Changes in body composition and fat distribution in response to weight loss and weight regain

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Karin van der Kooy

Changes in body composition and fat distribution in response to weight loss and weight regain

Proefschrift
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in de landbouw- en milieuwetenschappen,
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Stellingen

1. Verschillende, indirecte technieken om bij gewichtsverlies de verandering in lichaamssamenstelling te meten, geven niet dezelfde resultaten.
   dit proefschrift

2. Beeldvormende technieken, gebaseerd op computer-tomografie en kernspin-resonantie, zijn geschikt om veranderingen te meten in de totale hoeveelheid vet in de buikholte. Anthropometrie is daar minder geschikt voor.
   dit proefschrift

3. De vetverdeling over het lichaam verandert door gewichtsverlies.
   o.a. dit proefschrift

4. 'In the meantime we are in the position where any fool can make a subject lose weight but that possibly there are times when *only* a fool would try.'
   Munro & Cantley, International Journal of Obesity 1992;16:S53-S57

5. Het geclaimde 'succes' van veel afslankprodukten strookt niet met het feit dat nog veel mensen ontevreden zijn met hun gewicht.

6. In het ballet Groosland van choreografe Maguy Marin worden alle schoonheids-idealen van het academisch ballet met voeten getreden en is dik minder lelijk dan de hedendaagse reclameboodschappen het publiek willen doen geloven.
   En Rond, Het Nationale Ballet, 1992

7. Het aantal deelnemers aan wetenschappelijke experimenten dat voortijdig stopt, is omgekeerd evenredig met het enthousiasme van de onderzoeker.

8. 'Bij werkelijke onderzoeksinnovaties speelt toeval (serendipiteit) een minstens zo grote rol als doorzettingsvermogen en methodologische nauwkeurigheid.'
   Klazinga & Everdingen, Mediator 1993;4(1):9-10

9. Voor omschakeling naar meer milieubewuste vormen van land- en tuinbouw is een breed pakket chemische bestrijdingsmiddelen een noodzakelijk kwaad.

10. Afval scheiden is goed, ook wanneer er per afvalstroom (nog) onvoldoende verwerkingsmogelijkheden zijn.

11. Een voedingskundige eet niet per definitie gezond.

Stellingen behorend bij het proefschrift van Karin van der Kooy, 'Changes in body composition and fat distribution in response to weight loss and weight regain'.

Wageningen, 28 april 1993.

Aan mijn ouders

Contents

Abstract

Changes in body composition and fat distribution in response to weight loss and weight regain

PhD Thesis, Department of Human Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands, 28 April 1993.

Karin van der Kooy

This thesis describes the effects of weight loss and subsequent weight regain on body composition, fat distribution and resting energy expenditure in moderately obese men and moderately obese premenopausal women. Participants were subjected to a controlled 4.2 MJ/day energy deficit diet for 13 weeks, and re-examined more than one year after weight loss intervention. Five techniques to assess the changes in body composition after weight loss (on average 12.2 ± 3.7 kg (mean ± SD)) were compared. The results from densitometry (hydrostatic weighing) and the deuterium oxide dilution technique were similar, whereas, bioelectrical impedance and two anthropometric methods (skinfold thicknesses and body mass index) showed larger reductions in fat-free mass (FFM) than estimated by densitometry and the dilution technique. These findings were similar in both sexes. Magnetic resonance imaging (MRI) was used to assess the reductions after weight loss in the visceral and subcutaneous abdominal fat depots and the subcutaneous fat depot at trochanter level. The proportional reduction of fat was largest in the visceral depot (men 40%, women 33%) and less fat was lost subcutaneously, especially at trochanter level (men 29%, women 26%). The reductions in visceral fat as measured by MRI were compared with changes in anthropometric measurements. The change in waist-to-hip ratio (WHR) was not related to the change in visceral fat, and the change in sagittal-to-transverse abdominal diameter ratio was only moderately associated with visceral fat loss in both sexes. During the follow-up of 67 weeks after weight loss, 80% of the weight lost was regained on average. In men but not in women, the reduction in resting metabolic rate (RMR) after weight loss was larger than expected from the losses of FFM and fat mass. The RMR returned to baseline level in both sexes after weight regain. The reduction in RMR was not related to later weight regain. Percentage body fat and amount of visceral fat also nearly returned to the level similar to that before weight loss.
It is concluded that bioelectrical impedance and anthropometric measurements are not as good as densitometry or the deuterium-oxide dilution method for the evaluation of changes in body composition. Only approximate estimates of visceral fat can be achieved by anthropometry. As a consequence, the assessment of changes in visceral fat by anthropometry is limited. Finally, one weight cycle as observed in this study does not lead to a permanently reduced RMR, nor to a greater body fatness nor to an increase in visceral fat compared with initial levels.

Chapter 1

Introduction

Obesity is a common condition in affluent countries. For example, the prevalence of moderate obesity (body mass index between 25-30 kg/m^2) in Dutch men and women with ages between 37 and 43 years, was 40-45% and 23-30% respectively, in the period 1974-1980.[1] The prevalence of more severe obesity in the Netherlands (body mass index greater than 30 kg/m^2) in young adults is similar to that in some other Western European countries (5-6%), but is lower than in the United States.[1,2] The prevalence of obesity does not seem to increase in the overall population of industrialised countries[1,2], but, increases appear in specific countries and groups of people.[3] There is convincing evidence that obesity is associated with an increased risk for mortality and morbidity, particularly, of cardiovascular and coronary heart disease and non-insulin-dependent diabetes mellitus.[3-5] The distribution of body fat plays a critical role in this context. An abundance of visceral fat (i.e. intra-abdominal fat) is a stronger predictor of specific metabolic aberrations than overall body fatness.[6-8]

It has been proposed that weight loss strategies should be aimed primarily to those obese individuals who have metabolic disorders such as hypertension, elevated serum lipid levels, atherosclerosis, non-insulin-dependent diabetes mellitus etc., which may improve with weight loss. In addition, those who have an increased risk for complications due to, for instance, family history of coronary heart disease or diabetes, should also be encouraged to lose weight.[9,10] Abdominal obesity is another characteristic that may indicate the need for weight loss.[6,7,11,12]

There is conflicting evidence, however, whether or not an unfavourable fat distribution is changed by losing weight[13,14], and whether or not this reduces morbidity and mortality. Furthermore, dieting histories of many obese subjects show multiple cycles of weight loss and weight regain indicating that the long-term prognosis of weight maintenance after slimming is poor.[15] It has been suggested that repeated weight cycles have adverse consequences on body composition[16], energy expenditure[17], fat distribution[18], and mortality and morbidity.[19,20] Because of the inconsistent findings and the continuing debate about advantages and disadvantages of weight loss treatment, the study described in this thesis focused on the effects of weight loss and subsequent weight regain, on body composition, fat distribution and resting energy expenditure in healthy moderately obese men and women.

For the evaluation of weight loss and subsequent weight regain (i.e. how much and where fat and fat-free mass have been lost or gained), different methods are available varying from sophisticated, technically advanced techniques such as dual photon or dual energy X-ray absorptiometry and imaging techniques, to relatively simple and readily applicable techniques like bioelectrical impedance and anthropometry.[21-26]

The most commonly used and easily applicable methods have been developed and tested cross-sectionally in large population samples that varied in age, body fatness and fat distribution. These methods have usually not been evaluated with respect to the assessment of changes in body composition and fat distribution for which they are frequently used. Reliable assessments of changes in body composition are also very important for the interpretation of changes in energy expenditure due to weight loss or weight regain, because the composition of the body is closely related to energy expenditure.[27] The fat-free mass, which includes muscle mass and organs, represents the metabolically active part of the body and it determines to a large extent the energy expenditure of the body.

The interpretation of consequences of weight changes on fat distribution, body composition and energy expenditure may largely be influenced by the methods used to assess the changes in fat distribution[14] and body composition.[28] Different measurement techniques were therefore used in this study for the assessment of changes in body composition and fat distribution, and their results have been compared.

The main questions of research of the study described in this thesis were:
- Do different measurement techniques for the assessment of changes in body composition and fat distribution lead to similar results?
- Does fat distribution change when weight is lost and what are the consequences of subsequent weight regain, with special emphasis on visceral fat accumulation?
- Finally, are body fatness and resting energy expenditure unfavourably affected by a weight cycle?

Body composition measurements

Research on body composition started more than hundred years ago and is still an area of basic research in which new methodologies and physiological concepts are developed.[21,26,29] A traditional way of classifying methods to assess body composition is dividing them into direct and indirect techniques.[22-24] Direct measurement methods determine specific components of the body such as water, fat or adipose tissue, muscle mass or specific chemical elements. Cadaver analysis, imaging techniques and neutron activation analysis are examples of these methods. Indirect measurement techniques are derived from direct methods and are dependent on the relationship between the component measured by the indirect method and components known from direct measurements as determined in usually small selected groups of subjects.

It will be clear that cadaver analysis is no option for most research purposes. In the past, few human cadavers were analyzed and several assumptions have been derived from the results. For example, the fat-free mass of the body can be calculated from total body water assessed by isotope-dilution techniques assuming that 73% of the fat-free mass consists of water.[30] The percentage body fat can be determined by estimation of the density of the body using hydrostatic weighing. The method is based on a two-compartment model that assumes that the body consists of a fat and a fat-free part with constant densities of 0.9 kg/l and 1.1 kg/l respectively.[31] The variety of assumptions that underlie the indirect methods limits their application and gives rise to measurement errors other than pure technical measurement errors.[25,32] The various techniques to measure body composition have frequently been reviewed. For detailed descriptions and explanations of principles, these reviews should be consulted.[21-26]

In the present study, five indirect methods to assess body composition were compared (densitometry, isotope-dilution, bioelectrical impedance, and two anthropometric methods, skinfold thicknesses and a weight for height index). The techniques were chosen because of their relatively easy application and frequent use in studies with human volunteers.

Fat distribution measurements

Research on fat distribution started about 50 years ago and it has become an important field of interest in obesity research.[4,6-8] From the beginning, relatively simple methods such as photographs, circumference measurements and skinfold thickness ratios have been used to distinguish between different types of fat distribution.[4] A drawback of these methods is that the overall shape of the body is evaluated with photographs and circumferences which include contributions of muscle, fat and skeletal dimensions, whereas skinfold thicknesses only describe subcutaneous fat patterning. None of these methods is able to quantify the visceral fat depot which is the fat depot associated with health hazards.[6-8]

In the early 1980's, computerized tomography scanning was introduced in fat distribution research and this technique allows the quantification of visceral fat.[33] A disadvantage of computerized tomography is X-ray exposure, particularly in longitudinal studies with repeated measurements of total body scanning. Magnetic resonance imaging has been found to be another imaging technique without X-ray exposure, but with the same potential.[34]

A comprehensive description of techniques to assess fat distribution and especially to assess visceral fat is given in this thesis. Furthermore, magnetic resonance imaging and anthropometric measurements were compared in examining changes in fat distribution.

Weight cycling

The majority of obese people have a long history of dieting with successful weight losses, but also with disappointing relapses.[15,35] It has been suggested that repeated weight cycles may lead to an increase in body fatness[16] and to a permanent reduction in energy expenditure, which may cause difficulty in weight maintenance and an inability to lose further weight.[17] Additionally, fat deposition may shift to a more abdominal type of fat distribution[18] and, perhaps as a consequence, weight cycling has been associated with an increased risk for coronary heart disease.[19,20]

Similar findings were not observed in all studies. Obese subjects matched for body weight and divided according to their self-reported history of weight cycling, were comparable in body fatness, fat distribution expressed as waist-to-hip ratio and resting metabolic rate, as well as in changes in body composition and resting metabolic rate due to energy restriction and physical exercise.[36] Moderately obese women subjected to three weight cycles (three two-week periods with a very-low-calorie diet alternated with four-week periods with free diet), also did not show adverse consequences on body composition and resting energy expenditure.[37] In the latter study, only small weight changes (3-5 kg) were achieved and the rate of weight loss declined throughout the experiment due to reduced dietary compliance.

In the present study, body composition, resting energy expenditure and visceral fat accumulation were investigated after successful weight loss followed by weight regain that occurred during a follow-up of more than one year.

Outline of the thesis

Changes in body composition after weight loss assessed by five different methods are described in Chapter 2. In Chapter 3, techniques to measure visceral fat are reviewed. The principles of techniques, their accuracy and reproducibility as well as aspects of costs and safety are discussed. Effects of weight loss on fat distribution measured by magnetic resonance imaging and anthropometry are reported in Chapter 4 and Chapter 5. In Chapter 6, the results of weight loss and subsequent weight regain on body composition and energy expenditure are given, and the consequence of one weight cycle on visceral fat deposition is described in Chapter 7. Finally, overall conclusions and implications for future research are discussed in Chapter 8.

The study was part of an extensive project in which the effects of an energy restricted diet and weight maintenance diets varying in amount and composition of dietary fat, on energy expenditure, body composition, fat distribution, serum lipids and lipoproteins, and several hormones were investigated (Figure 1). Moderately obese men and women with a considerable range in fat distribution participated in the project. The part of the project described in this thesis focused on the changes in body composition, resting energy expenditure and fat distribution due to weight loss and subsequent weight regain more than a year after weight loss intervention. Other aspects of the project (results of the dietary interventions preceding the energy restricted diet, consequences of fat distribution on weight loss and energy expenditure, and the associations between fat distribution, sex hormones and serum lipids and lipoproteins) are described and discussed elsewhere.[38]

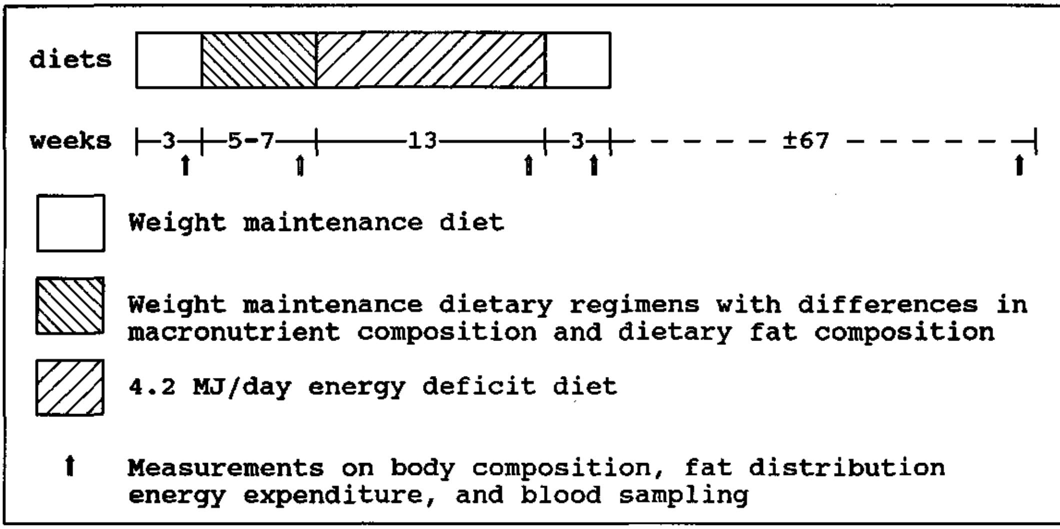

Figure 1. Study design.

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Chapter 2

Changes in fat-free mass in obese subjects after weight loss: a comparison of body composition measures

Karin van der Kooy, Rianne Leenen, Paul Deurenberg, Jacob C. Seidell, Klaas R. Westerterp and Joseph G.A.J. Hautvast

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Abstract
Estimates of body composition by densitometry were made in 84 apparently healthy subjects (42 men, 42 women) with a mean age of 40 ± 6 years (mean ± SD), before and after weight loss. The initial body mass index (BMI) was 30.7 ± 2.3 kg/m^2 and the achieved weight loss on a 4.2 MJ/day energy deficit diet for 13 weeks was 12.2 ± 3.7 kg. The results from densitometry were compared with estimates obtained by four other techniques: deuterium oxide dilution, skinfold thickness, bioelectrical impedance (three equations) and BMI (two equations). The fat-free mass (FFM) loss estimated by densitometry in men and women was 2.8 ± 1.8 kg and 1.3 ± 1.3 kg respectively. The dilution technique gave results comparable with densitometry. The losses of FFM assessed by skinfold thicknesses, BMI and impedance equations were almost similar, but significantly larger than the reduction in FFM measured by densitometry. These deviations were mainly the result of significantly larger differences from densitometry before compared to after weight loss. No correlation was found between change in FFM by densitometry and change in resistance measured by the bioelectrical impedance method in both sexes. It is concluded that application of published prediction formulae in weight loss studies are less appropriate and will lead to changes in FFM that are significantly different from the changes estimated by densitometry or deuterium oxide dilution.

Introduction
In studies on the effects of treatments aimed to lose weight, there is a need to have accurate information on the changes in body composition. This information is important in evaluating the effect of the prescribed treatment and determining which part of the weight reduction consisted of fat and which part of fat-free mass.[1-4] In obese subjects the evaluation of weight loss regimens often leads to contradictory findings which are, according to Grande[1], partly attributable to the methods available to assess the composition of the lost weight.

The method of choice for the assessment of body composition depends on the subjects studied, what is to be quantified, the study design and the availability and feasibility of equipment.[5] Sophisticated methods such as dual photon absorptiometry, neutron activation analysis, computerized tomography and magnetic resonance imaging are restricted to specialized research institutes and are difficult to apply to large numbers of subjects or in the follow-up of weight loss treatments.[4-6] The same practical limitations hold for energy and nitrogen balance measurements which were found suitable for measuring changes in body composition.[1,3,7-10] Densitometry and deuterium oxide dilution are more widely available and are commonly used as a reference to validate other techniques.[2,11-18] They are also applied in weight loss studies.[2,3] However, these methods do require subject cooperation, advanced equipment and skill of the investigator which also limits their applicability. In contrast, bioelectrical impedance is a relatively easy method to perform and has been investigated intensively during recent years.[7,13-15,17-23] The conclusions about the feasibility of this technique in different populations and in studies on weight changes are conflicting.[7,19-23] Anthropometry, a method that also requires little subject cooperation, has been investigated by many groups as well. It is, however, doubtful whether this method can be used in assessing changes in body composition because of its low precision and accuracy. [4,5,8,24-27]

The most commonly used prediction equations to assess body composition were derived from large studies in which the subjects varied in age and percentage body fat. [2,11-16] Proper validation of these formulae to assess changes in body composition is lacking. In the present study changes in body composition estimated with five methods (body mass index, skinfold thicknesses, bioelectrical impedance, deuterium oxide dilution and densitometry) were investigated. Densitometry was used as the reference. These methods were applied before and after weight loss in a group of obese individuals.

It should be stressed that the estimates of changes in body composition here, may differ from the true changes because there is no *in-vivo* method available that can be regarded as a "gold standard". A validation of methods is therefore always relative. The findings, however, do provide necessary knowledge of sources of variation in separate body composition measurements and show how far the results of the easy-to-measure techniques can be compared.

