VAT accumulation. The umbilical and waist girth were also found to be highly correlated with VAT mass (r = 0.78 and 0.77 respectively) in the present study. Ross et al. (1992) also found the waist girth to have a good correlation with VAT area at L4-L5 intervertebral disc region (r = 0.82). The umbilical and waist girth, measured by anthropometry could explain about 60% of the variation in VAT mass. The umbilical girth had a slightly better correlation with VAT mass than the waist girth. This suggests that waist and umbilical girths are moderately good predictors of VAT. But as mentioned previously waist and umbilical girths only give an estimate of overall adiposity and not adipose distribution. This is confirmed in this study in that the waist and umbilical girths have a higher correlation with the TAAT mass than the VAT mass (r = 0.86 and r = 0.77 respectively).
Relationship between WHR and AT measured by MRI

WHR is also often used in clinical settings as a quick indicator of cardiovascular risk (Larson et al., 1984; Haarbo et al., 1989). In this study the WHR was found to have a better correlation with VAT mass (r = 0.83) than waist girth (r = 0.77). WHR has been found to be correlated significantly with the ratio of visceral adipose tissue area to SAAT area (Svendsen et al., 1993) and is an independent predictor of cardiovascular disease (Lapidus et al., 1984).

One factor that may partially explain this study's finding of a correlation between VAT mass and WHR was the level of waist circumference chosen to calculate WHR. The levels of circumference measurements reported in the literature vary considerably, especially for waist level (Seidell et al., 1988). Small differences in this level may yield different results in the calculation of ratios of waist-to-hip or waist-to-thigh and this has important implications for the classification of an individual's fat distribution when using these ratios (Seidell et al., 1988; Houmard et al., 1991; Seidell et al., 1992). The levels for measuring waist and hip circumferences recommended by the World Health Organization are based on skeletal reference points (Seidell et al., 1988; WHO document, 1988). The WHO defines the waist as midway between lower rib margin and the suprailiac and the hips as the widest point over the greater trochanter). Ross et al. (1994), and this study, define the waist as the narrowest point (i.e. minimum girth) between the ribs and suprailiac.

WHR values obtained using a waist circumference at the umbilicus may decrease the relationship between WHR and VAT mass. For example, Peiris et al. (1987) observed that a WHR calculated using a waist circumference obtained at the level of minimum girth remained significantly correlated to VAT after adjusting for age and body mass index. Thus
future studies that would assess the ability of WHR to predict VAT might benefit from calculating WHR values by use of more than one waist circumference; however, waist circumference should be obtained using accepted standards (i.e., level of minimum girth and umbilicus). Furthermore, it would be ideal if these studies would simultaneously assess metabolic parameters to evaluate whether the WHR that is best related to VAT mass is also associated with clinical abnormalities or metabolic risk factors.

**Development of Prediction Equations to Estimate VAT Mass**

The first regression equation to predict VAT mass used: trunk fat measured by DXA, the subscapular skinfold and the sum of seven skinfolds (Equation #6). The $r^2$ value for this equation was 0.95 with a SEE of 8.00%. The subscapular skinfold was also used in an equation by Ross et al. (1994), who used WHR, subscapular skinfold and the triceps skinfold to estimate VAT area.

After the trunk fat measured by DXA and subscapular skinfold entered the regression equation the sum of seven skinfolds best predicts the variability in VAT mass. Even though the variability that the sum of seven skinfolds explains (partial $r^2 = 0.05$) the sum of seven skinfolds variable was significant. This may be due to a high degree of multicollinearity between other variables or measurements related to the trunk region to the trunk fat measured by DXA and the subscapular skinfold. For the same reason the partial correlation of variables that entered the equations to predict VAT mass after the TFDXA was entered were very small and even smaller for the third variable that entered the equations.

It was then decided to remove the sum of seven skinfolds from the list of available independent variables because it may not always be practical to do seven skinfolds on each
subject; especially for large populations. A new forward stepwise multiple regression analysis was performed to estimate VAT mass from the L1-L5 vertebrae. The resulting equation to estimate VAT mass (Equation #7), contained one skinfold and two DXA measurements. The DXA measurement variables (in Equation #7) is very similar to the equation derived by Goran et al. (1998) except that Goran et al. (1998) used the abdominal skinfold instead of the subscapular skinfold (used in this study) with the two DXA measurements.

Another interesting point to note is the minus sign in front of the SUBSCAPSF, SOSSF and BFDXA variables in the derived equations. This may suggest that as VAT mass has an inverse relationship with these variables. Further research is required in order to look at this relationship in middle aged men.

The trunk fat mass measured by DXA, which was able to explain by far the most variation in the equation, is measured indirectly. Prediction of VAT mass by the equation with trunk fat mass measured by DXA might therefore be less population specific than equations using solely anthropometric variables which are doubly indirect measurements. However, this must be confirmed by cross-validation in other populations.

To date this is the best equation to predict VAT mass (measured with adjacent MRI scans from the L1 to L5 vertebrae region of the abdomen) in middle aged men using the combination of DXA and anthropometry. This study contradicts the findings by Jensen et al. (1995) who found that the combination of anthropometry and DXA was a suboptimal predictor of CT-measured volume of VAT. Jensen et al. (1995) suggested that the combination of a single CT slice should also be included (to assess the ratio of VAT to TAAT) with the DXA-measured abdominal fat to estimate VAT mass measured by CT.
Recently prediction equations using solely anthropometric variables have been found to have an $r^2$ value of 0.82 with a SEE of 31.6% (Goran et al., 1998). For this study using anthropometric measurements alone the only significant variable that entered the stepwise regression automatically (i.e. didn't have to be forced in) was the WHR ($r^2 = 0.69$). This study confirms the findings of other studies (Bonora et al., 1995; Owens et al., 1999) that found that simple anthropometric measurements alone were not able to accurately predict VAT mass. This may be due to the suggestion that predictive equations using solely anthropometric variables are population-specific (Svendsen et al., 1991) and in order in to reduce sample specificity it is recommended to use variables which incorporate ratios of girths and sum of skinfolds.

In this study, the abdominal sagittal diameters (measured anthropometrically) were not significant predictors of VAT mass in middle aged men ($r = 0.69$). This is in contrast to other studies by Krist et al. (1988) and Treuth et al. (1995) who found the sagittal diameter to have a good correlation with VAT ($r = 0.85$ and 0.90 respectively).

**Cross-Validation with Other Studies in the Literature**

The linear correlation between the VAT measured in the eleven men by MRI in this study and that predicted by equations reported by other authors in the literature were reported in Table 8. Most of the cross-validations were poor, which is what would be expected because of the different populations that the equations were derived from. For example, Svendsen et al. (1993) used very obese post-menopausal women in their study and the trunk fat measured by DXA was only at the L1-L4 region and not the whole trunk as used by this study. Most prediction equation are population specific and regression equations developed
by one population will generally not work on another population (Jensen et al., 1995). The equation derived by Han et al. (1997) to estimate VAT mass (L1-L5) best cross correlated with the values measured in this study which was not surprising because their subjects and region of interest was very similar to the present study.

Correlational Analysis of BF Measured by DXA

The highest correlation with BFDXA was with the sum of seven skinfolds \( r = 0.98 \). As expected the trunk fat measured by DXA had a very high correlation \( r = 0.97 \) with the BF measured by DXA (Table 10). The suprailiac skinfold could explain about 92% of the variation in the body fat.

Development of Prediction Equations to Estimate BF by Anthropometry

An interesting finding from this study was the ability to estimate body fat determined by DXA using anthropometry alone. The best prediction equation resulted from using the sum of seven skinfolds and the abdominal skinfold \( r^2 = 0.98, \text{SEE} = 5.22 \% \). The minus sign in front of the abdominal factor in the equation may suggest that as BF increases the abdominal skinfold may only increase at a slower rate. It may also mean that AT may be deposited more internally (i.e. VAT) or more at the extremities with increased body fat.