Subjects and Methods

Subjects

Subjects included were men and women aged between 25 and 51 years. They were recruited by advertisements. Ninety-six subjects (48 men and 48 women) were selected if

i) their BMI was between 28 and 38 kg/m^2,
ii) they were apparently healthy as evaluated by a medical examination and
iii) they did not use any medication known to influence variables measured.

Twelve participants did not complete the study (3 women and 2 men for personal reasons, 1 woman and 2 men due to illness, 2 men because of poor compliance and 2 women in whom underwater weighing could not be performed). Results presented here include 84 subjects who completed all parts of the study successfully. The study was carried out with the approval of the Medical Ethical Committee of the Department of Human Nutrition. After all procedures had been explained to the subjects, their written informed consent was obtained.

Body composition was studied in weight stable periods before and three weeks after a 13 week period in which the subjects received a 4.2 MJ/day energy deficit diet (42 energy percentage (E%) carbohydrate, 25 E% protein and 33 E% fat). Individual energy requirements on which the energy restricted diet was based were estimated from resting metabolic rate and physical activity pattern.[28] During the weeks before and after the energy restricted diet the energy intake was adjusted individually to prevent weight changes. During the study 95 E% of the food was provided by the Department. The remaining 5 E% was chosen by the subjects themselves, from a list of products free of fat and cholesterol, such as fruits, vegetables, beverages ect. These free food items were reported in a diary and the average nutrient composition of these 5 E%, as assessed by the Dutch computerized food composition table[29], was 15 E% protein, 84 E% carbohydrate and 1 E% fat.

Body composition measurements

The measurements before and after weight loss were performed at the same time of the day with the subjects in a non-fasting state. Body weight was measured to the nearest 0.05 kg using a digital scale (Berkel ED60-T, Rotterdam, The Netherlands) with the subjects wearing swimming costumes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated from weight and height (kg/m^2). The percentage body fat (%FAT) was estimated from BMI according to the age and sex specific equations of Womersley and Durnin[12] and the equation of Deurenberg et al.[16]

Triceps, biceps, subscapular and supra-iliac skinfolds were measured to the nearest 0.2 mm as described by Durnin and Womersley[11] by one of two well trained observers using a Harpenden skinfold caliper (Holtain Ltd. Crymmych, UK). The skinfold thicknesses were measured twice on two different occasions in the weight stable periods. The average of these two measurements was used in the statistical analysis. In five subjects, one or more skinfolds could not be measured because the thicknesses of the skinfolds exceeded the size of the caliper (>45 mm). In one woman, both subscapular and supra-iliac skinfold measurements were missing; in two women, the subscapular measurement was missing and in one woman and one man, the supra-iliac measurement was missing. Percentage body fat was calculated using the sum of skinfolds in the age and sex specific equations of Durnin and Womersley.[11] In the subjects in whom the sum of four skinfolds was not available the specific equations for the sum of the remaining skinfolds were used.

Resistance was measured with a body composition analyser (BIA-101, RJL-Systems, Inc, Detroit, Michigan, USA) to the nearest ohm at the left side of the body with the electrodes placed as described by Lukaski et al. [30] after voiding. The FFM in kg was assessed by using the equation of Lukaski et al.[13], by using the age, sex and fatness-specific equations of Segal et al.[14], and by using the equation of Deurenberg et al.[15]

Total body water (TBW) was estimated from deuterium oxide concentration in urine from a second voiding, after 5.5 h equilibrium time, and corrected for a 4% overestimation of the dilution space.[31] The subjects were given deuterium oxide (99.8% $^{2}H_2O$) at a dose of 0.08 g/kg FFM as determined by underwater weighing. Isotope abundance in the urine sample after dilution was measured with an Aqua-SIRA mass spectrometer (VG Isogas Ltd. Middlewich, UK).[32] FFM was calculated assuming 73.2% of the FFM to be water.[33]

Whole body density was determined by underwater weighing.[34] Underwater weight was measured to the nearest 0.05 kg (Sartorius 3826MP 81, Göttingen, Germany). Residual lung volume was measured simultaneously by a helium dilution technique[35] (Spiro-Junoir, Jaeger GmBH, Würtzburg, Germany). The measurements were done in quadruplicate and the average density was used in the statistical analysis. Percentage body fat was calculated according to Siri's formula[34]:

$$\%FAT = 495 / \text{body density} - 450$$

When %FAT was the result of a method or equation, the FFM was calculated as the difference between body weight and fat mass (FM). Table 1 shows the reliability of each measurement technique except for TBW in ten subjects, five women and five men, who were measured twice in the weight stable period before weight loss. The TBW measures were not repeated in these subjects for practical reasons. The coefficient of variation in the analysis of deuterium oxide in urine was found to be 0.2% in this study.

Statistical analysis

The reliability of the methods was calculated using the intra-class correlation coefficient, the residual standard deviation and the residual coefficient of variation assessed by two-way analysis of variance after adjusting for differences between subjects within each method. Differences between variables and changes in variables were tested using a two-sided paired *t*-test. The relative validity for the alternative methods was evaluated using the procedures described by Bland and Altman.[36] Pearson's product-moment correlation coefficients were calculated for the changes in body composition measured by densitometry and the changes in body weight, TBW, resistance and sum of skinfolds.
P values less than 0.05 were considered to be significant and the results are expressed as mean ± standard deviation (SD).

Results

Table 1 shows the reliability of the measurement techniques before weight loss. The residual coefficients of variation (CV) for BMI, resistance, sum of skinfolds and density led to CVs in FFM estimates from both BMI-equations[12,16] of 0.11%; from the impedance equations of Segal et al.[14], Lukaski et al.[13] and Deurenberg et al.[15] of 0.37, 2.02 and 0.84% respectively, from the formulae of Durnin and Womersley[11] of 0.77%, and from densitometry of 1.57%. The CVs for the separate skinfold measurements varied between 6 and 17% before weight loss and between 6 and 15% after weight loss. The CV of the sum of skinfolds was 4.73% after weight reduction. It is assumed that the reliability of measurements of density, resistance and BMI are comparable before and after weight loss. The measurements were carried out by the same observers.

The physical characteristics and variables used to assess body composition before weight loss are summarized in Table 2. Table 3 shows the changes in the body composition variables after weight loss.

Table 1. Reliability of the variables used to estimate body composition in ten subjects measured twice before weight loss within two weeks.

Measurements	Residual standard deviation	Residual coefficient of variation (%)	Intra-class correlation coefficient
Density (kg/l)	0.0020	0.20	0.97
Body mass index (kg/m^2)	0.09	0.28	0.99
Resistance (ohm)	12.38	2.83	0.93
Sum of skinfolds (mm)[a]	3.54	3.39	0.98

[a] Sum of triceps, biceps ,subscapular and supra-iliac skinfolds.

Table 2. Characteristics and body composition variables of subjects before weight loss.

Characteristics	Women (n=42)		Men (n=42)	
	mean	SD	mean	SD
Age (years)	39	5	40	6
Height (m)	1.66	0.06	1.79	0.06
Weight (kg)	85.5	8.4	97.1	7.5
Body mass index (kg/m^2)	31.1	2.5	30.4	2.1
Density (kg/l)	1.0035	0.0095	1.0260	0.0089
FFM[a] (kg)	48.3	4.7	65.4	5.7
%FAT[a]	43.3	4.7	32.5	4.2
Total body water (l)	36.2	3.5	48.3	4.0
Resistance (ohm)	480	55	409	33
Sum of skinfolds (mm)[b,c]	113.4	15.5	100.0	19.4

[a] Calculated by equation of Siri[34]: %FAT=495/body density-450.
[b] Sum of triceps, biceps ,subscapular and supra-iliac skinfolds.
[c] Women (n=38) and men (n=41) because of missing skinfolds.

Table 3. Changes in body composition variables after weight reduction.

Variables	Women (n=42) mean	SD	Men (n=42) mean	SD
Weight (kg)	-11.9	4.0†	-12.5	3.5†
Body mass index (kg/m^2)	-4.3	1.5†	-3.9	1.1†
Density (kg/l)	-0.0156	0.0064†	-0.0148	0.0063†
Total body water (l)	-1.1	1.1†	-2.0	1.2†
Resistance (ohm)	+22.0	22.0†	+16.0	19.0†
Sum of skinfolds (mm)[a,b]	-36.2	14.4†	-34.5	15.1†

[a] Sum of triceps, biceps ,subscapular and supra-iliac skinfolds.
[b] Women (n=38) and men (n=41) because of missing skinfolds.
† $P<0.0001$ before *versus* after weight loss.

Table 4. The change in FFM measured by densitometry compared with the alternative methods and the change of FFM as a percentage of weight loss[a].

Methods	Women (n=42) Δ[b]FFM (kg) mean	SD	ΔFFM/Δweight ×100%	Men (n=42) ΔFFM (kg) mean	SD	ΔFFM/Δweight ×100%
Densitometry	1.3	1.3	11	2.8	1.8	22
D_2O-dilution	1.5	1.5	13	2.8	1.6	22
Body mass index						
Womersley[12]	3.5	1.5†	29	5.1	1.6†	40
Deurenberg[16]	3.3	1.4†	27	4.9	1.6†	39
Sum of skinfolds[c]						
Durnin[11]	3.5	1.9†	29	3.7	1.7§	30
Impedance						
Segal[14]	3.2	2.0†	27	5.2	2.3†	42
Lukaski[13]	2.1	2.1‖	20	2.4	2.9	21
Deurenberg[15]	4.1	1.4†	35	4.4	1.6†	36

[a] All reductions in FFM estimated by the different methods were significant: $P<0.0001$.
[b] Change in variable (Δ,before weight loss - after weight loss).
[c] Sum of triceps, biceps ,subscapular and supra-iliac skinfolds.
† $P<0.0001$, § $P<0.01$, ‖ $P<0.05$ densitometry *versus* alternative methods.

The loss of FFM as measured by densitometry compared with the alternative methods is shown in Table 4. The reduction of FFM as a percentage of weight loss is also given for each method. The change in FFM assessed by deuterium dilution was comparable with the estimate by densitometry in both sexes. The prediction formulae with BMI, resistance and the sum of skinfolds resulted in corresponding changes in FFM, however, they were significantly larger than those assessed by densitometry, with the exception of the prediction from the impedance equation of Lukaski et al[13] in men.

The mean differences in FFM estimates between densitometry and the alternative methods before as well as after weight loss are summarized in Table 5. Significantly larger deviations from densitometry were found before weight loss compared with after weight loss, except for the estimates from deuterium dilution in both sexes and for the predicted FFM from the sum of skinfolds and from the equation of Lukaski et al[13] in men. The extent of the standard deviations of the differences can be interpreted as a degree of agreement between the alternative methods and densitometry[36] In men and women, the standard deviations of the differences from the BMI, impedance and skinfold prediction formulae were considerable, varying in men from 2.4 kg after weight loss to 4.1 kg before weight loss and in women from 2.5 kg after weight loss to 3.8 kg before weight loss. The FFM estimated by, for example, the BMI equations of Womersley et al[12] in men before weight loss, differed by up to 12.6 kg compared with the estimate of FFM by densitometry.

Table 5. Mean differences between FFM measured by densitometry and FFM measured by the alternative methods before and after weight loss[a].

	Women (n=42)				Men (n=42)			
	Mean difference FFM (kg)				Mean difference FFM (kg)			
	(before)[a]		(after)		(before)		(after)	
Methods	mean	SD	mean	SD	mean	SD	mean	SD
D_2O-dilution	-1.2	2.1‡	-1.0	1.7‡	-0.6	2.3	-0.5	2.0
Body mass index								
Womersley[12]	-3.3	3.4†	-1.0	3.2‖**	-2.5	4.1‡	-0.2	3.2**
Deurenberg[16]	-2.1	3.5‡	-0.1	3.2**	-2.8	4.0†	-0.7	3.3**
Sum of Skinfolds[2]								
Durnin[11]	-2.6	3.8†	-0.4	2.8**	-0.2	4.1	+0.8	2.4*
Impedance								
Segal[14]	-2.1	3.0†	-0.1	2.5**	-3.7	3.1†	-1.3	2.4§**
Lukaski[13]	-4.5	3.6†	-3.6	3.9†*	-4.6	2.7†	-4.9	3.5†
Deurenberg[15]	-2.8	2.6†	+0.1	2.6**	-2.2	2.6†	-0.5	2.5**

[a] Difference = $FFM_{densitometry}$-$FFM_{alternative\ method}$; [b] Sum of triceps, biceps, subscapular and supra-iliac skinfolds; † $P<0.0001$, ‡ $P<0.001$, § $P<0.01$, ‖ $P<0.05$ densitometry *versus* alternative methods; ** $P<0.001$, * $P<0.05$ before *versus* after weight loss.

In Table 6 the correlation coefficients between changes in FM and FFM estimated by densitometry, and the changes in weight, TBW, resistance and the sum of skinfolds are given. No statistically significant correlations were found between the change in resistance and the change in FFM in both sexes. The change in FFM in men showed a positive correlation with the change in TBW. However, in women the changes in FFM as well as in FM were weakly associated with the change in TBW ($r=0.29$, $P=0.06$ and $r=0.30$, $P=0.06$ respectively).

Table 6. Pearson correlation coefficients between changes in variables used in the prediction equations, and changes in FFM and FM measured by densitometry after weight loss.

	Women (n=42)		Men (n=42)	
Variables[a]	Δ[a]FFM (kg)	ΔFM (kg)	ΔFFM (kg)	ΔFM (kg)
Weight (kg)	0.39 ‖	0.94 †	0.35 ‖	0.86 †
Total body water (l)	0.29	0.30	0.52 ‡	0.06
Resistance (Ohm)	0.11	-0.08	-0.10	-0.01
Sum of skinfolds (mm)[b,c]	0.24	0.52 ‡	0.08	0.69 †

[a] Change in variable (Δ, before weight loss - after weight loss); [b] Sum of triceps, biceps, subscapular and supra-iliac skinfolds; [c] Women (n=38) and men (n=41) because of missing skinfolds; † $P<0.0001$; ‡ $P<0.001$; § $P<0.01$; ‖ $P<0.05$.

Discussion

The present study shows that the choice of method used to assess changes in body composition greatly affects the assessed composition of the lost weight. The loss of FFM as a percentage of weight reduction assessed by the different methods varied in women from 11 to 35% and in men from 21 to 42% The estimates of changes in body composition by deuterium oxide dilution were in good agreement with those of densitometry, in contrast to the estimates from the prediction formulae with BMI, resistance and sum of skinfolds.

The finding that in both sexes the change in FFM measured by densitometry and the change in resistance were not correlated is surprising. Slightly higher correlations were found between the change in resistance and TBW for men and women ($r=-0.33$, $P=0.03$; $r=-0.28$, $P=0.07$ respectively). This may be the result of the more direct relationship between resistance and TBW compared to resistance and FFM.[23,30] Recently, Vazquez and Janosky[7] reported that resistance did not change in 16 obese women after weight loss while lean body mass assessed by nitrogen balance decreased significantly. On the contrary, previous studies concluded that bioelectrical impedance was effective in detecting changes in body composition.[19,22,23] Deurenberg et al.[20,21] found an under-estimation of the change in FFM measured by impedance compared with densitometry. These studies[20,21] and previous mentioned studies[19,22,23] are in contrast with the present study. This may be due to the timing of impedance measurements, the diet[7] or duration of slimming. In this study, the subjects were measured in a weight stable period while in the studies of Deurenberg et al.[20,21] the final measurements were performed with the subjects still in a negative energy balance. Changes in water and glycogen stores might have affected the estimates of FFM.[21]

The impedance measurements could also have been disturbed by an expansion of the extra-cellular water (ECW) compartment and a higher value of the ratio of extra- to intra-cellular water (ECW/ICW) in the obese state.[37] Deurenberg et al.[38] showed that the distribution of water over the ECW and ICW compartments affected the resistance. The impedance analyser with a frequency of 50 kHz presumably measures the whole ECW compartment and only a part of the ICW compartment.[21,38,39] A shift in the ratio ECW/ICW after weight loss by a decrease in the ECW compartment may result in a resistance that can not simply be compared with the resistance measured before weight loss, based on another distribution of the body water.

When body weight, age and sex are included in the impedance equations besides resistance and height, more precise results could be expected.[7,15,40,41] This may explain the large deviations between FFM estimates from densitometry and from the equation of Lukaski et al.[13] before as well as after weight loss.

The FFM loss predicted by BMI and skinfolds was also significantly larger than the reduction in FFM as assessed by densitometry. Skinfold thicknesses and BMI may not be appropriate for the follow-up of weight loss treatment.[8,12,16,24-26,42] Skinfold measures have large inter- and intra-observer variability and are difficult to measure in the obese [8,24-26] The better relationships between changes in weight or BMI and skinfolds with the change in FM compared to FFM assessed by densitometry, might be due to the larger range of changes in FM than in FFM. In addition, skinfold thicknesses and BMI are more directly related to overall fatness.[12] The discrepancies of the prediction equations with densitometry might be explained by the fact that the equations used were derived from a range of lean to moderately obese subjects and are not necessarily valid in more severely obese subjects with %FAT higher than the validation population mean. This could have resulted in the larger deviations and systematic differences in estimates before compared with after weight loss in this study. A reduction in weight of about 12 kg improved the application of the equations.

The deuterium dilution technique resulted in estimates of changes in FFM comparable to densitometry, although in women the estimates of FFM by dilution showed a slightly but significantly larger FFM before as well as after weight loss. In contrast, in other studies[18,43,44] a lower FFM was found using TBW compared with densitometry, but this under-estimation was more pronounced in lean than in obese women.[18] The change in TBW and FFM as assessed by densitometry correlated less strongly in women than in men. In women, the change in TBW was also weakly associated with change in FM. This may be due to the relatively small changes in TBW in women and the fluctuations in body water during the menstrual cycle.[45]

Densitometry, the reference in this study, could have limitations as well. Assumptions of relationships between the different compartments of the body may vary between and probably within individuals as they lose or gain weight. It has been suggested that the fraction of total body water, and therefore the hydration of the FFM, is relatively increased in the obese subjects.[37,46] This could lead to discrepancies between estimates of changes in FFM assessed by densitometry and total body water[46-48], but in the present study these methods were in good agreement. Consequently, application of equations including both TBW and body density[47] did not result in a different assessment of compositional change of weight loss compared to assessments by TBW or density separately ($\Delta FFM_{TBW\ \&\ density}$ = 3.0 ± 1.6 kg in men and 1.5 ± 1.2 kg in women).

Calculation of the water fraction of the FFM ($TBW/FFM_{densitometry}$) in the present study, did not differ across the range of fatness in men before and after weight loss. However, in women in the upper tertile of fatness (%FAT >45) before weight loss, the water fraction was significantly higher than the assumed hydration factor (73.2% [30]) (results not shown). As a consequence, the assumption of a constant water fraction of the FFM may be violated in more severely obese subjects.[37,41,46-48]

Further research is needed on applicable techniques to assess changes in body composition, particularly for obese subjects. Calibration of techniques against methods and models that consider variability in FFM composition are necessary to approximate as well as possible the changes that occur.

In summary, the results of this weight loss study showed that application of prediction formulae with BMI, skinfolds or resistance led to different estimates of changes in body composition compared to assessments from body density or total body water. The prediction formulae tended to under-estimate percentage body fat, especially before weight loss. Densitometry and the deuterium oxide dilution method are recommended when changes in FFM are investigated. The BMI-formulae are still the most suitable alternatives, because of the simple practical performance and relatively low costs of measuring weight and height compared to skinfolds and impedance. However, results of all techniques have to be interpreted with caution, particularly in the grossly obese.

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Chapter 3

Techniques for the measurement of visceral fat: a practical guide

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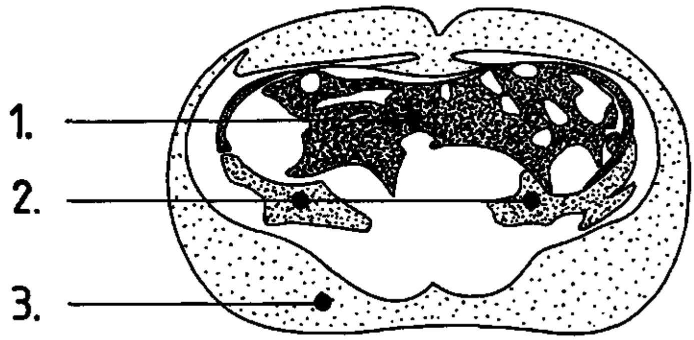
Abstract

The measurement of fat distribution has become an important issue in obesity research. Numerous techniques have been developed to assess visceral fat because this fat seems to be most strongly associated with metabolic disorders. This review focuses on methods for the direct and indirect assessment of visceral fat ranging from multiple-scan computerized tomography to anthropometric measurements. The principles of techniques, their accuracy and reproducibility as well as aspects of costs and safety are discussed. Comparison of the different methods shows that imaging techniques, such as computerized tomography and magnetic resonance imaging, are the optimal techniques available for accurate assessment of visceral fat. Anthropometric measurements can be useful to classify subjects into different types of fat distribution, and for general application in epidemiological studies. Methods other than imaging techniques have limited potential in the measurement of changes in visceral fat deposition. The choice of a particular technique should be based on the aim of the study and on a balance of practical and financial considerations. Involvement of ionizing radiation exposure may be an important element in the decision-making process.

Introduction

Human obesity is associated with a large number of diseases and metabolic complications such as heart disease, diabetes mellitus, hypertension, gallbladder disease and some types of cancer.[1] After the pioneering work of Jean Vague[2] it has become increasingly clear that it is not just the amount of the fat deposited in the body but especially its localization which is responsible for the increased health risks associated with obesity. Currently it is understood that the fat stored in the abdominal cavity, in particular the 'portal' tissues stored as mesenteric and omental fat, are responsible for a major part of the health hazards.[3,4] Figure 1 shows the different abdominal fat depots with the nomenclature as used in the literature. In this review equivalent terms will be used interchangeably throughout the text.

It has been suggested that an increased deposition of subcutaneous fat in the gluteal or femoral region may actually be associated with a favourable risk profile.[5] It remains to be determined whether this is a true biological phenomenon rather than a mathematical



1 : omental and mesenteric fat
(= portal fat = intraperitoneal fat)
(dark shading)

2 : retro or extraperitoneal fat
(intermediate shading) +

1+2 : visceral fat
(= intra-abdominal fat = deep abdominal fat)

3 : subcutaneous fat
(light shading)

Figure 1. Nomenclature and anatomy of abdominal fat depots.

artefact because when body fat is predominantly stored in these subcutaneous regions there will be less fat stored in the abdominal depots. If, in future studies, some subcutaneous fat depots are shown to be truly protective against diseases, it will be important also to quantify these depots with accuracy. At present, however, visceral fat deposition seems to be of prime interest and this review will predominantly focus on methods that attempt to quantify this fat with different degrees of accuracy. The technical and practical possibilities and limitations of the available techniques will be discussed as well as the principles of the techniques, their reproducibility and accuracy, the costs in terms of time, money and labour, and their safety and discomfort for the subjects.

Figure 2 shows an overview of techniques to measure intra-abdominal fat depots from high-tech measurements with high accuracy and reproducibility to low-tech measurements with limited accuracy and reproducibility.

Aim of measurement:
to quantify amounts of mesenteric, omental
and retroperitoneal adipose tissue

Techniques in descending order of accuracy:

Cadaver analysis
↓
Multi-slice computerized tomography (CT)
or (less precise)
Multi-slice magnetic resonance imaging (MRI)
↓
Single-slice CT or MRI
↓
Dual energy X-ray absorptiometry (DEXA)
or dual photon absorptiometry (DPA)
↓
Ultrasound
↓
Anthropometry
(abdominal diameters, circumferences
and skinfold thicknesses)
↓
Eye-ball examination of subjects

Figure 2. Techniques for measuring visceral adipose tissue.

Measurement of visceral fat

Cadaver analysis

It is clear that cadaver analysis is usually no option for research purposes. Nevertheless, direct measurement of abdominal adipose tissue is the 'gold standard' for other techniques. One study has been carried out in which two male cadavers were sectioned in a way that made it possible to perform planimetric measurements in cross-sectional slices and to compare these with computerized tomography (CT).[6]

In addition, dissection seems to be the only available way to clearly distinguish between intraperitoneal (mesenteric and omental) and retroperitoneal fat. This distinction is important because only the intraperitoneal fat depots can be considered as 'portal' tissues which are associated with metabolic disorders.[3,4] In the study by Rössner et al.[6] it was found that the percentage of visceral fat as retroperitoneal fat was 41% and 25% in the two cadavers respectively.

In another validation study of magnetic resonance imaging (MRI)[7], dissection and chemical analysis of cross-sectional slices of 12 pigs were compared with 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. The distinction of measurements at the body, tissue, cellular, molecular or atomic level is of great importance as has been shown by Wang et al.[8] and is summarized for the techniques discussed in this review in Table 1.

Table 1. Overview of techniques and their level of measurement.

TECHNIQUE	LEVEL OF MEASUREMENT	WHAT IS MEASURED ?
Cadaver analysis	Tissue (dissection) or atomic (chemical analysis)	Separate intra-abdominal fat depots in grams of tissue (dissection) or grams of lipid (chemical analysis)
Computerized tomography	Tissue	Total intra-abdominal fat tissue area in cm^2 (with multiple scans integration to cm^3, and with assumptions to grams)
Magnetic resonance imaging	Atomic (spins of protons)	Total intra-abdominal tissue area in cm^2 (see CT)
Dual photon absorptiometry or dual energy X-ray absorptiometry	Molecular	Total abdominal fat mass as all fatty elements of abdominal soft tissues in grams
Ultrasound	Tissue	Intra-abdominal depth in cm
Anthropometry (diameters, circumferences, skinfolds)	Body	body dimensions (diameter in cm, circumferences in cm, and the subcutaneous fat layer by skinfolds in mm)

Computerized tomography (CT)

Computerized tomography is an imaging technique commonly used for diagnostic purposes in medicine but it is also suitable for measuring body composition. The CT system consists of an X-ray tube and detectors aligned at opposite poles of a circular gantry. The X-ray beam is rotated around the subject located in the centre of and perpendicular to the gantry, and information about the intensity of the attenuated X-ray beams is recorded and stored. The scanner computer reconstructs the information to give cross-sectional images. Clear differences in attenuation intervals between bone, adipose tissue and fat-free tissues make this technique appropriate for quantification of separate fat depots and for the assessment of whole body composition.[9-11]

A CT scan can be compared with a black and white newspaper photograph constructed from dots with varying shades of grey in a range from white to black. A typical scanner creates images that contain 256x256 'dots' (pixels in CT jargon). The pixels represent signal intensities, which are translated into shades of grey and scaled as CT-numbers in Hounsfield units (HU). The number of pixels (256x256) combined with a field of view of say 400 mm implies that one pixel covers 2.4 mm^2. 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. Figure 3 shows a region of interest with its accompanying histogram.

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 these pixels 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

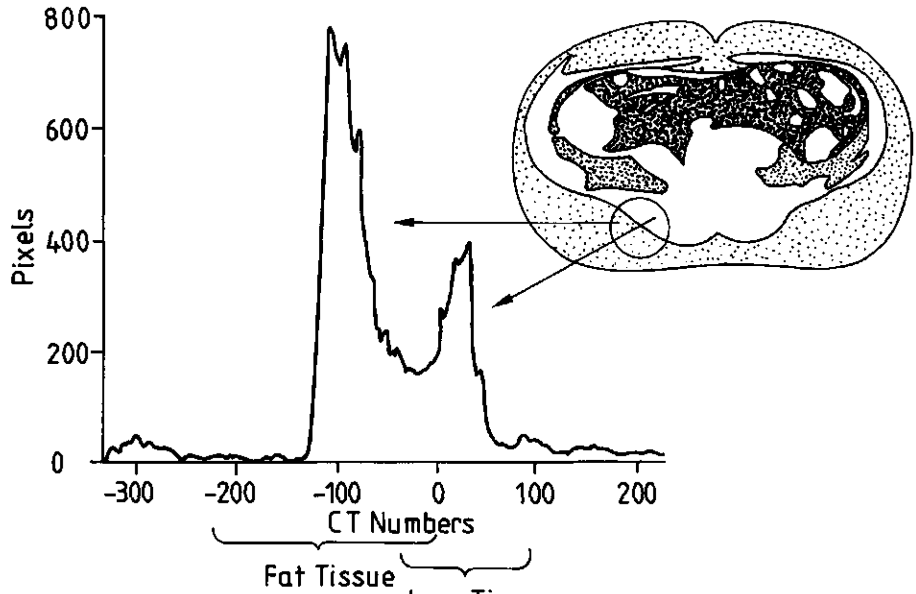


Figure 3. Schematic representation of a region of interest and its histogram of CT attenuation scores (Hounsfield units) obtained from an abdominal scan (adapted from Rössner et al.[6]).

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 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 the quantification of adipose tissue[9,12-14] and it may be that the limits vary with the type of scanner used and that they differ in different individuals.[15] 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.[6,13] Varying the upper limit (-30 HU) with ± 10 HU or varying the lower limit (-190 HU) with ± 10 HU resulted in a change in area determinations of 5-6% and <1% respectively.[6]

The validity of the technique has been studied by Rössner et al.[6] As discussed in the previous section, adipose tissue areas assessed planimetrically on frozen sections from cadavers were highly correlated with CT-assessed adipose tissue areas in the same slices (r coefficients around 0.90). CT has also been proven to be highly reproducible. The variability found for integrated total fat areas from multiple scans ranged from 0.6%[12] to 1.4%[16]. The reproducibility of visceral fat areas solely, calculated from single or multiple scans, has not been reported to our knowledge.

Technical problems that one may encounter when using CT for measurement of fat 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.[13], but this is a tedious procedure introducing unknown errors. Furthermore, it is difficult to make an accurate distinction between retroperitoneal and intraperitoneal fat areas using CT because the peritoneum is not visible.[6,15] Ashwell et al.[17] 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. Our experience is that on many scans the blood vessels and the colon boundaries are poorly visible.

The number of scans performed determines the duration of the procedure. One scan usually takes less than 10 seconds. A pre-scan 'scout' view allows the investigator to precisely define the anatomic location of each scan. An increasing number of scans is undoubtedly associated with higher precision of the assessment of the visceral fat volume, but also with increased exposure to ionizing radiation. Kvist et al.[12] reported that the radiation dose to skin surface in each slice was found to be 25-50 mSv and the effective dose equivalent for 22 scans was 2-4 mSv. Fortunately, visceral fat areas from a single scan taken at the level of L3-L4 or L4-L5 (approximately umbilicus level) were shown to be highly correlated to the total visceral fat volume (r coefficients >0.95).[18,19] This suggests that for many purposes it may suffice to make a single abdominal scan for visceral fat determinations.

Magnetic resonance imaging (MRI)

In magnetic resonance imaging, a subject is placed in a strong magnetic field and irradiated by radio frequency pulses. The emitted signals are collected by the MR receiver coil, stored in a computer and processed in an image in a fashion similar to CT. Magnetic field gradients are used to allow the information to be collected from spatially defined parts of the body.[20]

The emitted signal intensity is governed by the concentration and relaxation properties of water and fat protons in the tissues being imaged. Differences in relaxation times result in different signal intensities. The visualisation of adipose tissue by MRI is usually based on the fact that adipose tissue has a considerably shorter longitudinal relaxation time (T1) compared with other soft tissues with a higher water content.[20,21] Differences in resonance frequency between water and fat protons can also be used (chemical shift).[22]

In order to distinguish between adipose tissue and non-adipose tissues, MRI experiments have been designed which give an optimal contrast between tissues.[15,19,22-25] These experiments are characterized by specific sequences of radio pulses.[20] Table 2 gives a summary of some of the used sequences and parameters in this context.

Table 2. Sequences and accompanying parameters used for measurement of fat areas with MRI.

REFERENCE	FIELD STRENGTH SCANNER (tesla)	SEQUENCE:	*repetition time* (msec)	*echo-time* (msec)	*inversion time* (msec)
Seidell et al.[15]	1.5	Inversion-recovery	820	20	300
Fowler et al.[24]	0.08	Inversion recovery	370	?	170
Fowler et al.[7]	0.04	Saturation-recovery + Inversion recovery	1000	?	200
Gray et al.[25]	1.5	Spin-echo	516-533	17-20	-
Ross et al..[19]	1.5	Spin-echo	500	20	-
Staten et al.[23]	0.5	Spin-echo	500	17	-
Sobol et al.[22]	1.5	Water suppression	800	30	-

Sobol et al.[22] compared their sequence with the one used by Seidell et al.[15] and found that the fat areas determined by both sequences were very similar and highly correlated. In addition, useful results can be achieved with different magnetic field strengths, as shown in Table 2.

The emitted signal intensity is reflected in a degree of pixel shading and expressed in arbitrary units from a minimum value (black on reconstructed images) to a maximum value (white on reconstructed images). The range of arbitrary units that represents adipose tissue is usually determined from inspection of histograms of pixel intensities as described for CT.

The range of signal intensities that represents adipose tissue may vary between and within images. There may be differences between scans because the sensitivity of the receiver is optimized for every scan and may differ from scan to scan.[15,19] The translation of signal intensities is done for every scan sequence independently (relative scaling). The signal intensities associated with an 'adipose tissue signal' may differ within a scan because of system and object related imperfections (e.g. non-uniform excitation and signal detection, magnetic field inhomogeneity, flow in and movements of object that is scanned and partial volume effects)[15,19,22-26] For all these reasons, the ranges have to be determined visually for each image by selecting the level producing the best fat and non-fat delineation. This procedure is subject to measurement error and may contribute to the inter-observer variability of the technique.

The acquisition time for one scan depends mainly upon 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 0.5 T [23], whereas an inversion-recovery sequence may take 10 minutes by 1.5 T .[15] Movements of the abdomen due to breathing and intestinal movements are a source of error when using scan sequences with a relatively long acquisition time (increasing the partial volume effects). This may be a problem, in particularly, for the assessment of visceral fat. The reproducibility of visceral fat areas measured by MRI (coefficient of variation (CV)) of about 10-15%[15] is due to the potential measurement errors as mentioned above, which are larger compared to measurement errors by CT.

The quantification of adipose tissue with MRI has been shown to be accurate in pigs.[7] MRI assessed percentage fat in slices of pigs correlated well with percentage fat of the same slices estimated by dissection and chemical analysis, as previously discussed. In two other studies, MRI was validated by comparing measurements of fat areas by MRI with those obtained by CT in human volunteers.[15,22] Although in both studies CT and MRI yield different absolute values for abdominal fat areas, the ranking of individuals on the basis of their fat areas was similar by both methods. As for CT, it is also difficult in MRI images to distinguish between retroperitoneal and intraperitoneal fat.[6,15]

Figure 4 shows abdominal MRI images of the same person before and after weight loss. In this figure, adipose tissue is represented by white areas.

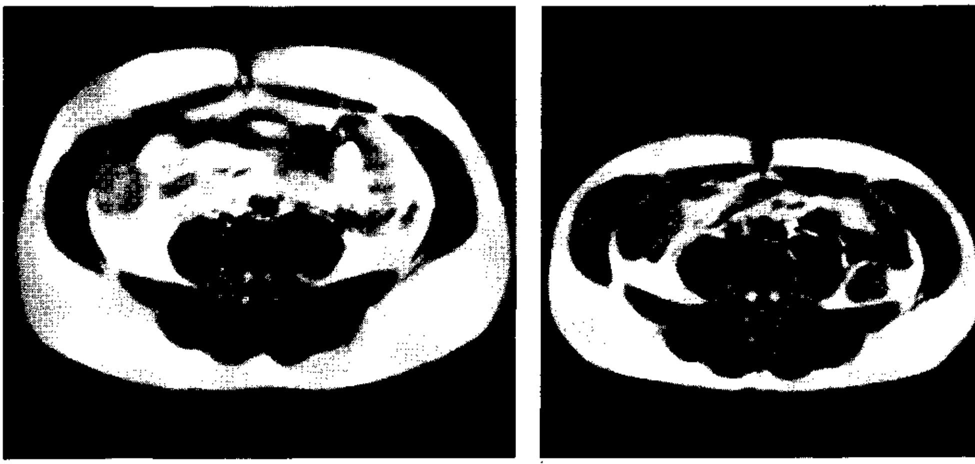


Figure 4. MRI scans at abdominal level before (left) and after weight loss (right).

Dual photon absorptiometry (DPA) and dual energy x-ray absorptiometry (DEXA)

Dual photon absorptiometry (DPA) and dual energy x-ray absorptiometry (DEXA) were originally designed to measure bone mineral content, and are still most widely used for this application. It has been shown that these methods may also be used to estimate the composition of soft tissue.[27]

Dual photon (153-Gd) absorptiometry (DPA) 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 (Figure 5).[28] 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 tissues.[27] 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 (DEXA) is based on a similar principle to DPA by using dual energy determination for soft-tissue. DEXA measurements are made with a scanner that uses a constant potential X-ray source at 12.5 fJ and a K-edge filter (cerium) to achieve a congruent beam of stable dual energy radiation.[29] The effective energies are 6.4 and 11.2 fJ. Whole body scanning from top to toe is done as described for DPA. Mazess and colleagues[29] reported scanning times of 10-20 minutes for the total body determination. In the same report these authors wrote that radiation doses were 0.05 and 0.10 μGy for the 10 and 20 minute scanning procedures respectively.