Another prediction equation derived from this study to estimate body fat with an \( r^2 \) value of 0.96 (SEE of 7.18%) used the suprailliac skinfold and waist girth. A study by Lean et al. (1996) found that triceps skinfold and waist girth could explain 86.6% (SEE 3.2%) in men compared to under water weighing determination of body fat. These findings are important because in a very short period of time anthropometry can now be used in clinical
settings to estimate body fat very quickly and accurately, thereby avoiding the need to use an expensive DXA machine, which also exposes the subjects to a small amount of ionizing radiation.

Cross Validation with Other Equations to Estimate BF

The highest correlation ($r^2 = 0.82$) with the cross-validation was with the Durnin & Womersley (1974). The results from the Yuhaz equation (Carter 1982) to estimate body fat correlated very well with the BF measured in this study by whole body fat mass measured by DXA ($r = 0.77$). In fact the Yuhaz equation and the Equation #9 derived in this study are very similar. Yuhaz equation from Carter (1982) is % fat = 0.01051 x sum of six skinfolds (triceps, subscapular, suprailiac, abdominal, front thigh, medial calf).

Development of Prediction Equations to Estimate TF by Anthropometry

Another interesting finding from this study was the ability to estimate trunk fat determined by DXA using anthropometry alone. The suprailiac skinfold had the highest correlation with TF measure by DXA ($r = 0.96$). A forward stepwise multiple regression analysis was performed to develop prediction equations to estimate trunk fat had an $r^2$ value of 0.95 (SEE of 8.80%). The resulting equation for predicting the TFDXA had the same variables in Equation #12 to estimate body fat (suprailiac skinfold and waist girth).
Limitations of This Study

Because the equations developed in this study were derived from regression analysis they may reflect unique characteristics of the study population. All of the subjects were 50 years of age and older and the prediction equations developed by this study may only be accurate with middle aged men of similar ages and BMI. But the variables used in the equations are dimensionally consistent and may help improve its external validity.

Subjects

Another limitation of this study was that the subjects were not a random sample. The subjects were selected to be over 50 years of age and have a wide range of BMI’s. It is not known how representative this sample is of the whole population of men between the ages of 50-69 years of age.

DXA machines and software

One of the limitations of the Lunar software used is the fact that the trunk fat by DXA includes the entire thoracic and abdominal region. This may have compromised the accuracy of DXA predication equations since more specific analysis of anatomic locations within the DXA scan (for example, fat at the umbilicus or the L1 to L5 region) may improve the accuracy of prediction of adipose mass measured by MRI from the L1 to L5 vertebrae.

But trying to isolate specific sections of the abdomen with DXA might also introduce some estimation and subjectivity and thus intertester error. Different technicians may select different landmarks for the cutoff for the trunk region. This study wanted to develop equations where the areas were easy to distinguish and most commonly used. Thus it was
decided to identify the trunk region for DXA evaluation as the whole body less the head, neck, arms and legs. The cutoff area for the trunk region measured by DXA were the neck, shoulder and hip joint (see Appendix B). In studies of adults that have used DXA to predict VAT (Treuth et al., 1995), specific locations within the trunk region have been analyzed for fat content and used as predictor variables. But, Treuth et al. (1995) found that by dividing the trunk region into three specific regions did not improve the estimation of VAT.

Different software and different DXA machines used for the data analysis may not be consistent with all populations may also introduce an error in the estimation of BF and TF.