As for DPA, 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 with a calibration line based on

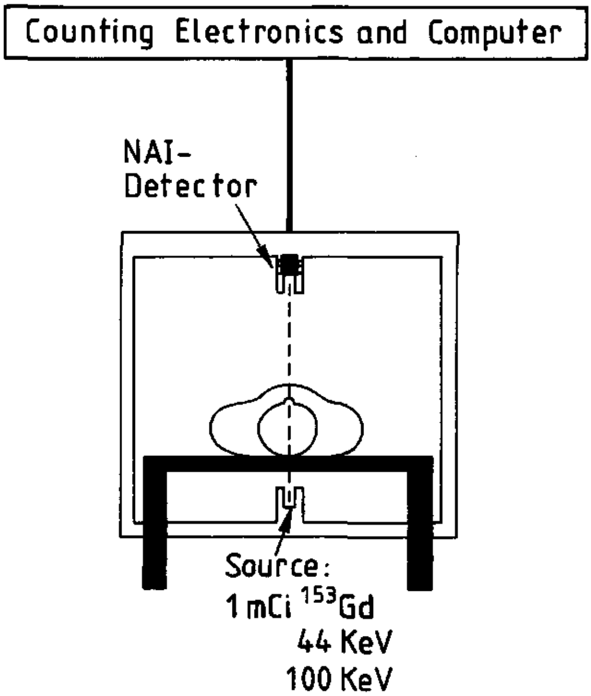


Figure 5. The experimental DPA set-up with the subject lying supine on the scan table with the source moving below and the detector above (adapted from Schlemmer et al.[28]).

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 reproducibility of the DPA measurement of the abdominal fat mass is about 12% (CV).[28] The DEXA method has been reported to have better reproducibility compared with DPA and is considerably faster as well.[29] A DEXA measurement does, however, involve X-ray exposure of subjects, including the thyroid and gonadal regions when scanning the whole body.

The measurement 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. In addition, a common disadvantage of the DPA and DEXA methods is that no distinction can be made between visceral and subcutaneous lipid, only information about the total abdominal fat mass can be obtained.

Ultrasound

Ultrasound allows sonographic imaging of the internal body. A beam of ultrasound penetrates the body and echoes reflect at tissue interfaces. An A-mode instrument uses a single beam of ultrasound resulting in a depth measurement. The B-mode developed later produces a two-dimensional picture of underlying tissue, representing tissue interfaces as bright lines or areas. Ultrasound instruments are portable and are therefore suitable for use in clinical and field applications. The time of one measurement is very short (several seconds). The assessment of relatively thick subcutaneous fat layers seems to be easier with ultrasound than with skinfold calipers.[30] However, the variability has been found to be comparable to the variability in skinfold measurements.[31-33] In addition, comparison of subcutaneous fat thickness assessed by imaging and ultrasound revealed that the absolute scores differed, but that within a site they were highly correlated.[31,33]

Armellini et al.[34] developed an ultrasound procedure to measure the intra-abdominal depth as an indicator of visceral fat. An ultrasound transducer of 3.5 MHz was used to assess this depth which is the distance from the internal face of the rectus abdominis muscle (linea alba) and the posterior wall of the aorta. The intra-abdominal depth was moderately associated with the amount of visceral fat as assessed by CT ($r = 0.67$).[34] In addition, changes of this depth after weight loss only explained 22% of the variance of the CT-assessed reduction in visceral fat.[35]

A difficulty and source of error of the ultrasound technique is that the tissue interfaces are not as clearly delineated as with imaging techniques[31,33] and the aorta is not always visible in the reconstructed images.[36] Consequently, the reproducibility and accuracy of the assessment of the intra-abdominal depth was found to be poor.[36] This method seems to be more appropriate to assess the thickness of subcutaneous fat layers.

Anthropometry

Anthropometric measurements are relatively easy to perform and cheap. They are therefore attractive for clinical settings and for use in epidemiological studies. Anthropometric measurements have been used to describe fat distribution or fat patterning for many decades. Historically the use of anthropometric measurements for this purpose shifted from skinfold thicknesses[37-40] to circumferences[41-44] and most recently abdominal diameters have been introduced as well.[18,45,46] 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.[18,19,45,47-50] 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. [46,51-53] In addition, the reported prediction formulae are not generally applicable across populations[50] and coefficients of variation of predicted visceral fat areas are on average 25%.[45,46] The advantages and disadvantages of the separate anthropometric measurements will be discussed.

Anthropometry: abdominal diameters

Sjöström and colleagues[18,54] 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 of visceral fat.[18,45,46,55] Figure 6 shows where the abdominal diameters are usually measured.

According to the above-mentioned principle,[18,54] 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. Measurements can also be made in standing subjects by a whole body caliper. Until now, in most studies, the diameters were not measured anthropometrically but derived from CT or MRI images (Figure 6).[18,45,46,55] 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).[46,50] Correlation coefficients between visceral fat area or volume and the sagittal diameter derived from the same scan, ranged between 0.46 to 0.96, with the lowest correlation coefficients in the most obese subjects.[18,45,46,50]

To our knowledge, 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%[56]). 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.[18,46] However, accurate assessment of the thickness of the subcutaneous fat layer is problematic with conventional anthropometric measurements such as skinfold thicknesses. Particularly in obese subjects, the measurement of abdominal skinfold thicknesses is not accurate or even not possible.[46,56,57] Combinations of ultrasound measurements of subcutaneous fat with abdominal diameters or circumferences may yield more satisfactory results.

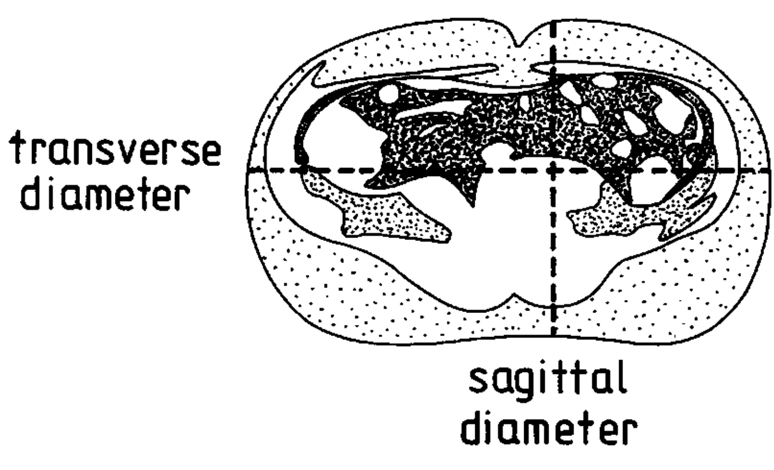


Figure 6. Sagittal and transverse abdominal diameters.

Anthropometry: circumferences

The most commonly used measure of fat distribution is the waist-to-hip circumference ratio. Ashwell et al.[58] and, several years later, Seidell et al.[59] 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 to visceral fat area were not always significant and sometimes the waist alone or the waist-to-thigh ratio were stronger correlates of visceral fat.[49,59]

The great advantage of circumference measurements is of course that they are relatively easy to perform. In addition, the reproducibility of circumference measurements was found to be good (CV about 2%).[56] The disadvantage of the waist circumference as well as waist-to-hip and waist-to-thigh ratio is that they do not allow accurate distinction between the amount of visceral fat and subcutaneous abdominal fat. Nevertheless, the use of waist-to-hip ratio as a diagnostic tool to distinguish between different types of fat distribution is extremely useful, especially, because of the appeal it now has to public health policy makers, as well as to the public itself.

The levels of circumference measurements reported in the literature vary considerably, in particular for waist level.[60] 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.[60-62] The World Health Organization recommended levels, based on skeletal reference points, for the measurement of waist, hip and thigh circumferences.[60,63] The recommended levels are indicated in Figure 7.

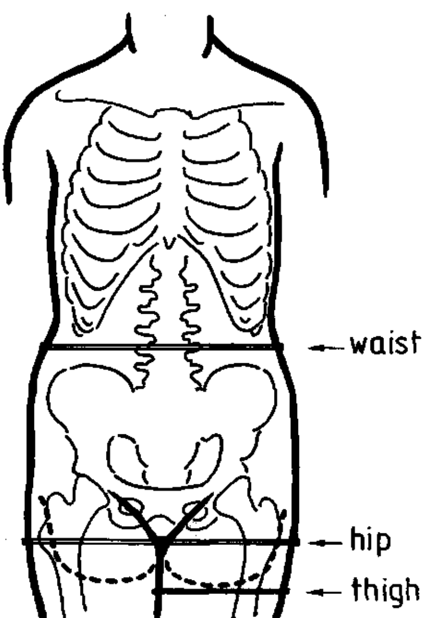


Figure 7.
Schematic representation of the levels at which waist, hip and thigh circumferences should be measured according to the recommendations of the WHO.

Waist: midway between the lower rib margin and the iliac crest;
Hip: widest circumference over the great trochanters;
Thigh: highest level at the level of the gluteal fold

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.[64,65] Kannel et al.[64] reported that the two ratios were correlated ($r=0.75$). The 'conicity' index, developed by Valdez[66], 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 circumference ratios.

Another advantage of circumference measurements is the possibility of instructing subjects to measure the circumferences themselves. The validity of self-reported circumferences appeared to be reasonable.[67,68]

Anthropometry: skinfolds

Skinfold measurements were originally used to assess the fat percentage of a body,[69,70] but they have also been used for the description of subcutaneous fat patterning by comparing skinfold thicknesses of different sites of the body.[37-40] Attempts to describe indirectly an abdominal fat distribution with ratios of extremity to trunk skinfolds revealed that waist circumference or waist circumference ratios were more strongly related to metabolic disorders associated with an abdominal fat distribution than the skinfold ratios.[61,71] 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.[72]

The most commonly used skinfold thicknesses to assess the subcutaneous fat patterning are the biceps and triceps skinfolds on the arm, and the supra-iliac, subscapular and para-umbilicalis skinfolds on the trunk. The coefficients of variation of repeated skinfold measurements by the same trained observer is approximately 5%,[69,70] and between observers 10-20%.[73,74] Factors affecting the reproducibility are the determination of the anatomical site for the measurement of a specific skinfold, the way of picking up a skinfold, time of scale reading, and last but not least, the experience of the observer.[69,70,73,74] In obese subjects, skinfold thicknesses are often difficult or impossible to measure 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.[56,57] 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.

Visceral fat and fat distribution assessment in children

Practically all the work on visceral fat determination has been performed in adults. CT imaging is usually no option in healthy children. MRI scanning may be problematic in small children because they have difficulty in lying motionless even for several minutes.

De Ridder et al.[75] showed that in early and late pubertal girls there was little visceral fat. In this study, conventional anthropometric measurements, such as waist-to-hip or waist-to-thigh ratio were not related to the visceral fat area. The waist circumference was significantly related to visceral fat but its correlation to the subcutaneous fat area was stronger. In addition, visual inspection of the scans suggests that the majority of visceral fat in children is retroperitoneal (unpublished observations).

Anthropometric measurements have frequently been used to measure fat distribution (waist-to-hip) or fat patterning (skinfold ratios) in children. Waist-to-hip ratio decreases with age, especially in girls, due to both an increase in pelvic diameters as well as predominant fat deposition in the gluteal area.[75-78] Despite the limitations for the interpretation of circumference ratios in growing children, waist-to-hip ratios have been shown to be correlated with plasma lipid concentrations and other potential risk factors of diseases associated with abdominal fat deposition.[79-81]

In children aged 12-17 years, Sangi and Mueller[82] propose that only among the sexually mature adolescents can fat distribution be separated from overall body fatness, which is in agreement with the findings of Deurenberg et al.[78] It is not clear which index (skinfolds or circumference ratios) is preferable in this age range.[82] It may be that they estimate different aspects of fat distribution.[78,82]

Although much work remains to be done we can tentatively conclude that visceral fat cannot be accurately assessed in prepubertal children from anthropometric measurements. Fat distribution probably becomes relevant only after puberty, but up till now it has seemed impossible to evaluate visceral fat accumulation without using imaging techniques such as CT or MRI.

Conclusions

Figure 2 and Table 3 (next page) summarize the accuracy, reproducibility, costs in terms of money and time, and safety of the various methods discussed.

Obviously, choosing the optimal method for a study is a trade-off between the different aspects of a study. For an epidemiological survey only anthropometric measurements such as circumferences and diameters, will be feasible. On the other hand, if the aim is to study changes in visceral fat accumulation in clinical or intervention studies there are no good alternatives to imaging techniques.[52,53] The choice between CT and MRI is usually is based on ethical problems; CT is clearly preferable to MRI in terms of accuracy, reproducibility, financial costs and scanning time, but it does involve X-ray exposure. If the aim of the study is primarily to rank or classify individuals according to types of fat distribution, anthropometric measurements may be adequate, especially if more precise and widely applicable regression equations can be developed for the prediction of visceral fat.[50]

Table 3. Some aspects of techniques used to measure visceral fat.

METHOD: WHAT IS MEASURED	REPRO-[1] DUCIBILITY	COSTS OF ONE MEASURE[2]	TIME OF ONE MEASURE	IONIZING RADIATION
Cadaver analysis: retro- and intra-peritoneal adipose tissue or lipid	-	Unknown	Several days	No
CT: visceral fat area	<2 %[3]	Expensive	10 seconds	Yes
MRI: visceral fat area	10-15 %	Very expensive	1-10 minutes	No
DPA : DEXA: total abdominal fat	< 12 % < 10 %	Expensive Expensive	50-90 10-20 minutes	Yes
US: intra-abdominal depth	10-15 %[4]	Moderate	10 seconds	No
Anthropometry: sagittal diameter waist circumference abdominal skinfold	 ? 2% 10-15 %	Cheap	30 seconds	No

CT, computerized tomography; MRI, magnetic resonance imaging; DPA, dual photon absorptiometry; DEXA, dual energy X-ray absorptiometry; US, ultrasound.
[1] Intra-observer variability; [2] Costs of one measurement (not including buying the apparatus);[3] Reproducibility for total body fat mass of multiple scans; [4] Reliability coefficient 85-90%.

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Chapter 4

Waist-hip ratio is a poor predictor of changes in visceral fat

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Abstract
Magnetic resonance imaging was used to study the effect of weight loss on three fat depots: the visceral and subcutaneous abdominal depots and the subcutaneous depot at trochanter level. Changes in fat depots were compared with changes in circumference measures and the waist-hip ratio (WHR) in obese men (n=38) and women (n=40). Mean weight loss was (mean ± SD) 12.9 ± 3.5 kg ($P<0.001$). The proportional reduction of fat was largest in the visceral depot (men 40%, women 33%). Less fat was lost subcutaneously, especially at trochanter level (men 29%, women 26%). WHR decreased significantly in both sexes ($P<0.001$). Change in WHR was not significantly related to the absolute reduction in visceral fat. Total body-fat loss showed a stronger association with subcutaneous fat loss than with visceral fat loss. The findings suggest that fat distribution may change with weight loss, particularly by the loss of visceral fat, but changes in WHR are not appropriate for evaluating changes in this fat depot.

Introduction
An abundance of visceral fat is a stronger predictor of cardiovascular disease and other metabolic complications of obesity, such as non-insulin-dependent diabetes, than overall fatness.[1-3] To counteract the adverse health consequences of a predominance of fat accumulated in the abdomen, it is important to know whether weight loss is accompanied by a reduction of this fat depot. Several groups have investigated the effect of weight loss on fat distribution, usually assessed by anthropometric measurements, but the results are contradictory.[4-14] This might be due to the methods used to define and estimate fat distribution[8,11,14-16], the amount of weight loss[3] or the characteristics of the study population.[16] Two studies[5,8] included both men and women, in the other experiments only women were examined.

Recently, results of a magnetic resonance imaging (MRI) study[13] and computerized tomography (CT) studies[11,12,14] were reported in which various fat depots were estimated in obese women before and after weight loss. The results of these imaging studies showed that abdominal fat depots, especially the visceral depot, were reduced with weight loss. These observations are in agreement with findings from *in-vitro* experiments that showed that abdominal adipocytes are more lipolytically active than gluteal and femoral adipocytes.[17,18]

The aim of this study was to investigate the effect of weight loss in obese women as well as in obese men on three fat depots: the visceral and subcutaneous abdominal depots, and the subcutaneous depot at trochanter level. Magnetic resonance imaging was used to estimate the changes in fat depots, which were then compared with changes in circumference measurements and the waist-hip ratio. In previous experiments MRI was found to be an appropriate technique for the estimation of fat distribution.[13,19-21] It has the advantages over CT scanning of not exposing subjects to ionizing radiation and the absence of beam-hardening effects when measuring areas containing cortical bone, such as thigh and hips.

Subjects and methods

Subjects

Participants were recruited by advertisements in local newspapers. Ninety-six obese subjects, 48 men and 48 premenopausal women aged between 25 and 51 years, were selected for the weight loss study when their body mass index (BMI: in kg/m^2) was between 28 and 38 and after a medical examination ascertained their healthiness. Subjects with an abdominal and gluteal-femoral fat distribution, based on WHR, were matched for BMI and age in each sex. Before treatment all subjects gave their written informed consent to participate in the study, which was approved by the Medical Ethical Committee of the Department of Human Nutrition. Eight subjects withdrew because of intercurrent illness unrelated to the intervention (2 men, 1 woman) or for personal reasons (2 men, 3 women). Results from two men were excluded from statistical analysis because of possibly poor compliance with the dietary regimen.

Diet

The weight loss treatment consisted of a 4.2 MJ/day energy deficit diet (42% of energy as carbohydrate, 25% as protein and 33% as fat) for 13 weeks. The food composition of the diet was calculated by using the Dutch computerized food composition table.[22] Special dieting products and ordinary foodstuffs were combined in the diet. Individual energy deficits were based on estimated daily energy requirements calculated from resting metabolic rate in combination with the physical activity pattern[23] and on the energy intake in a 3-week weight-stable period preceding the weight loss treatment. Energy intakes in the weight-stable period were adjusted to prevent weight changes when needed. Ninety-five percent of the energy of the food was provided by the Department during the whole study. The remaining 5% was chosen from a list of products free of fat and cholesterol. Throughout the study compliance was checked by dieticians. The participants were asked to report illness, medication, deviations from the diet and habitual activity pattern, and the free food items they had chosen in a diary. They visited one of the dieticians every two weeks. During the visit body weight was measured and the diary checked.

Body composition

Body composition was assessed before and after weight loss. Body weight was measured to the nearest 0.05 kg with a digital scale (Berkel ED60-T, Rotterdam, The Netherlands) with the subjects wearing swimming gear. Height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. BMI was calculated as weight (kg) divided by height squared (m^2). Whole body density was determined by underwater weighing[24] with simultaneous measurement of the residual lung volume by a helium-dilution technique.[25] The measurements were done in quadruplicate and the average density was used for the assessment of the percentage body fat, which was calculated according to Siri's formula.[24] Percentage body fat of two women was determined from weight and total body water assessed by deuterium oxide dilution, by assuming 73.2% of the fat-free mass to be water.[26] These women were afraid of complete immersion.

Fat distribution

Body-fat distribution was assessed by circumference measures and MRI scanning before and after the weight-loss treatment. Waist circumference was measured midway between the lower rib margin and the iliac crest at the end of a gentle expiration. The hip circumference was measured at the level of the widest circumference over the great trochanters. The circumferences were measured to the nearest 0.1 cm with the subject standing upright. Waist-hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

MRI scans were performed with a whole-body scanner (GYROSCAN S15, Philips Medical Systems, Best, The Netherlands) with a 1.5 T magnetic field (64 MHz) by using an inversion recovery pulse sequence (inversion time 300 ms, repetition time 820 ms, and echo time 20 ms).[19] Slice thickness was 10 mm. Two anatomic locations were chosen comparable with the levels where waist and hip circumferences were measured. A transverse scan was taken halfway between the lower rib margin and the iliac crest (abdominal level) with the subjects lying supine. This site was determined by palpation and for most subjects similar to the umbilicus level and L4-L5 region. During the experiments, however, it appeared that some subjects moved a little or that the site was misjudged. As a consequence, abdominal scans of four men and four women differed in location before compared with after weight loss. These subjects were excluded from statistical analysis to separate the effect of weight loss from measurement error.

In a subgroup of 26 men and 24 women, a MRI scan at the level of the great trochanters (hip level) was performed as well. No statistically significant differences were found in baseline characteristics between the subgroup in whom hip-scans were made and the remaining group (unpaired t-tests).

Image analysis to determine the fat areas in squared centimeters was carried out as described by Seidell et al.[19] The visceral-subcutaneous fat ratio (VSR) was calculated as the visceral-fat area divided by the subcutaneous-fat area at abdominal level. Previous studies showed that the visceral-fat area assessed from a single scan taken at L4-L5 level was highly correlated to the volume of visceral fat estimated from multiple scans ($r>0.95$).[27,28]

The reproducibility of the fat-area determinations was assessed by repeating the estimation of the visceral- and subcutaneous-fat areas in a random sample of 37 abdominal scans before and 45 abdominal scans after weight loss. The reproducibility expressed as coefficients of variation (two-way analysis of variance), was 5.0% for the visceral-fat area before weight loss and 5.7% after weight loss, and 2.2% for the subcutaneous-fat area before weight loss, and 2.0% after weight loss.

Statistics
Results from 78 subjects were used for statistical analysis. In women, the distributions of the visceral-fat area before and after weight loss and the change in visceral fat were slightly skewed. Transformations were applied but this resulted in findings principally identical to analyses based on variables not transformed. Therefore, results of analyses without transformation are presented.

Effects of weight loss on variables were tested by using paired t-tests, whereas differences between the sexes were tested by unpaired t-tests. Pearson's product-moment correlation coefficients and multiple-regression analyses were performed to assess the relationships between the different fat areas and the circumference measures, including the WHR, and between changes in fat areas and changes in circumference measures, WHR, and loss of weight and fat mass. Mean percentage changes were calculated as the average of individual changes. Two-tailed P values less than 0.05 were considered to indicate statistical significance. Results are expressed as mean ± SD. The analyses were performed on the SAS statistical package (SAS Institute, Cary, NC, USA).

Results
Body composition and fat distribution before weight loss
Initial body-composition and fat-distribution variables of the subjects are given in Table 1. The men had on average more visceral fat, a higher VSR and a higher WHR compared with women. The women, on the other hand, had more subcutaneous fat at abdominal and hip level and a wider hip circumference, illustrating the known phenomenon that men and women store fat differently. The associations between abdominal-fat areas, and age and anthropometric variables before weight loss are given in Table 2. In men but not in women, the WHR was correlated with the subcutaneous abdominal fat depot and only in women was WHR positively associated with the VSR. Hip circumference was positively correlated with the subcutaneous fat depot on hip level ($r=0.57$ in men and $r=0.87$ in women, $P<0.01$).

Changes in body composition and fat distribution after weight loss
Men and women lost similar amounts of weight and fat mass. The changes in body composition and fat distribution variables are given in Table 1. The relative decreases in fat areas, expressed as a percentage of their initial level, are illustrated in Figure 1. Men lost significantly more visceral fat than women in absolute areas (61 cm^2 *versus* 37 cm^2, $P<0.01$), but at borderline significance in relative areas (39.8% *versus* 33.3%, $P=0.07$). As shown in Figure 1, within each sex the percentage change of the visceral fat

Table 1. Body-composition and fat-distribution variables at baseline and after weight loss*.

Variables		Initial values				Decreases from initial values			
		Women		Men		Women		Men	
		mean	SD	mean	SD	mean	SD	mean	SD
Age	(years)	39	6	40	6	-		-	
Body mass index	(kg/m^2)	31.3	2.3	30.7	2.3	4.6	1.4 §	4.2	1.0 §
Weight	(kg)	86.5	8.7	98.3	7.2	12.6	3.9 §	13.3	3.0 §
Fat mass	(kg)	37.9	6.5	32.6	5.3	10.9	3.0 §	10.3	2.7 §
Waist circumference	(cm)	99.2	7.4	107.6	6.5	12.0	4.6 §	14.6	3.8 §
Hip circumference	(cm)	113.7	6.6	109.4	3.8	8.8	3.2 §	6.8	2.1 §
Waist-hip ratio		0.87	0.07	0.98	0.05	0.04	0.04§	0.08	0.03§
Visceral fat	(cm^2)	106	50	154	40	37	29 §	61	25 §
Subcutaneous abdominal fat	(cm^2)	395	102	318	67	118	56 §	110	45 §
Subcutaneous hip fat†	(cm^2)	418	72	261	43	109	33 §	74	22 §
Visceral-subcutaneous fat ratio‡		0.28	0.14	0.50	0.16	0.02	0.07	0.05	0.10‖

* n=40 women, 38 men;

† Subcutaneous hip fat was measured in a subgroup of the population, n=24 women, 26 men;

‡ Abdominal visceral-subcutaneous fat ratio;

Significant change after weight loss, § $P<0.001$, ‖ $P<0.01$.

Table 2. Pearson correlation coefficients between abdominal-fat areas, and age and anthropometric variables before weight loss.

		Visceral fat (cm²)	Subcutaneous abdominal fat (cm²)	Visceral-subcutaneous fat ratio
Women (n=40)				
Age	(years)	0.42 ‡	-0.08	0.39 §
Body mass index	(kg/m²)	0.38 §	0.52 †	0.13
Total fat mass	(kg)	0.29	0.66 †	-0.04
Waist circumference	(cm)	0.64 †	0.53 †	0.31
Hip circumference	(cm)	-0.15	0.53 †	-0.33 §
Waist-hip ratio		0.68 †	0.11	0.51 †
Men (n=38)				
Age	(years)	0.44 ‡	0.01	0.39 §
Body mass index	(kg/m²)	0.42 ‡	0.66 †	-0.06
Total fat mass	(kg)	0.56 †	0.71 †	0.02
Waist circumference	(cm)	0.64 †	0.68 †	0.10
Hip circumference	(cm)	0.25	0.53 †	-0.10
Waist-hip ratio		0.56 †	0.42 ‡	0.17

† $P<0.001$; ‡ $P<0.01$; § $P<0.05$

depot was larger compared with the two subcutaneous depots, with the smallest decrease in the subcutaneous depot at hip level. A similar comparison was made after dividing men and women separately into groups with initially low and high levels of visceral fat (below and above the median initial visceral fat area, for women 95 cm^2 and for men 155 cm^2). Men and women with relatively high amounts of visceral fat showed a similar ranking in relative changes in fat depots compared with the total group. In men and women with relatively low amounts of visceral fat, the difference between the relative changes in visceral and subcutaneous abdominal fat disappeared, but the changes were still larger than the relative change in subcutaneous hip fat (results not shown).

Relationships between changes in fat areas as measured by MRI and changes in waist and hip circumferences and WHR are presented in Table 3. Despite a significant change in WHR with weight loss and a positive correlation between initial amount of visceral fat and WHR, the change in visceral fat showed no statistically significant correlation with change in WHR in either sex. The strongest associations were found between the reductions in the two subcutaneous depots and the changes in waist and hip circumferences.

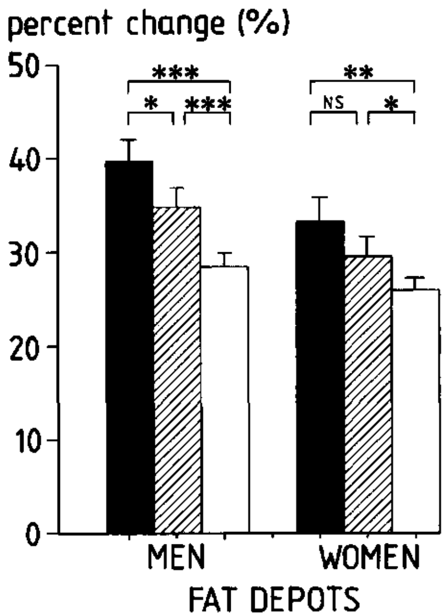

Figure 1. Percentage change in visceral (black bars), subcutaneous abdominal (hatched bars) and subcutaneous hip fat areas (open bars) (mean ± SEM). * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Test between %-change in visceral fat and in subcutaneous abdominal fat, n=38 men and n=40 women. Tests between %-change in subcutaneous hip fat and the other fat depots, n=26 men and n=24 women.

Table 3. Pearson correlation coefficients between changes in fat areas (cm^2) and changes in waist and hip circumferences (cm) and the waist-hip ratio*.

	Change in:		
Change in:	Waist circumference	Hip circumference	Waist-hip ratio
Women (n=40)			
Visceral fat	0.14	-0.14	0.21
Subcutaneous abdominal fat	0.58 †	0.68 †	0.23
Subcutaneous hip fat*	0.52 ‡	0.70 †	0.06
Men (n=38)			
Visceral fat	0.33 §	0.27	0.18
Subcutaneous abdominal fat	0.63 †	0.61 †	0.37 §
Subcutaneous hip fat*	0.49 §	0.46 §	0.24

* Subcutaneous hip fat was measured in a subgroup of the population, n=24 women, 26 men;† $P<0.001$; ‡ $P<0.01$; § $P<0.05$.

In men and women the loss of fat in the three depots was related to the initial fatness of each depot, with the strongest correlation being between change in visceral fat and its initial fat amount (Table 4). Regression analysis showed that the initial amount of visceral fat accounted for 60% of the variance in reduction of visceral fat in women and for 37% of the variance in men. Table 4 gives the correlation coefficients between fat areas and reductions in weight and fat mass with and without adjustment for the initial amount of fat for each depot separately. The reductions in the two subcutaneous fat depots correlated with each other ($r=0.68$ in men and $r=0.49$ in women, $P<0.01$) and they showed stronger positive relations with weight loss and fat loss than change in visceral fat did, particularly in women.

Table 4. Pearson correlation coefficients between changes in fat areas (cm^2) and their initial amounts and between changes in fat areas, and loss of weight (kg) and fat mass (kg) with and without adjustment for the initial fat area*†.

		Change in:			
		Weight		Fat mass	
Change in:	Initial fat area per depot	Adj*.	*N*Adj*.	Adj*.	*N*Adj*.
Women (n=40)					
Visceral fat	0.78 ‡	0.29	0.14	0.37 ‖	0.22
Subcutaneous abdominal fat	0.50 ‡	0.75 ‡	0.80 ‡	0.74 ‡	0.79 ‡
Subcutaneous hip fat†	0.57 §	0.69 ‡	0.72 ‡	0.65 ‡	0.70 ‡
Men (n=38)					
Visceral fat	0.61 ‡	0.62 ‡	0.44 §	0.54 ‡	0.36 ‖
Subcutaneous abdominal fat	0.43 §	0.70 ‡	0.70 ‡	0.67 ‡	0.72 ‡
Subcutaneous hip fat†	0.39 ‖	0.68 ‡	0.59 §	0.69 ‡	0.69 ‡

* Adj.= adjustment for initial fat area, *N*Adj.= no adjustment for initial fat area;
† Subcutaneous hip fat was measured in a subgroup of the population, n=24 women, 26 men; ‡ $P<0.001$; § $P<0.01$; ‖ $P<0.05$.

Discussion

Two major findings emerged from this study. First, in men as well as in women, the proportional reduction of visceral fat is larger than the reduction of subcutaneous fat, especially compared with subcutaneous fat loss at trochanter level. This suggests a preferential reduction in abdominal fat, which is associated with reduced health risks.[2,6,9,12] Second, the changes in visceral fat are not related to the changes in waist-hip ratio.

The baseline values of the fat areas as assessed by MRI in this study were similar to those of other imaging studies, with mean visceral-subcutaneous fat ratios at abdominal level varying from 0.53 to 0.72 in lean and moderately obese men[4,29,30] and from 0.22 to 0.33 in overweight and obese women.[4,13,14] The reproducibility of the fat-area determination in obese subjects before and after weight loss was comparable with other studies as well.[13,21] Comparison of the present study with a MRI-CT validation study[19] showed that the reproducibility is, as expected, better in subjects with a higher percentage body fat. This is in agreement with the results of Staten et al.[21]

The finding that the size of the visceral fat depot decreased relatively more compared with the subcutaneous depots is in accordance with the results of previous imaging studies.[11-14] In those studies, however, only women varying in age were investigated. Stallone et al.[14] examined only postmenopausal women. The postmenopausal women had considerably more visceral fat than the premenopausal women in the present study, although their mean percentage body fat was almost similar. The postmenopausal women also had a greater average loss of visceral fat and body weight than the premenopausal women in this study. According to the results of the present study, body-weight loss is only weakly related to visceral fat reduction. The loss of visceral fat seemed to be predominantly determined by the initial fatness of the visceral depot, which was also observed by Gray et al.[13] The differences between post- and premenopausal women may be explained by hormonal status and age dependency of visceral fat accumulation.[16,18,31,32] Differences in hormonal status between men and women probably also reveal why men have a larger visceral fat depot[17,18] and reduced relatively more in visceral fat than women did in this study.[16]

The relatively large reduction of visceral fat may be partly the result of the higher lipolytic capacity of this abdominal fat depot compared with subcutaneous depots.[17,18] In this study, nevertheless, the difference between the proportional reduction in visceral and subcutaneous fat at abdominal level was small but significant in men, whereas in women no statistically significant difference was found. In men as well as in women, larger differences in relative reductions were found between the visceral fat depot and the subcutaneous depot at hip level and between the two subcutaneous depots. A diminished lipolytic activity in the gluteal and femoral adipocytes compared with abdominal adipocytes may be responsible for these results.[16-18,31]

In contrast to the other imaging studies[13,14], we found that weight and fat loss were better predictors of change in subcutaneous fat than change in visceral fat. This was more pronounced in women than in men after adjustment for initial fat amount per depot. These findings might be in accordance with an overfeeding study in twins[33], in which it was observed that increases in total body fat correlated with gains in truncal subcutaneous fat as measured by skinfold thicknesses, and not with gains in visceral fat as measured by CT. This comparison should be viewed cautiously because other physiological processes are involved during weight gain compared with weight loss. Besides, the overfed twins were young lean men with little visceral fat (54 cm^2 after overfeeding).

There may be differences in adaptation in lipoprotein lipase activity and lipolyse activity in adipose tissues at the various sites of the body in response to energy restriction.[17,34] This may cause different rates of fat loss in the different fat depots and, possibly also during the time course of intervention. Differences in energy-restricted diets and duration of dieting could possibly explain the discrepancies in relationships of visceral fat loss with loss in weight and fat mass between this study and other imaging studies.[13,14]

WHR is an accepted index of fat distribution in cross-sectional studies, however, its use to assess changes in fat distribution is questionable.[8,10,11,15] The lack of an association between change in WHR and change in visceral fat may have important implications for the interpretation of studies that failed[4,8] or found only small changes (<7%) in the WHR[5-7,9,10] after weight reduction. The relative stability of the WHR after weight loss may be explained by: 1) the sites where waist and hip circumferences were measured[8,15,16]; 2) a relatively small weight loss, less than 10 kg[3]; 3) an insufficient number of subjects to detect the relatively small changes in circumference measures and their ratio; and 4) the initial fat pattern of the study population.[16] Wadden et al.[10] found in a sample of 68 obese women a small but significant change in WHR, with the largest reductions in subjects with a high initial WHR. This latter finding was also observed in experiments with relatively small numbers of subjects (n<25) in which the populations were divided in groups with high and low WHRs.[5-7,9] At the group level, a reduction in WHR might be interpreted as a reduction in visceral fat. However, the present study showed that evaluation of changes in the WHR at the individual level are not predictive of changes in the visceral fat depot.

There is only limited knowledge about lipolytic activity of the separate visceral fat depots (mesenteric, omental, i.e. 'portal fat', and retroperitoneal fat). The omental and mesenteric fat cells seem to have an elevated lipolytic activity compared with retroperitoneal and subcutaneous abdominal adipocytes in *in-vitro* experiments.[17,18] It is not, however, possible to make an accurate distinction between these depots in MRI images.[4,19] Therefore, no conclusions can be drawn here about reductions in the different visceral depots. It may be assumed that the large reduction in the total visceral fat depot (>30%) also reflects a decrease in portal fat. The reduction of portal fat is considered to be favorable in terms of changes in risk factors.[1,2]

This study has demonstrated that fat distribution may change in obese subjects with weight loss, particularly in those with abdominal obesity, by a relatively larger reduction of visceral fat compared with subcutaneous fat. This potential beneficial change in fat distribution is relevant for the treatment of abdominal obesity. Furthermore, changes in waist-hip ratio are not appropriate for the evaluation of changes in visceral fat. More specific anthropometric measures have to be developed for this purpose because imaging techniques are scarcely available and impractical in population studies.[27,28]

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Chapter 5

Abdominal diameters as indicators of visceral fat: comparison between magnetic resonance imaging and anthropometry

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Abstract

The aim of this study was to investigate the usefulness of abdominal diameters to indicate visceral fat, their relationship with serum lipids and their capability of detecting changes in visceral fat. Before and after weight loss, visceral and subcutaneous abdominal fat and the sagittal and transverse abdominal diameters were measured by magnetic resonance imaging (MRI) in 47 obese men and 47 premenopausal obese women with an initial body mass index of 31.0 ± 2.4 kg/m^2 (mean ± SD). In a subsample (n=21), diameters were also measured by anthropometry in standing and in supine position. They were strongly correlated with the diameters derived from the MRI scans. Serum levels of total- and HDL-cholesterol and triglyceride were measured before weight loss. In men but not in women, the sagittal diameter was correlated with serum lipids. In women, the sagittal diameter correlated less strongly with visceral fat than anthropometrically measured waist circumference and waist/hip ratio (WHR). In men, these associations were almost similar. Changes in visceral fat with weight loss were more strongly correlated with changes in the sagittal diameter and sagittal/transverse diameter ratio (STR) than with changes in waist circumference or WHR in men. In women, changes in the anthropometric variables and the separate diameters were not associated with visceral fat loss, except for STR. It is concluded that the sagittal diameter and STR may have advantages over waist circumference and WHR in men, particularly in assessing changes in visceral fat, but this could not be demonstrated in women. The ability to predict visceral fat from circumferences and diameters or their ratios is, anyhow, limited in obese men and women.