**MRI**

As for CT, another limitation of this study, is the inability of MRI to clearly differentiate between intraperitoneal (portal) adipose and extraperitoneal (retro) adipose tissue (Rossner et al., 1990; Seidell et al., 1990). This distinction is important because only the intraperitoneal AT depots can be considered as 'portal' tissues which are associated with the risk factors for cardiovascular disease (Bjorntorp et al., 1990; Depres, 1992). There is a possibility that intraperitoneal fat, which drains into the portal circulation, may have unique effects on hepatic intermediary metabolism (Abate et al., 1997). Finally, as with CT, efforts to discriminate between retroperitoneal and intraperitoneal AT must be viewed with caution since the relevant methods have not been fully validated (Rossner et al., 1990; Seidell et al., 1990; Ross et al., 1994).

The values for adipose tissue in this study were found to be slightly lower than found by other researchers with subjects of similar BMIs (Han et al., 1997). This may be due to the level on the histogram that was set for the cutoff mark (i.e. 170). With a previous pilot study
it was determined that by using a cutoff threshold of 170 (on a scale of 0 – 255) on the histogram and increasing the contrast by 75% would result in the best representation and distinction of adipose tissue from other tissues. In this study it was decided not to increase the contrast too much (i.e. changing anything gray to white) or lower the limit of the attenuation value that represented the lower limit of adipose tissue because progressively lower cut off levels would have included greater amounts of nonfat soft tissue as adipose tissue. Theoretically, if only tissues with attenuation values within two SDs below the mean of pure adipose tissue are included, < 2.5% of adipose tissue volume should be missed and the problem of falsely characterizing nonfat soft tissue as adipose tissue should be minimized (Jensen et al., 1995). This study increased the contrast of the MRI images by 75% and if the contrast was increased higher an inflated amount of adipose tissue measured may have resulted. It is possible that our choice of the lower limit of attenuation was too conservative, in an effort to avoid including substantial amounts of nonfat tissue as adipose tissue, and as a result some adipose tissue may have been excluded.

Adipose vs. fat

Thomas et al. (1998) stated that conversion of adipose volume to fat (lipid) mass requires the use of certain assumptions. These assumptions are based on the lipid content or lipid fraction in the adipose tissue. As Martin et al. (1994) stated the lipid fraction is not always constant among all individuals and increases with adiposity. Thomas et al. (1998) have shown that these assumptions on lipid content of adipose tissue can significantly affect the absolute levels of body fat estimated for a subject. Thus the VAT mass measured in this study may not be directly comparable to other studies if the assumptions of lipid content are
Type 1 and Type 2 errors

In research there is a possibility of rejecting the null hypothesis when in fact it is true (Type 1 error) or accepting it when in fact it is false (Type 2 error). The probability of making a Type 1 error is equal to the level of significance, which was 0.05 in this study. Therefore, there was a probability of 0.05 (i.e. p < 0.05) that the sample data was extreme enough to reject the null hypothesis when it was actually true. For example, with a very small sample, and a researcher taking many measurements may find by chance that the length of the subjects' nose has a very good correlation with body fat. Most likely this is not true and the results may be due to chance. Then to reduce the possibility of a Type 1 error, future research should reduce alpha (i.e. level of significance) as the number of statistical tests increases.

One way for a researcher to get an idea of expected correlations is to look at other researchers studies in the same area (with larger sample sizes) and see if similar conclusions were found. With the previous example, it is very unlikely that any other researchers have found the length of the nose as having a good correlation with body fat and that the finding was most probably by chance. Despite the small sample size of this present study it can be seen in Table 7 the correlation coefficients found in this study were very similar to those found by Treuth et al. (1995) who had a very large sample size of 151 women.
**Future research**

Future studies of a similar nature should look at a region larger than L1 to L5, perhaps a measure from the diaphragm to the top of the trochanter (or symphysis pubis bone). But as mentioned earlier, in order to reduce artifacts caused by respiratory movements, a signal averaging technique as mentioned by Ross et al. (1992) would be required and take a longer acquisition time for the MRI analysis superior to the L1 vertebra. Having the subjects hold their breath during the acquisition of images near the diaphragm may also reduce the movement artifacts and would also be recommended for future analysis.