Introduction

Body fat distribution is an important feature to consider in the associations between obesity and cardiovascular disease[1,2] and between obesity and metabolic complications such as insulin resistance, hyperinsulinemia and diabetes mellitus.[3] The amount of visceral fat plays a critical role in the relationships between regional fat distribution and metabolic complications.[1-4]

Imaging techniques like computerized tomography (CT) and magnetic resonance imaging (MRI) allow a precise and reliable measurement of visceral fat.[5-7] However, these imaging techniques are expensive, not generally available and, in case of CT, expose subjects to ionizing radiation. Estimation of the amount of visceral fat from simple anthropometric measurements would therefore be useful in clinical settings and also in epidemiological research studying the hazards of 'visceral obesity'. A common and simple method for the assessment of fat distribution is the determination of the waist/hip ratio (WHR). This ratio was found to be more strongly related to visceral fat than to subcutaneous fat.[8,9] Nevertheless, the WHR has limitations because it is not able to distinguish quantitatively between subcutaneous and visceral abdominal fat[10,11], and its use to assess changes in visceral fat is controversial.[11,12] It has been suggested that the abdominal diameters, in particular the sagittal diameter, represent good predictors of the amount of visceral fat.[13]

The aim of the present study was to investigate the usefulness of abdominal diameters as predictors of visceral fat in comparison with waist circumference and WHR in obese men and women. The relationships between abdominal diameters and serum lipids and the ability of the diameters to detect changes in visceral fat were studied in this population as well.

The results suggest that the sagittal diameter is comparable to waist circumference and WHR as indicator of visceral fat in obese men. In obese women, however, waist circumference and WHR seem to be better indicators than the diameters or their ratio. For the assessment of changes in visceral fat, the sagittal diameter in men and the diameter ratio in both sexes were superior to waist circumference and WHR. Overall, the circumferences, the diameters or their ratios were no more than rough indicators of visceral fat.

Subjects and methods

Subjects and study design

Ninety-six obese subjects, 48 men and 48 premenopausal women aged between 25 and 51 years, were selected for the study. The subjects were apparently healthy, as assessed by a medical history, a physical examination, a blood screening and an urine test. They had a body mass index (BMI) between 28 and 38 kg/m^2. All volunteers gave their written informed consent to participate in the study that was approved by the Medical Ethical Committee of the Department of Human Nutrition.

Before the weight loss treatment, body composition and anthropometric measurements were performed in the same week that blood samples were taken. The MRI scans were performed within five weeks after body composition was assessed. Body weight was kept stable during this five week period. Data of one man were missing due to illness and data of one woman could not be used because of technical problems with MRI scanning.

Weight loss was achieved by using a 4.2 MJ/day energy deficit diet (42 energy percentage (E%) carbohydrate, 25 E% protein and 33 E% fat) for 13 weeks. The composition of the diet was calculated using the Dutch computerized food composition table.[14] Special slimming products and ordinary foodstuffs were combined in the diet. Individual energy deficits were based on estimated daily energy requirements calculated from resting metabolic rate measured by a ventilated hood system, in combination with the physical activity pattern.[15] Throughout the study, dietary compliance was checked by dieticians by means of interviews and measuring body weight every two weeks. After weight loss treatment, MRI scans, body composition and circumference measurements were repeated.

Seventy-eight of the subjects (40 women, 38 men) completed all parts of the weight loss programme successfully. Seven subjects withdrew due to intercurrent illness unrelated to the intervention (1 man, 1 woman) or for personal reasons (2 men, 3 women). Results of two men were excluded because of suspicion of poor dietary compliance. In addition, in seven subjects (4 men, 3 women) only the MRI scans before weight loss could be used due to measurement errors after weight loss (see description MRI).

Magnetic resonance imaging

MRI scans were performed with a whole-body scanner (GYROSCAN S15, Philips Medical Systems, Best, The Netherlands) with a 1.5 T magnetic field (64 MHz) by using an inversion recovery pulse sequence (inversion time 300 ms, repetition time 820 ms, and echo time 20 ms).[6] Slice thickness was 10 mm. The performance of one measurement took 10 minutes. One single transverse scan was taken halfway between the lower rib margin and the iliac crest with the subjects lying supine. This site was determined by palpation and the location was approximately on the L4-L5 level. During the experiments, however, it appeared that some subjects moved a little or that the site was misjudged. As a consequence, abdominal scans of seven subjects differed from location before compared with after weight loss. These subjects were excluded from statistical analysis to separate the effect of weight loss from measurement error.

Image analyses to determine the abdominal fat areas were carried out as described by Seidell et al.[6] The reproducibility of the fat area determination was assessed by repeating the estimation of the visceral and subcutaneous fat areas in a random sample of 37 abdominal scans before and 45 abdominal scans after weight loss. The reproducibility, expressed as coefficient of variation (two-way analysis of variance), was 5.0% for the visceral fat area before weight loss and 5.7% after weight loss, and for the subcutaneous fat area 2.2% before weight loss, and 2.0% after weight loss. The sagittal and transverse diameter were obtained from the MRI scan as described by Kvist et al.[13]

Body composition and anthropometry

Body weight was measured to the nearest 0.05 kg using a digital scale and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer with the subjects wearing a swimming suit. BMI was calculated as weight (kg) divided by height (m^2).

Waist circumference was measured midway between the lower rib margin and the iliac crest and hip circumference was measured at the level of the widest circumference over the great trochanters. Both circumferences were measured at the end of a gentle expiration while subjects were standing. The variability of duplicate measurements in a subsample of the population (n=46) of the waist and hip circumference was 1.4% and 0.7% respectively.

Whole body density was determined by underwater weighing[16] with simultaneous measurement of the residual lung volume by a helium dilution technique.[17] The measurements were done four times and the average density was used for the calculation of the percentage body fat.[16] Percentage body fat of two women was determined from body weight and total body water as assessed by deuterium oxide dilution assuming 73.2% of the fat-free mass to be water.[18] These two women were afraid of complete immersion. Comparison between densitometry and the deuterium oxide dilution technique in this population showed good agreement.[19]

Three skinfold measurements (Harpenden skinfold caliper ,Holtain Ltd. Crymmych, UK) were taken: the supra-iliac and the subscapular skinfold[20] and the para-umbilicalis.[9] The variability of these skinfold measurements in a subsample of the population ($n=39$) was 6.6%, 5.0% and 6.7% respectively. The supra-iliac skinfold thickness could not be measured in four subjects before weight loss, because the skinfold thickness exceeded the wide of the caliper (> 45 mm). The subscapular skinfold measurement in four subjects and the para-umbilicalis skinfold measurement in eight subjects were missing for the same reason.

In a subsample of twenty-one subjects (10 men, 11 women) the abdominal diameters were, besides by MRI, also assessed by anthropometry in both a standing and supine position. The standing diameters and supine transverse diameter were measured by a caliper. The supine sagittal diameter was determined by a stadiometer as the distance between abdomen and back while lying on a couch. All diameters were assessed at the end of a gentle expiration at the same level as where the MRI-scan was taken (halfway between the lower rib margin and the iliac crest) and this site was determined by palpation. Reproducibility of the anthropometric diameters was not assessed.

Serum lipids

Two blood samples were taken with an interval of two days after an overnight fast. The mean value of the two samples was used in statistical analysis. Total serum cholesterol and high-density-lipoprotein (HDL)-cholesterol after precipitation by dextran sulfate-Mg^{2+} [21], were determined using an enzymatic method.[22] Low-density-lipoprotein (LDL)-cholesterol was calculated using the equation of Friedewald.[23] Serum triglyceride was determined as described by Sullivan et al.[24] The within-run coefficient of variation of control sera was 1.4% for total cholesterol, 1.6% for HDL-cholesterol and 1.7% for triglycerides. Accuracy for total cholesterol and triglycerides was checked by analysis of serum pools of known value provided by the U.S. Centers of Disease Control (Atlanta, GA, USA). The mean bias with regard to these target values was +0.13 mmol/l for total cholesterol and -0.02 mmol/l for triglycerides. Accuracy for HDL-cholesterol was checked by serum pools of known value produced by Solomon Park Research (Kirkland, WA, USA). The mean bias with regard to the target value was +0.08 mmol/l HDL-cholesterol.[25]

Results of blood analyses of three subjects were excluded from statistical analysis because one man and one woman were diagnosed as having subclinical hyperthyroidism and another woman appeared to have hyperinsulinemia (> 100 μU/ml) afterwards.

Statistical analyses

Linear regression analysis and the methods described by Bland & Altman[26] were used to compare the agreement between the diameters assessed anthropometrically and those derived from the MRI scans. Differences between men and women were tested with the Student's *t*-test. Pearson's product-moment correlation coefficients were used to quantify the associations between variables after checking the normality of the distributions of the variables. Logarithmic transformed values were used for triglycerides to achieve a normal distribution. Although the distribution of visceral fat area, visceral/subcutaneous fat area ratio, change in visceral fat area and change in visceral/subcutaneous fat area ratio with weight loss in women were slightly skewed, we do not present results with transformed variables as none of the transformations improved the strength and linearity of associations. Partial correlation coefficients were computed for associations between serum lipids and fat distribution variables with age and fat mass as covariates. Effects of weight loss on variables were tested using a paired *t*-test. Two-sided P values were considered statistically significant at $P<0.05$. The SAS statistical package (SAS Institute, Cary, NC, USA) was used.

Results

The characteristics of the subjects and fat distribution variables measured by MRI and anthropometry are presented in Table 1. Men had, on average, more visceral fat than women, absolute as well as relative expressed as the visceral/subcutaneous fat area ratio ($P<0.001$).

Table 1. Descriptive characteristics of the subjects.

		Women (n=47)		Men (n=47)		
		mean	SD	mean	SD	
Age	(years)	39	6	40	6	
Body weight	(kg)	86.4	8.7	98.4	8.7	*
Height	(m)	1.66	0.06	1.79	0.06	*
Body mass index	(kg/m^2)	31.3	2.4	30.7	2.4	
Fat mass	(kg)	37.6	6.4	32.6	6.3	*
Visceral fat area†	(cm^2)	108	47	156	43	*
Subcutaneous fat area†	(cm^2)	391	100	316	78	*
Visceral/subcutaneous ratio		0.29	0.13	0.52	0.17	*
Sagittal diameter†	(cm)	23.5	2.2	25.0	1.9	*
Transverse diameter†	(cm)	36.2	3.1	36.0	2.0	
Sagittal/transverse ratio		0.65	0.05	0.69	0.04	*
Waist circumference‡	(cm)	99.4	7.3	107.2	6.8	*
Waist/hip ratio		0.87	0.07	0.98	0.05	*

† Derived from abdominal MRI scan; ‡ Measured by anthropometry;
* $P<0.001$, difference between men and women.

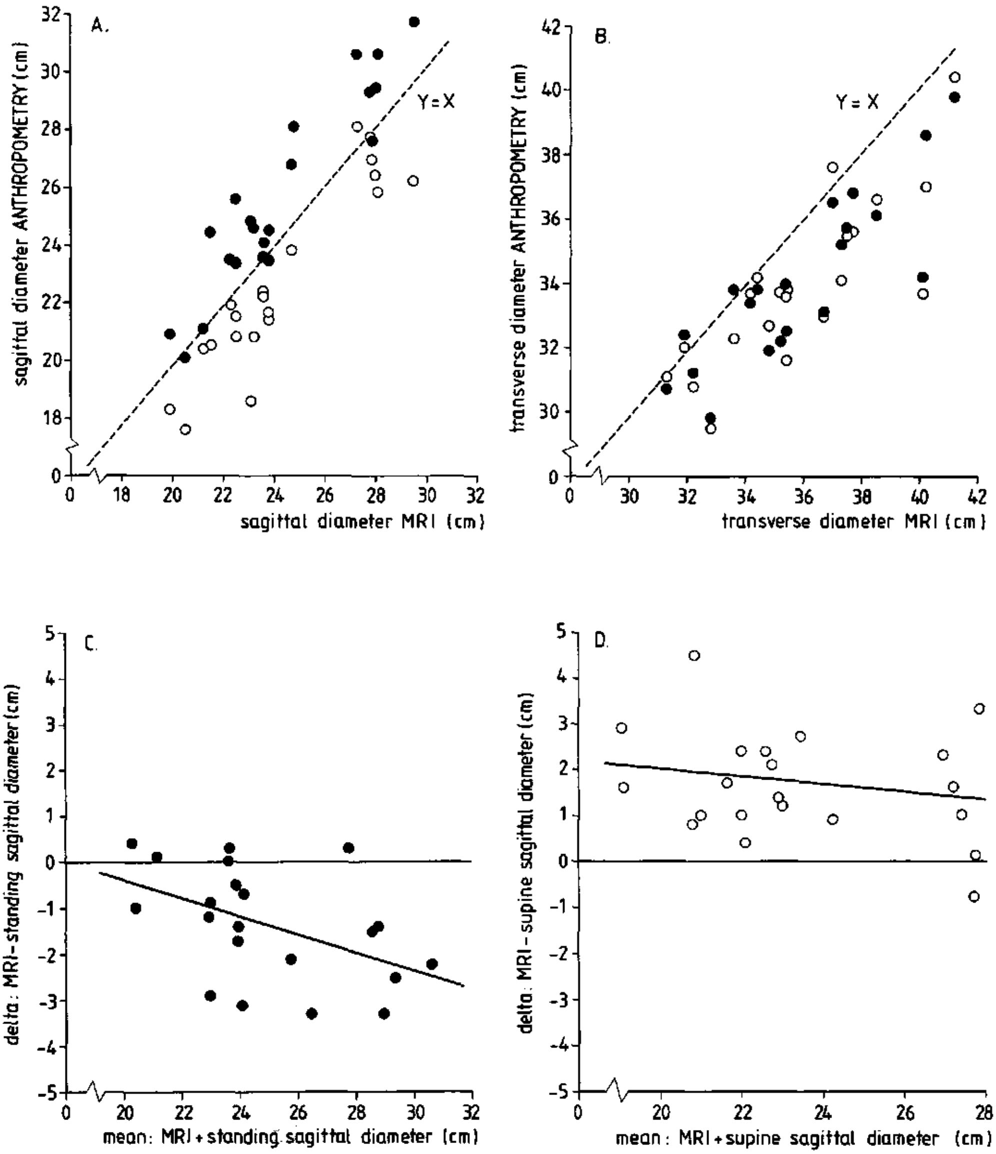

Figure 1. The relationships between abdominal diameters (sagittal (A) and transverse (B)) derived from MRI, and anthropometric diameters in standing (●) and in supine (○) position, and the relationships between the difference and mean of the anthropometrically assessed standing (C) and supine (D) sagittal diameter with the MRI derived diameters in a subsample of 21 subjects. A: standing r=0.94, $P<0.001$ and supine r=0.93, $P<0.001$; B: standing r=0.85, $P<0.001$ and supine r=0.82, $P<0.001$; C: r=-0.46, $P=0.03$, and D: r=-0.28, $P=0.22$.

It was checked whether the diameters from the MRI scans were comparable to the anthropometrically assessed abdominal diameters. Figure 1A and 1B illustrate the correlations between the abdominal diameters measured in standing and in supine position, and the MRI derived diameters. No differences in correlations and deviations were found between the sexes, therefore, the data of men and women were combined. The standing and supine anthropometric sagittal diameter differed systematically from the sagittal MRI diameter (MRI minus anthropometry, -1.4 cm (SEM 0.3) and +1.6 cm (SEM 0.3) $P<0.001$ respectively) which is illustrated in Figure 1C and 1D. The standing and supine transverse anthropometric diameter were both, on average, smaller than the transverse MRI diameter (MRI minus anthropometry, +1.8 cm (SEM 0.3) and +1.9 cm (SEM 0.4) $P<0.001$ respectively). In Figure 1C, it can be seen that the difference increased between the MRI and standing sagittal anthropometric diameter in subjects with large sagittal diameters. This was not found for the supine sagittal anthropometric diameter (Figure 1D) and for the standing and supine transverse anthropometric diameter with their MRI equivalents ($r=0.18$, $P=0.45$ and $r=0.15$, $P=0.52$ respectively).

In Table 2, correlations are given between abdominal fat areas, and the sagittal and transverse diameter, their ratio (STR), waist circumference and waist/hip ratio (WHR). In women, waist circumference and WHR showed the strongest correlations with visceral fat with WHR differentiating the best between visceral and subcutaneous abdominal fat. In men, the sagittal diameter, waist circumference and WHR were comparably associated with visceral fat, but they were all associated with subcutaneous fat as well. The relative amount of visceral fat (visceral/subcutaneous fat area ratio) was only in women weakly associated with STR and WHR. Strong interrelations were found between waist circumference and both diameters (sagittal and transverse, $r=0.84$, $r=0.82$ respectively in men and $r=0.76$, $r=0.71$ respectively in women, $P<0.001$), but a weak interrelations between STR and WHR ($r=0.37$, $P=0.01$ in men and $r=0.23$ $P=0.11$ in women).

The associations between visceral fat and the sagittal diameter improved as expected when adjustments were made for the thickness of the subcutaneous abdominal fat layer, which also could be obtained from the MRI scan ($r=0.72$ in men and $r=0.86$ in women, $P<0.001$). We did an attempt to adjust the diameters for this abdominal fat layer with the sum of trunk skinfolds (supra-iliac, subscapular and para-umbilicalis skinfold) and with the trunk skinfolds separately. The associations with visceral fat did, however, not improve (results not shown).

In both sexes, age and body fat mass contributed to the relationships between abdominal fat areas and anthropometric fat distribution variables. Age was significantly correlated with visceral fat in men ($r=0.53$, $P<0.001$) and in women ($r=0.39$, $P=0.006$). Fat mass was correlated with visceral fat in men ($r=0.52$, $P<0.001$), but only weakly in women ($r=0.26$, $P=0.08$). When the relationships were adjusted for age and fat mass, WHR remained the best predictor of visceral fat in women ($r=0.60$, $P<0.001$) and did not show a significant correlation with subcutaneous fat ($r=0.23$, $P=0.13$). In men, the sagittal diameter as well as STR were the strongest correlates of visceral fat ($r=0.39$, $P=0.008$ and $r=0.38$, $P=0.01$ respectively).

Table 2. Pearson correlation coefficients between abdominal fat areas, and diameters, circumferences and their ratios.

	Women (n=47)			Men (n=47)		
	Visceral fat area† (cm^2)	Subcutaneous fat area† (cm^2)	Visceral/ Subcutaneous ratio	Visceral fat area† (cm^2)	Subcutaneous fat area† (cm^2)	Visceral/ Subcutaneous ratio
Sagittal diameter† (cm)	0.51***	0.68***	0.09	0.61***	0.65***	0.08
Transverse diameter† (cm)	0.27	0.89***	-0.18	0.45**	0.68***	-0.05
Sagittal/transverse ratio	0.35*	-0.14	0.32*	0.39**	0.22	0.15
Waist circumference‡ (cm)	0.60***	0.55***	0.23	0.57***	0.73***	0.01
Waist/hip ratio	0.64***	0.16	0.42**	0.55***	0.30*	0.22

† Derived from abdominal MRI scan; ‡ Measured by anthropometry; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

The associations of serum lipids with visceral fat and the potential visceral fat predictors are shown in Table 3 (next page). Adjustments for age and fat mass were made to evaluate the independent role of abdominal fat distribution on the lipid profile. In women, the correlations of HDL-cholesterol and triglyceride with the sagittal diameter or the diameter ratio (STR) were much weaker than the correlations found with visceral fat, waist circumference and WHR. In men, on the contrary, the sagittal diameter as well as STR were positively related to total cholesterol and triglyceride, while visceral fat showed only a weak correlation at borderline significance with total cholesterol ($P=0.06$). Neither waist circumference nor WHR was significantly associated with any of the serum lipids in men, but WHR showed similar trends with the serum lipids as visceral fat did.