Although the results from this study are very optimistic these observations must be viewed with caution however because they are based on a small number of subjects. Even though some authors have suggested that using MRI and DXA increases the power of the study due to the nature of the indirect measurements, in the future a larger population with more obese subjects (BMI > 30) would also be desired. Given that MRI is not subject to the restrictions associated with CT, larger numbers of subjects could be assessed and more robust prediction equations developed.

Another suggestion would be to measure sagittal diameter of the subjects while they are in the supine position or measure the values from the MRI images.

Schreiner et al. (1996) suggested using quadratic terms to estimate VAT. These authors suggested that VAT is quadratically related with BMI, waist circumference, weight, and subscapular skinfold and linearly related to WHR. Schreiner et al. (1996) also suggested that subjects with a low body weight will have a higher proportion of VAT/body weight and as body weight increases that the VAT/bodyweight ratio will level off. These authors also stated that SAAT may be linearly related to anthropometric variables.
One of the potential benefits of assessing abdominal adiposity by MRI or CT is the development of mathematical equations from external anthropometry that can predict MRI-measured VAT. The variance in the cross validation analysis between the measured and predicted values may be partially explained by the fact that the independent variables may have been obtained at slightly different anatomic locations.
CHAPTER 6: CONCLUSION

This study is the first to demonstrate that prediction equations using DXA combined with anthropometry can accurately estimate VAT mass that was measured by a series of adjacent MRI scans from the L1 to L5 lumbar vertebrae region in middle aged men. The results of this study also show that the TF measurement from DXA is a good predictor of VAT mass and can increase the accuracy of equations developed to estimate VAT using anthropometry alone. These findings are important because DXA and anthropometry can provide a valid estimate of VAT mass in middle aged men, which could be used in studies with large numbers of subjects, to save time and costs, and reduce the risk of exposure to radiation (in the case of CT). Despite the limitations of this study the proposed equation appears to provide the best estimate of VAT mass to date in middle aged men.

This study concludes that:

1) Measurement of trunk fat by DXA can accurately predict VAT mass and that the predictions can be improved by including anthropometric variables into the estimation equation;
2) A single MRI scan at the L2-L3 intervertebral disk region was found to be the best predictor of VAT mass;
3) TAAT can be accurately estimated by trunk fat measured by DXA;
4) Body fat and trunk fat measured by DXA can be accurately estimated using anthropometry alone (especially the suprailiac skinfold and waist girth).
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APPENDIX A:

SUBJECT CONSENT FORM
**Background:**
There is growing evidence that the distribution of body fat influences metabolic abnormalities and cardiovascular risk factors such as insulin resistance, hyperlipidemia, hypertension, and hyperinsulinemia. Specifically, the accumulation of body fat in the abdominal region has been independently associated with diabetes, stroke, coronary heart disease, and related mortality; especially in elderly men. The distribution of fat to the buttocks area does not appear to increase the risk of metabolic abnormalities. However, all investigators are not in uniform agreement that abdominal obesity has a more detrimental effect on these metabolic abnormalities than the same amount of excess fat in other body locations. Other researchers have suggested that these metabolic abnormalities may be particularly associated with the accumulation of internal rather than subcutaneous (just under the skin) abdominal tissue. Much of this uncertainty derives from the lack of methodology for accurate determination of adipose tissue mass in the different regions of the body, especially abdominal fat. Quantities of subcutaneous adipose tissue can be determined with reasonable accuracy with techniques such as ultrasound or skin-fold calipers, whereas internal adipose tissue compartments are difficult to measure accurately. Advanced imaging techniques such as dual-X-ray absorptiometry (DXA) and Magnetic Resonance Imaging (MRI) offer new promise for the visualization and quantification of adipose tissue masses in different compartments MRI is particularly promising because of its lack of radiation exposure and its superior imaging of adipose tissue.