The weight loss treatment resulted in a similar mean weight reduction in men and women (13.3 kg (SD 3.0) and 12.6 kg (SD 3.9) respectively) of which, on average, 82% was loss of fat mass (10.3 kg in men and 10.9 kg in women). Table 4 shows the changes in body fat distribution variables after weight loss. Men lost, on average, more visceral fat than women, but the change in visceral/subcutaneous fat ratio did not significantly differ between the sexes ($P=0.22$).

Table 4. Reductions in body fat distribution variables after weight loss†.

Variables		Women (n=40)		Men (n=38)	
		mean	SD	mean	SD
Visceral fat area‡	(cm^2)	37	29	61	25**
Subcutaneous fat area‡	(cm^2)	118	56	110	45
Visceral/subcutaneous ratio		0.02	0.07ns†	0.05	0.10
Sagittal diameter‡	(cm)	3.3	1.6	4.4	1.4*
Transverse diameter‡	(cm)	3.9	2.6	3.2	1.2
Sagittal/transverse ratio		0.02	0.04	0.07	0.03**
Waist circumference§	(cm)	12.0	4.6	14.6	3.8*
Waist/hip ratio		0.04	0.04	0.08	0.03**

† Reductions in all variables were significant ($P<0.001$) except for the change in visceral/subcutaneous ratio in women, ns = not significant; ‡ Derived from abdominal MRI scan; § Measured by anthropometry; * $P<0.01$, ** $P<0.001$, difference between men and women.

Table 3. Serum lipid levels and their relationship with body fat distribution variables adjusted for age and fat mass.

	Lipid levels (mmol/l)		Visceral fat area† (cm^2)	Sagittal diameter† (cm)	Transverse diameter† (cm)	Sagittal/ transverse ratio	Waist girth‡ (cm)	Waist/ hip ratio
	mean	SD	Pearson correlation coefficients					
Women (n=45)								
Total cholesterol	5.59	0.88	0.01	0.14	0.08	0.07	0.03	-0.09
LDL-cholesterol§	3.78	0.73	-0.05	0.15	0.14	0.03	0.05	-0.05
HDL-cholesterol‖	1.23	0.25	-0.33*	-0.23	-0.22	-0.03	-0.52***	-0.53***
Triglyceride	1.27	0.50	0.49***	0.28	0.07	0.21	0.54***	0.41**
Men (n=46)								
Total cholesterol	5.81	0.97	0.28	0.31*	-0.09	0.33*	0.11	0.20
LDL-cholesterol§	4.01	0.84	0.26	0.25	-0.01	0.23	0.14	0.19
HDL-cholesterol‖	0.96	0.19	-0.08	-0.06	-0.06	-0.01	-0.09	-0.05
Triglyceride	1.86	0.71	0.21	0.31*	-0.27	0.44**	0.02	0.20

† Derived from abdominal MRI scan; ‡ Measured by anthropometry; § LDL= low density lipoprotein; ‖ HDL= high density lipoprotein;
* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Correlations between the reductions in abdominal fat areas, and abdominal diameters, waist circumference and WHR are presented in Table 5 (next page). In women, only the change in STR was weakly correlated with the change in visceral fat, whereas the reductions in the separate diameters and waist circumference were highly correlated with changes in subcutaneous fat. In men, the changes in both abdominal diameters, in particular the sagittal diameter, and the waist circumference were related to changes in visceral fat, however, they were also related to changes in subcutaneous fat. The change in the relative amount of visceral fat (visceral/subcutaneous fat area ratio) was not correlated with change in STR and WHR, in either sex.

Discussion

The comparison between anthropometrically assessed diameters and diameters derived from the MRI scans was important for the rationale of this study in which MRI diameters were available. Since a good agreement was found between anthropometric and MRI derived diameters, further analyses with MRI diameters was appropriate. Similar agreements between scan and anthropometric diameters were reported by Després[27] and Kvist[13] and their colleagues. The comparability of the diameters is also confirmed by the correlations found between the visceral fat area and the diameters in the subsample of twenty-one subjects. In women (n = 11), the correlation between visceral fat and the sagittal MRI diameter was $r=0.76$ ($P=0.007$), whereas the correlation between visceral fat and the anthropometrically assessed supine sagittal diameter was $r=0.72$ ($P=0.01$). These relationships in men (n = 10) were $r=0.66$ ($P=0.04$) and $r=0.61$ ($P=0.06$) respectively. A good agreement between the MRI and supine anthropometric abdominal diameters could be expected, because the MRI diameters were also measured in supine position. In standing position with increasing obesity, gravity[10] in combination with abdominal muscle strength and constitution of the abdominal adipose tissue mass may cause shifts in the abdominal fat mass. Therefore, supine diameters were expected to be preferable over standing diameters. This study showed that this may be true for the standing sagittal diameter (Figure 1C) but not for the standing transverse diameter and not, as reported by Ross et al.[28], for waist circumference.

It has been suggested that in supine position, an increased 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 gravity.[29] This was a reason to expect that the sagittal diameter and the sagittal/transverse diameter ratio were useful indicators of visceral fat, and why they were probably more specific than waist circumference and WHR. The results of this study showed, however, that the sagittal diameter was comparable to waist circumference and WHR as indicator of visceral fat in obese men. The changes in the sagittal diameter and in the diameter ratio were, on the other hand, better in detecting changes in visceral fat than waist circumference and WHR. In women, waist circumference and WHR were superior to abdominal diameters in assessing visceral fat. The cross-sectional associations between visceral fat and abdominal diameters were also revealed by the relationships of the diameters with serum lipids in both sexes.

All the potential visceral fat predictors examined in this study were only moderately

Table 5. Pearson correlations coefficients between changes in abdominal fat areas, and changes in diameters, circumferences and their ratios.

	Changes in abdominal fat areas					
	Women (n=47)			Men (n=47)		
Changes in:	Visceral fat area† (cm^2)	Subcutaneous fat area† (cm^2)	Visceral/ Subcutaneous ratio	Visceral fat area† (cm^2)	Subcutaneous fat area† (cm^2)	Visceral/ Subcutaneous ratio
Sagittal diameter† (cm)	0.10	0.76***	-0.29	0.56***	0.46**	0.16
Transverse diameter† (cm)	-0.18	0.71***	-0.39*	0.34*	0.43**	0.09
Sagittal/transverse ratio	0.32*	0.01	0.14	0.40*	0.27	0.09
Waist circumference‡ (cm)	0.14	0.58***	-0.18	0.33*	0.63***	-0.10
Waist/hip ratio	0.21	0.23	0.01	0.18	0.37*	-0.12

† Derived from abdominal MRI scan; ‡ Measured by anthropometry; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

associated with the amount of visceral fat. The present study was performed in obese subjects and this may partly explain why the correlations between visceral fat and anthropometric measures were relatively low compared with other studies. In previous studies, the correlations between visceral fat and WHR ranged between 0.55 and 0.85 in women[8,13,30] and between 0.60 and 0.88 in men.[13,27,28,31] A wider range in age and/or body fatness compared with the present study may have enhanced the previously reported associations.[27]

The ability to predict the amount of visceral fat by abdominal diameters improves when appropriate adjustments are made for the subcutaneous abdominal fat layer. Skinfold thickness measurements for assessing the subcutaneous abdominal fat layer were not found to be useful in this population confirming the results of Hayes et al.[32] Other techniques should be developed for this purpose.

Another phenomenon may have interfered with the strength of the associations presented here. In this study, the amount of visceral fat was assessed by an area of only one single scan. Although in previous studies was shown that the visceral fat area of a single scan taken at the L4-L5 level is highly correlated to the volume of visceral fat estimated from multiple scans[13,28], intestine volumes and partial volume effects[6] may have caused errors in the visceral fat estimation from a single scan. Such errors in the visceral fat area may have lowered the associations in the present study. Associations between changes in visceral fat areas and anthropometric variables will be attenuated even more, because of the possible errors in the visceral fat areas estimated before as well as after the weight change.[28,29] Despite of this limitation, the correlations between visceral fat loss and changes in both the sagittal diameter and STR in men, and in STR in women as well, were superior to the correlations with waist circumference and WHR.

In the present study, no prediction formulae were generated for the assessment of visceral fat, because of the population specificity of such formulae and the moderate associations we have found. In Table 6 (next page), a comparison is made between measured (MRI) and predicted amount of visceral fat in this study population, by using prediction-equations reported in the literature (for references see Table 6). All these formulae were generated by measuring abdominal fat areas or volumes by CT or MRI and choosing a set of anthropometric variables which resulted in the highest explained variance. Only the equation of Ferland et al.[30] was generated for obese women, whereas the other formulae were generated for populations varying in age and body fatness. The correlations between measured and predicted visceral fat areas were moderate. Some equations predicted systematically larger amounts of fat, whereas others predicted smaller amounts. The coefficients of variation for the formulae used were large and similar within a sex, in men around 20% and in women around 35%. The results confirm the conclusion of Després et al.[27] that the ability to predict visceral fat from anthropometry is limited.

In summary, the sagittal diameter and the diameter ratio may have advantages over waist circumference and WHR in men, particularly in assessing changes in visceral fat. In women, waist circumference and WHR were superior to abdominal diameters. The results indicate that simple anthropometric measurements, which differ between the sexes, can only provide rough information about the amount of visceral fat.

Table 6. Comparison between the visceral fat area derived from the abdominal MRI scan and visceral fat predicted from formulae reported in the literature.

	Comparison between measured and predicted visceral fat area									
	Women					Men				
	Number of subjects†	Measured-Predicted area (cm^2)		Correlation Measured with Predicted		Number of subjects†	Measured-Predicted area (cm^2)		Correlation Measured with Predicted	
References	n	mean	SE	r‡	CV%	n	mean	SE	r‡	CV%
Seidell et al. (9)	47	+ 16	5**	0.62	34	40†	+ 2	5	0.66	21
Kvist et al. (13)	47	− 9	6	0.51	37	47	− 31	5***	0.61	22
Ferland et al. (30)	43†	− 46	5***	0.68	33	-				
Després et al. (27)§	-					47	+ 1	4	0.75	19
Després et al. (27)‖	-					47	− 8	5*	0.69	20
Ross et al. (28)	-					47	+ 42	5***	0.52	23

n, number of subjects for comparison; SE, standard error; r, Pearson correlation coefficient; CV%, coefficient of variation.

† These comparisons were made with less subjects because of missing skinfolds;

‡ Correlation coefficients were all statistically significant, $P<0.001$;

§ Prediction formula including the sagittal diameter; ‖ Prediction formula without the sagittal diameter;

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, difference between measured and predicted area.

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Chapter 6

Resting metabolic rate and body composition in obese men and women losing and regaining body weight

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(Submitted for publication)

Abstract

Changes in resting metabolic rate (RMR) and body composition after weight loss and weight regain were investigated in obese men (n=31) and women (n=33). Body composition was measured by densitometry and RMR by indirect calorimetry. Average weight loss was (mean ± SD) 12.3 ± 3.5 kg as a result of a controlled 4.2 MJ/day deficit diet for 13 weeks. Men and women lost similar amounts of fat mass (FM) ($P=0.66$), but men lost more fat-free mass (FFM) ($P<0.001$) and reduced more in RMR ($P<0.01$) than women. The reduction in RMR in men (9.1%) was larger than expected from the losses of FFM and FM. In women, the decline in RMR (5.5%) could be fully accounted for by changes in body composition. On average, 80% of the weight lost was regained during 67 weeks of follow-up. Men had a larger increase in FFM ($P<0.005$) and RMR ($P<0.01$) than women. RMR returned to baseline level in both sexes. It is concluded that in men but not in women, larger reductions in RMR occurred than expected, and one single weight cycle did not lead to a permanent reduction in RMR in both sexes.

Introduction

Obesity is a common condition in affluent societies and many obese people try to lose weight by reducing their energy intake temporarily. Satisfactory weight reductions can be achieved, but the long-term prognosis is usually disappointing.[1,2] One of the reasons for failing to achieve long-term weight reductions may be a reduced resting metabolic rate (RMR) after weight loss.[1,3-5] This reduction in energy expenditure may suggest that metabolic adaptations occur after slimming, which are likely to contribute to weight regain.[6,7]

In several weight loss studies, the reduction in RMR could be fully explained by the decrease in fat-free mass (FFM)[5,8,9], whereas in other studies, the reduction in RMR was larger than expected from the change in FFM.[6,10,11] These contradictory findings may be caused by differences in study designs. In a number of experiments, the RMR after weight loss was measured with the subjects still in negative energy balance.[10,12] A larger decline in RMR than could be accounted for by changes in FFM might partly have been a consequence of caloric restriction.[5,13] Another reason for discrepancy may be the inappropriate use of the change in RMR (kJ/per day) divided by the change in FFM (kg), i.e. RMR/FFM ratio, as a measure of 'metabolic efficiency'.[6] Regression of RMR on FFM usually reveals a significant regression coefficient for FFM, but also a significant nonzero intercept.[12,14,15] This intercept needs to be included when predictions or comparisons are made.[11,16] In addition, several investigators[17-20] showed that the fat mass (FM) contributes to RMR independently of the FFM, particularly in obese women. The reduction in FM has also been found to account partly for the decline in RMR.[11]

The purpose of the present study was to investigate whether the changes in RMR accompanying weight loss and weight regain could be accounted for by the concomitant changes in body composition in healthy obese men and women.

Subjects and methods

Subjects

Participants were recruited by advertisements in local newspapers. Ninety-six obese subjects (48 men and 48 women) were selected on the basis of their body mass index (BMI) (between 28 and 38 kg/m^2), age (between 25 and 51 years), smoking behavior (< 5 cigarettes/day) and drinking behavior (< 2 alcoholic consumptions/day). The women were premenopausal and did not use oral contraceptives. The subjects selected were apparently healthy as evaluated by a medical history and a physical examination. None of the subjects received any medication known to affect the variables measured and none of them reported to have been on an energy restricted diet in the months prior to the study. All volunteers gave their written informed consent to participate in the study that was approved by the Medical Ethical Committee of the Department of Human Nutrition.

Eighteen subjects did not complete the weight loss intervention successfully (8 due to intercurrent illness, 5 for personal reasons, and 5 were excluded because of suspicion of poor compliance). In addition, another group of fourteen subjects did not participate in the final follow-up measurements more than one year after the weight loss intervention (3 due to illness, 10 for personal reasons such as removals or changing of jobs, and in one woman the underwater weighing could not be performed). We measured, however, body weight of those fourteen subjects more than a year after intervention. Their mean weight regain did not differ from the weight regain of the group who participated in all the final measurements (10.9 ± 1.9 (SE) kg and 9.2 ± 0.7 kg respectively, unpaired *t*-test, $P=0.33$). Additionally, the reductions in weight, FM, FFM, and RMR after weight loss were also similar in these two groups (analysis of variance, $P>0.05$). Results are presented from the subjects who participated in all measurements (n=64). This group did also not differ in baseline characteristics from the group that did not complete the weight loss intervention successfully, nor from the group that did not participate in the final follow-up measurements (analysis of variance, $P>0.05$). Table 1 lists the characteristics of the present study population before weight loss.

Table 1. Characteristics of the subjects before weight loss.

Characteristics		Women (n=33)			Men (n=31)		
		mean	SE	range	mean	SE	range
Age	(years)	39	1	27-48	40	1	28-51
Body weight	(kg)	84.9	1.4	70.7-97.1	96.3	1.5	82.2-115.9
BMI	(kg/m^2)	30.9	0.4	27.6-35.6	30.5	0.4	26.2-34.8
Percentage fat*	(%)	43.1	0.8	35.1-51.6	32.2	0.7	23.2-38.7
FFM*	(kg)	48.1	0.7	40.9-59.4	65.2	1.1	53.6-79.8
FM*	(kg)	36.8	1.1	26.6-47.2	31.1	0.9	20.7-38.8
RMR	(kJ/day)	6232	111	5060-7555	7657	103	6465-8961

SE, standard error; BMI, body mass index; FFM, fat-free mass; FM, fat mass; RMR, resting metabolic rate;* Derived from densitometry.

Experimental design and diet

The total study lasted for eighty-six weeks and consisted of a controlled dietary weight loss intervention (19 weeks) and a non-controlled follow-up (67 weeks) (Figure 1).

The subjects completed the weight loss intervention of thirteen weeks with at least a three week weight-stabilisation period before and after weight loss. No other diet or weight-maintenance counselling was provided after intervention. The subjects were subsequently followed for a period of, on average, sixty-seven weeks (ranging from 61 to 77 weeks) after the intervention.

Body composition and RMR were assessed before (week 3) and after weight loss (week 19), in the last week consuming the energy restricted diet (week 16), and after follow-up (week 86).

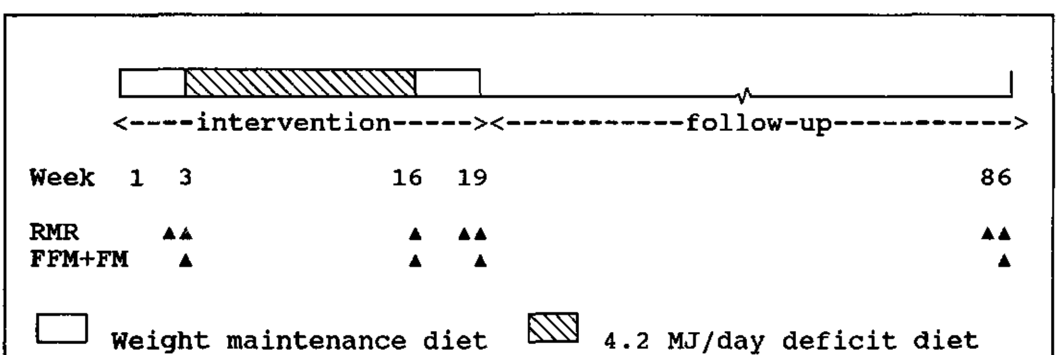

Figure 1. Study design, ▲; Assessment of body composition (fat-free mass, FFM; fat mass, FM) and resting metabolic rate (RMR).

During the first three weeks, a diet was given that met each person's energy requirement, which was estimated from RMR and physical activity pattern as described elsewhere.[21] Body weights were recorded twice a week by the subjects themselves and energy intakes were adjusted individually to prevent weight changes. For the next thirteen weeks, participants received a 4.2 MJ/day energy deficit diet (42 energy percentage (E%) carbohydrate, 25 E% protein and 33 E% fat). The individual amount of energy provided was equal to the daily energy intake at the end of the preceding weight stable period minus 4.2 MJ/day. Body weights then achieved were kept stable for another three weeks. Further details of the diets have previously been described.[22,23]

The subjects were instructed to maintain their habitual physical activity pattern and smoking habits during the study. They were asked to report illness, medication, and deviations from diets, habitual smoking and activity patterns in a diary. During the controlled intervention, dietary compliance was checked by dieticians by means of interviews every two weeks. During an interview, the diary was checked and body weight was measured.