Subjects are being invited to participate in this study in order to improve the estimation of visceral (internal) adipose tissue through more readily available and accurate means. Men between 45 and 70 years of age with a large range of abdominal fat will be required for this study.

**Purpose of study:**
There is a lack of methodology for accurate determination of adipose tissue mass in different regions of the body, especially abdominal fat. Quantities of subcutaneous adipose tissue (fat tissue just under the skin) can be estimated with reasonable accuracy with techniques such as ultrasound or skin-fold calipers, whereas internal adipose tissue compartments (fat tissue around internal organs) are difficult to measure accurately.

The aim of this study will be to derive a prediction equation for estimating visceral adipose tissue (VAT) by using DXA combined with anthropometric measurements and using MRI as the criterion method. A review of the literature has found that using MRI as the criterion method to develop prediction equations has never been done before.

**Study Procedures:**

**Study Design:**
This study will be a cross sectional correlational analysis (i.e. descriptive study with no control group). A broad crosssection of abdominal fat in the subjects will be desired to give a better range for the analysis. They will asked to under go anthropometric measurements, twenty MRI scans at the level of the abdominal region and a total body DXA measurement. The total time commitment will be about two hours for each subject. All measurements in
the same men will be performed on the same day. There will be 12 healthy male subjects 45-70 years in this study.

The Clinical Research Ethics Board of the University of British Columbia will approve the methods and procedures used in this investigation. All participants will also sign an informed consent before being tested.

**MRI:**
Magnetic Resonance Imaging. MRI uses a very strong magnet, radio waves and computers to create detailed images of your body. There is no radiation involved like x-rays. Subjects are required to lie on their backs with their arms stretched above their head in a somewhat confined space for ten minutes during the MRI scans. The time for the actual scanning procedure is about 5 minutes and 40 seconds per person.

**DXA:**
Dual Energy X-ray Absorptiometry is a machine similar to an X-ray machine. DXA was originally designed to measure bone mineral content, and are still most widely used for this application. But with recent advances in software and equipment, DXA can now be used to estimate the body composition of soft tissue with a very low radiation dose (i.e. about 1/30 of a chest x-ray). A total body DXA scan will be performed on each subject in order to estimate body composition. The DXA will provide a three-compartment equation of body composition: bone mineral, fat, and bone-free lean mass in different regions (arm, leg and trunk) and the total body. The total time for each subject to lie on their backs and remain still while the DXA machine scans up and down the body will be about 10 minutes.

**Anthropometry:**
Anthropometry is the quantitative measurement of the body and its parts. Height, weight, skinfolds (i.e. pinch tests with skinfold calipers), and circumferences are common anthropometric measurements. Height and weight will be measured with the subjects wearing light indoor clothes and no shoes. Total body fat will be estimated from measurements of skin-fold thickness at various sites of the body using calipers. The procedure is to grasp a fold of skin and subcutaneous fat firmly with the thumb and forefinger, pulling it away from the underlying muscle, after which the skinfold caliper is applied. Waist, hip, umbilicus circumference (circumference at level of the belly-button) will be measured with a metal tape measure. Skinfolds and circumferences will be taken at various locations of the body for each subject. Total time for anthropometric body measurements is about 5 minutes.

**Exclusions:**
Males who are apparently healthy and between 45 and 70 with a broad cross-section of abdominal fat in the subjects will be desired to give a better range for the analysis. Subjects must also be free of any metal implants and disease.
Risks to subject:
This study is considered to be very safe and we do not expect that you will have any side effects from doing this study. You can stop or withdraw from the study anytime you want to or if the study becomes uncomfortable or painful. There is virtually no discomfort, certainly no pain, no risk with MRI and a very low radiation dose (say 1/30 of a chest x-ray) with the DXA. If you do experience any side effects they should be reported to the Principal Investigators immediately.

Benefits of Study to Subject:
- Very accurate estimate of total body fat for the subjects interested in the estimation of how much fat one has so that a reference point can be made in order to set a reference to compare with in the future. Testing may also improve compliance, set goals and improve motivation for someone on an exercise program to lose weight.
- Very accurate estimation of the ratio of visceral adipose tissue versus subcutaneous adipose tissue. If one feels that they are over weight, estimating body composition can help determine if the excess weight is due to muscle or fat.
- Bone mineral density of whole body. Low bone mineral density may be a predisposition to osteoporosis of other bone or joint problems.
- Assess health risk for cardiovascular disease. It is healthier to have a greater percentage of fat situated subcutaneously. A large amount of VAT has been found to be associated with a greater risk for cardiovascular disease.
- There is growing evidence that the distribution of body fat to central abdominal sites is associated with risk factors for cardiovascular disease such as insulin resistance, hyperlipidemia, hypertension, and hyperinsulinemia. Abdominal fat has also been found to be associated with diabetes, stroke, coronary heart disease, and related mortality, especially in elderly men. Distribution of fat to the gluteal femoral area does not appear to increase risk. These metabolic abnormalities may be particularly associated with the accumulation of visceral (internal) rather than subcutaneous (just under the skin) abdominal tissue.

Confidentiality:
All the information, which identifies you, will be strictly confidential. The study records will be available only to the participant and the members of the study team. All documents will be identified only by code number and kept in a locked filing cabinet. You will not be identified by name, initials, or date of birth in any reports of the completed study.

Participation in the Study:
You are under no obligation to take part in this study. If you decide not to participate or if you decide to withdraw from the study before its completion, you are totally free to do so and such a decision will have no consequences for your medical care in the future.

Questions:
If you have any questions, concerns or desire further information with respect to this study
now or in the future, or you experience any adverse effects, please telephone Dr. Alan Martin, Professor, School of Human Kinetics at 822-9174.
If you have any concerns about your rights as a research subject you may contact the Director of Research Services at the University of British Columbia, Dr. Richard Spratley at 822-8598.
CONSENT

The study has been clearly explained to me and I have read and understood the information provided. I request to be enrolled in the study. I understand that participation in this study is entirely voluntary and that I have the right to decline to enter the study and to withdraw from it, for any reason, without any consequence to my present or future health care. I acknowledge that I have received a copy of this form for future reference.

I consent to participate in this study.

Subjects Signature: ___________________________ Date: ___________

Please print name: ___________________________

Witness Signature: ___________________________ Date: ___________

Investigators Signature: ___________________________ Date: ___________

Contact:
APPENDIX B

EXAMPLE OF DXA SCANS
## FAIRMONT BONE DENSITY

**Hologic QDR-4500W (S/N 48157)**
Whole Body V8.20a:5

<table>
<thead>
<tr>
<th>Region</th>
<th>BMC (grams)</th>
<th>Fat (grams)</th>
<th>Lean (grams)</th>
<th>Lean+BMC (grams)</th>
<th>Total (grams)</th>
<th>% Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>L Arm</td>
<td>222.9</td>
<td>1340.2</td>
<td>3237.1</td>
<td>3459.9</td>
<td>4800.2</td>
<td>27.9</td>
</tr>
<tr>
<td>R Arm</td>
<td>260.7</td>
<td>1181.0</td>
<td>3650.4</td>
<td>3911.2</td>
<td>5012.1</td>
<td>22.0</td>
</tr>
<tr>
<td>Trunk</td>
<td>695.2</td>
<td>13334.6</td>
<td>29976.1</td>
<td>38671.3</td>
<td>44085.9</td>
<td>30.3</td>
</tr>
<tr>
<td>L Leg</td>
<td>511.0</td>
<td>3288.3</td>
<td>9054.8</td>
<td>9565.8</td>
<td>12864.0</td>
<td>25.5</td>
</tr>
<tr>
<td>R Leg</td>
<td>576.0</td>
<td>3439.0</td>
<td>9669.3</td>
<td>10245.3</td>
<td>13604.3</td>
<td>25.1</td>
</tr>
<tr>
<td>SubTot</td>
<td>2265.8</td>
<td>22495.8</td>
<td>55587.7</td>
<td>60345.5</td>
<td>76836.4</td>
<td>28.0</td>
</tr>
<tr>
<td>Head</td>
<td>513.2</td>
<td>1066.8</td>
<td>4027.0</td>
<td>4540.3</td>
<td>5687.1</td>
<td>19.0</td>
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<tr>
<td>TOTAL</td>
<td>2779.0</td>
<td>23561.9</td>
<td>59614.7</td>
<td>62393.7</td>
<td>85955.6</td>
<td>27.4</td>
</tr>
</tbody>
</table>

- Assumes 17.8% brain fat
- LBM 73.2% water

### FAIRMONT BONE DENSITY

**a Whole Body**

<table>
<thead>
<tr>
<th>Age</th>
<th>18</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
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</thead>
<tbody>
<tr>
<td>B</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>M</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>D</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

No Curve Found

**BMD(WHOLE) = 1.178 g/cm²**

---

**Hologic QDR-4500W (S/N 48157)**
Whole Body V8.20a:5

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Comment:</td>
<td>RESEARCH</td>
</tr>
<tr>
<td>I.D.:</td>
<td>R6</td>
</tr>
<tr>
<td>S.S.#:</td>
<td></td>
</tr>
<tr>
<td>Ethnic:</td>
<td>U</td>
</tr>
<tr>
<td>ZIPCode:</td>
<td>Height: 172.72 cm</td>
</tr>
<tr>
<td>Operator:</td>
<td>TS</td>
</tr>
<tr>
<td>Weight:</td>
<td>87.00 kg</td>
</tr>
<tr>
<td>BirthDate:</td>
<td>1998 11-26 16:08</td>
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<tr>
<td>Physician:</td>
<td>WARREN</td>
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</tbody>
</table>

---

**FAIRMONT BONE DENSITY**

No Curve Found

**BMD(WHOLE) = 1.178 g/cm²**
APPENDIX C

EXAMPLE OF MRI SCANS
MRI “Scout scan” – sagittal image showing levels for transverse imaging between L1-L5 vertebrae. Adapted from Han et al. (1997) without permission.

Example of “Scout Scan” used in this study.
Transverse magnetic resonance image (MRI) image at the L4-L5 level. Adipose tissue (A) (subcutaneous and visceral adipose tissue) is clearly distinct from lean tissue (B). Adapted from Ross et al. (1992) without permission.

Transverse MRI image through midabdomen region. Arrow, region within subcutaneous adipose tissue that appears as a shadow. This is randomly occurring MRI artifact that must be corrected for after segmentation of the image. Adapted from Ross et al. (1992) without permission.
Two transverse MRI images of the lower (Panel A) and upper (Panel B) abdomen. For the MRI image in Panel A, the adipose tissue pixel intensities are uniform and thus the related histogram (below the image) reveals two unique pixel intensity peaks for lean and adipose tissue, respectively. The area indicated by the arrow in Panel B is the consequence of randomly occurring MRI artifacts, which as illustrated in the associated histogram, result in poorly defined thresholds for lean and adipose tissue. Adapted from Despres et al. (1996) without permission.
"Highlighting technique" where the VAT is delineated by drawing a line within the muscle wall surrounding the abdominal cavity. Adapted from Despres et al. (1996) without permission.
1 = omental and mesenteric adipose (portal adipose/intraperitoneal adipose)

2 = retro or extraperitoneal adipose

(1 + 2 = visceral adipose)

3 = subcutaneous adipose

Adapted from van der Kooy and Seidell (1993) without permission.
Adapted from Abate et al. (1994) without permission.

Adapted from Ross et al. (1992) without permission.