Energy expenditure

Energy expenditure was measured with an open-circuit ventilated hood system. Full details of the instruments measuring the O_2 and CO_2 gas exchange, and how corrections were made due to calibration of standard gases and movements of subjects during measurements have been reported elsewhere.[23]

RMR was calculated from rates of O_2 consumption and CO_2 production with the use of the equations of Weir.[24] The RMR value for a specific measurement period was the average of two measurements performed with an interval of one day, either in the morning or in the afternoon. In week 16, only a single measurement was performed. When RMR was measured during the morning, subjects were in a 12-14h postabsorptive state. Afternoon RMRs were measured with subjects in a 5-6h postabsorptive state after a light standardized breakfast of less than 2 MJ. The subjects were further instructed not to sleep, drink coffee or engage in strenuous physical activity, the morning before the afternoon tests. Previous studies in our laboratory showed that there are no significant differences between morning and afternoon measurements of RMR in the same subjects.[25] In the present study, RMR adjusted for body composition did not differ between the group measured in the morning and the group measured in the afternoon (analysis of covariance, $P>0.44$). In addition, subjects measured in the afternoon before weight loss were also measured afterwards in the afternoon, whereas, subjects measured in the morning had all their subsequent measurements in the morning.

The participants were taken to the laboratory for the measurements by car. After voiding they lay supine and were asked to remain motionless and awake during the measurement. After a rest period of fifteen minutes, RMR was measured for forty-five minutes while subjects watched video films. In this study, the within-person day-to-day coefficient of variation of the RMR in the various measurement weeks was better than 4.8% for women and 4.6% for men.

Body composition

Body weight was measured to the nearest 0.05 kg with a digital scale with the subjects wearing a swimming suit. Height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. BMI was calculated from weight and height (kg/m^2). Whole body density was determined by underwater weighing[26] with simultaneous measurement of the residual lung volume by a helium-dilution technique.[27] Percentage body fat was calculated according to Siri's formula[26] and fat-free mass (FFM) resulted from subtracting fat mass (FM) from total body weight.

Statistical analyses

Changes in RMR and body composition were tested using paired *t*-tests. Differences in RMR and body composition changes between the sexes were tested by unpaired *t*-tests. Multiple regression analysis was used to assess the relationship between RMR and body composition (i.e. FFM and FM) in both sexes. The regression equations before weight loss were used to predict the changes in RMR due to changes in FFM and FM, and the predicted changes in RMR were compared with the observed changes using paired *t*-tests. Two-tailed probabilities less than 0.05 were considered significant. Results are expressed as mean ± standard error (SE).

Results

Losses of body weight, FM and FFM after weight loss intervention are illustrated in Figure 2. The reductions were significant ($P<0.001$) in both sexes.

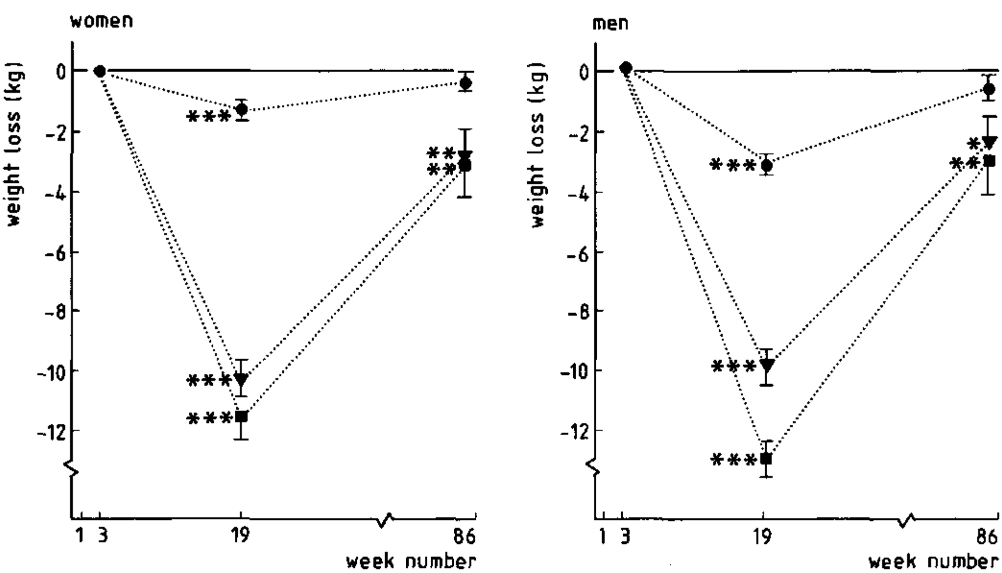

Figure 2. Changes in body weight (▪), fat-free mass (•) and fat mass (▾) after weight loss intervention (week 19 minus week 3), and after follow-up (week 86 minus week 3). Values are means ± SE. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Men lost significantly more FFM than women (3.1 ± 0.3 kg and 1.3 ± 0.2 kg respectively, $P<0.001$), whereas the reductions in fat mass (9.9 ± 0.6 kg and 10.3 ± 0.6 kg respectively, $P=0.66$) and weight (13.0 ± 0.6 kg and 11.6 ± 0.7 kg respectively, $P=0.12$) were almost similar. Figure 2 also shows the gains in weight, FFM and FM, which were measured sixty-seven weeks after weight loss. In men and women, body weight and FM were still significantly lower than baseline level, but FFM returned to baseline level. Men had a larger increase in FFM than women (2.4 ± 0.4 kg and 1.0 ± 0.3 kg respectively, $P<0.01$), but the gains in fat mass (7.6 ± 0.8 kg and 7.5 ± 0.8 kg respectively, $P=0.96$) and weight (10.0 ± 1.0 kg and 8.5 ± 1.0 kg respectively, $P=0.29$) did not differ significantly between the sexes.

The RMR measured in the last week of the weight loss intervention (during negative energy balance, week 16) was significantly lower than the RMR measured after three weeks weight stabilisation (week 19) (Table 2). Data of men and women were combined here, because the changes in RMR, FFM and FM from week 16 to week 19, did not differ significantly between the sexes ($P>0.64$). A small but statistically significant increase in FFM and decrease in FM during weight stabilisation could be observed, but could not explain the increase in RMR after weight stabilisation. The changes in RMR after weight loss and weight regain were calculated for additional analyses by using the RMR measured in the weight stable period after weight loss (week 19).

Table 2. Effect of negative energy balance (during dieting) on resting metabolic rate and body composition.

		Measurement period				Difference		
		during dieting (week 16)		in energy balance (week 19)		(week 19-week 16)		
		mean	SE	mean	SE	mean	SE	P^*
RMR	(kJ/day)							
Observed		6198	110	6392	104	189	68	<0.01
Adjusted for FFM+FM		6222	68	6368	67	159	67	<0.03
Weight	(kg)	78.1	1.2	78.1	1.2	0.0	0.1	0.73
FFM	(kg)	53.7	1.1	54.2	1.2	0.5	0.1	<0.001
FM	(kg)	24.4	0.8	23.9	0.8	-0.5	0.1	<0.001

SE, standard error; FFM, fat-free mass; FM, fat mass; RMR, resting metabolic rate;
* P=probability, paired t-test.

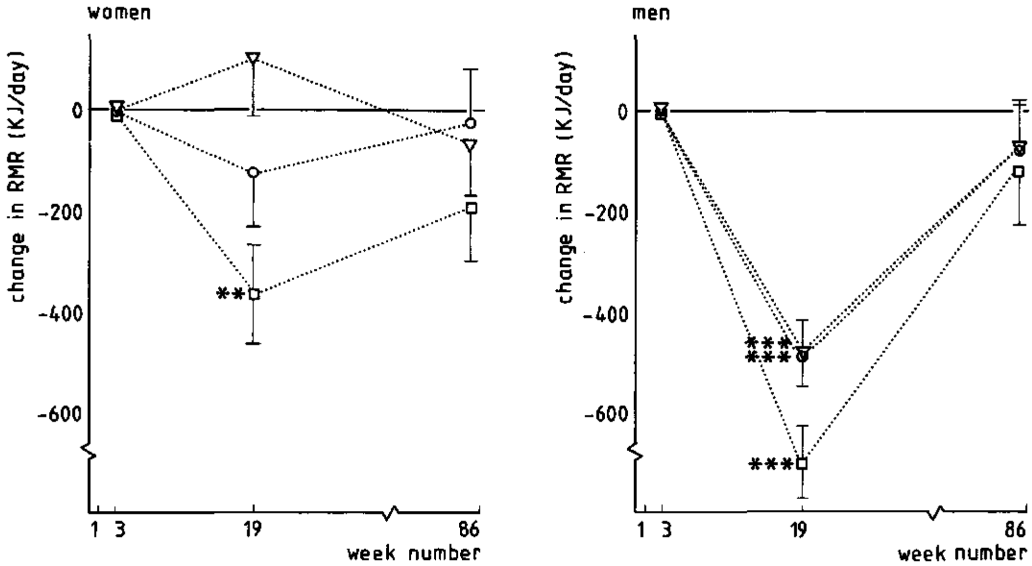

Figure 3. Changes in resting metabolic rate (observed (□), adjusted for changes in fat-free mass (○), and adjusted for changes in both fat-free mass and fat mass (▿)) after weight loss intervention (week 19 minus week 3), and after follow-up (week 86 minus week 3). Values are means ± SE. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

The observed decline in RMR after weight loss was significantly larger in men than in women (704 ± 70 kJ/day (9.1%) and 367 ± 104 kJ/day (5.5%) respectively, $P<0.01$) (Figure 3). In addition, the observed RMR of men and women after follow-up did not significantly differ from the RMR before weight loss.

Regression analysis within the sexes before weight loss showed that FM, independently of FFM, significantly contributed to the RMR in women, but not in men. In women, FM added 13% ($P<0.02$) to the explained variance of RMR in a model containing FFM. In men, FM accounted for only 1% ($P=0.96$) to the explained variance. The regression equations before weight loss for men and women separately were;

Men: $$\text{RMR (kJ/day)} = 2983 + (71 \times \text{FFM}_{kg}) + (1 \times \text{FM}_{kg}) \quad (1)$$
$r^2=0.54$, SEE=405 kJ/day (CV=5.3%).

Women: $$\text{RMR (kJ/day)} = 1567 + (69 \times \text{FFM}_{kg}) + (36 \times \text{FM}_{kg}) \quad (2)$$
$r^2=0.36$, SEE=527 kJ/day (CV=8.5%).

(r^2, explained variance; SEE, standard error of estimate; CV, coefficient of variation)

The regression coefficients for FFM were significant in both sexes ($P<0.01$). The regression equations were used to predict the changes in RMR as a consequence of changes in both FFM and FM after weight loss and weight regain. RMRs corrected for the changes in body composition are also plotted in Figure 3. In women, the RMR after weight loss adjusted for losses in FFM and FM, was not significantly different from the RMR before weight loss ($P=0.33$). In men, the adjusted RMR after weight loss was still significantly lower than before weight loss ($P<0.001$). After follow-up, the observed and the adjusted RMRs were not significantly different from baseline level in both sexes.

To illustrate the effect of correction for loss of FM, RMRs only adjusted for changes in FFM are plotted in Figure 3 as well. In men, additional adjustment for loss of FM had no effect, as could be expected from the non-significant contribution of FM in equation one. In women, on the other hand, adjustment for loss of FM explained part of the decline in RMR after weight reduction. In women, the RMR after weight loss adjusted for only loss of FFM was already not significantly different from the RMR before weight loss ($P=0.22$).

Discussion

The present study showed that the reduction in RMR after weight loss in women was fully accounted for by the changes in body composition, whereas the reduction in RMR in men was larger than expected from the changes in FFM and FM. The FM contributed to the RMR of obese women, independently of the FFM. Furthermore, the RMR in both sexes returned to the initial level, when weight, which had been lost, was almost completely regained.

The initial body composition and RMR of women of the present study is comparable to those measured in moderately obese women of other studies.[28-30] Such comparisons are more difficult for men, because they have not been studied as frequently as obese women. Men examined by Owen et al.[31] with a similar range of BMI as the men in this study, had levels of FFM, FM and RMR comparable to the men of the present study.

It has been argued that the commonly used equations to predict RMR from body composition are not appropriate in moderately and severely obese subjects.[32] The majority of equations were generated from population samples that varied widely in body fatness and age, and the data of men and women were often combined.[14,33] As a consequence, the variability in RMR could be largely explained by the variations in both FFM and age.[34] The group of obese subjects in the present study was a relatively homogeneous sample with respect to variation in age, FFM and FM. Age was, probably therefore, not associated with RMR and only a relatively small part of the variation in RMR was explained by body composition.[34] Also, we used different equations for men (equation 1) and women (equation 2) because of the dissimilar contributions of the FM to the RMR. Differences in FFM composition (the FFM of women has a larger proportion of metabolically active tissues, i.e. organs, than the FFM of men) may be another reason to use sex-specific formulae.[15,33-35] However, the regression coefficients for FFM estimated in the present study for men and women were of similar magnitude.

The contribution of the FM to the RMR, independently of the FFM, in obese women confirms previous findings.[17-20,33] The impact of FM on RMR is relatively small. Previous studies reported that the FFM has a 3 to 7-fold greater contribution to the RMR than the FM.[17-20,33] This relatively small effect in combination with relatively small numbers of obese subjects in certain population samples may be responsible for the fact that FM was not found to play a significant role in some other studies.[33,34] The FM of the obese men of the present study was probably also too small to detect its effect on energy expenditure. Bernstein et al.[17] studied men who were more obese compared with the present sample of obese men (mean BMI of 38.7 kg/m^2 *versus* 30.5 kg/m^2 respectively), and they found a significant positive association between FM and RMR adjusted for FFM. Fat cell size and fat cell number were also found to be correlated with RMR[17] and this could also be partly responsible for differences between the sexes in contribution of the FM to RMR.

Men and women of the present study lost similar amounts of weight on the 4.2 MJ/day energy deficit diet. The loss of FFM of women was rather small, on average, 11.4% of the weight lost, which is less than generally observed (approximately 25%).[5,36] In men, the loss of FFM was 23.6% of the weight lost, and this was significantly larger than in women ($P<0.01$). Explanations for this sex difference and relatively small loss of FFM in women might be due to the fact that women were more obese than the men in terms of FM or percentage body fat. The initial fat percentage of the men and women studied differed by more than 10%. A fatter person may lose less FFM and more FM than a lean person using the same energy restricted diet.[5,12,37]

The reduction in RMR after weight loss has to be evaluated as the difference in RMR between weight stable periods before and after intervention, since energy restriction itself induces a decline in RMR as was shown in previous studies[3,5,13] and which is confirmed in this study. Significant changes in the FM and FFM, however, were observed during weight stabilisation after weight loss. These changes might have been slightly under-estimated due to an under-estimation of percentage body fat (i.e. FM) during negative energy balance. The density of the FFM might have been larger than that used in Siri's formula[26], because of depletion of glycogen and water associated with this glycogen. This may have led, on the one hand, to an under-estimation of the effect of energy restriction on RMR, because changes in the FFM and especially in the FM, may indicate that the subjects were still in negative energy balance during the 3 week period of weight stabilisation. On the other hand, larger changes in the FM, and especially in the FFM, might have explained a greater part of the difference between RMR before and after weight stabilization. It is clear that the results are influenced by the methods (and its assumptions) used to assess body composition. We have chosen to apply densitometry (hydrostatic weighing) because it has been argued that this method is, despite its own limitations, more reliable than other readily applicable methods such as bioelectrical impedance or anthropometry, to assess relatively small changes in body composition.[22]

In the women of the present study, the observed reduction in RMR was small (5.5%) and could be fully accounted for by the accompanying changes in body composition confirming previous observations.[8,9,13,29] In men, on the contrary, the observed reduction in RMR (9.1%) could only be partly explained by the loss of FFM. Comparison of the observed reduction in RMR with predicted declines in RMR using published equations derived from a male population[31] or from population samples with a fair proportion of men[14,17,38], gave similar results (not shown). This suggests that energy-sparing adaptations might have occurred in the post-obese men, which might protect them from further weight loss under energy-restricted conditions, and theoretically would make them prone to regain weight. No correlation was, however, found between decrease in RMR after weight loss adjusted for changes in body composition and weight regain ($r=0.08$, $P=0.67$). Additionally, the men in this study did neither lose, nor regain more in weight than the women.

The decline in RMR in both sexes seems to be only temporarily. The RMR after weight regain returned to the initial level suggesting that a weight cycle as observed in the present study, does not lead to a permanent reduction in RMR. This finding is in agreement with studies in which repeated weight cycles were examined.[29,39-41]

For the interpretation of the present results on an individual level, i.e. to determine whether a specific loss of FFM and FM predict the change in RMR accurately, we re-analyzed the data in a similar way as Heshka et al.[11] They quantified the relationship between change in RMR and changes in body composition after weight loss by regression analysis, and revealed that besides reduction in FFM also the reduction in FM contributed to the decrease in RMR. They[11] reported that 35% of the reduction in RMR was accounted for by the losses of both FFM and FM. This could not be confirmed in the present study. No significant correlations were found between changes in RMR and changes in FFM or FM as a result of weight loss and weight regain. The relatively small absolute changes and limited variability in changes of RMR and FFM in the population studied may have obscured these associations.[34] The large between-subject variation in RMR of individuals of similar body composition may have played a role as well.[42] The present findings should therefore be interpreted with caution at the individual level.

In summary, the main findings of the present study are that the FM contributes independently of FFM to the RMR of obese women. The mean reduction in RMR after a moderate weight loss is in men, but not in women, larger than could be accounted for by changes in body composition. In addition, this reduction in RMR is not associated with subsequent weight regain and does not persist after weight regain.

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Chapter 7

Effect of a weight cycle on visceral fat accumulation

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(Submitted for publication)

Abstract

Magnetic resonance imaging was used to study the effect of a weight cycle on three fat depots: the visceral and subcutaneous abdominal depots and the subcutaneous depot at trochanter level. Obese subjects (17 men, 15 women) were examined before and after weight loss intervention for 13 weeks, and 67 weeks after intervention. They lost on average (mean ± SD) 12.9 ± 3.3 kg during intervention and regained 11.9 ± 5.1 kg during follow-up. Weight regain did not result in a greater body fatness than before weight loss. Regained fat mass was similarly distributed compared with initial fat distribution. There was no indication for a preferential deposition of visceral fat after weight regain. On the contrary, there was a slight tendency to accumulate subcutaneous fat at the expense of visceral fat. It is concluded that weight loss followed by weight regain neither leads to a greater body fatness nor to a larger amount of visceral fat compared with before weight loss.

Introduction

Obese subjects frequently lose and regain body weight because of their failure to maintain a reduced weight. Recent studies indicate that this process, which is called weight cycling, may lead to an increased risk for morbidity and mortality, primarily from coronary heart disease (CHD).[1-3] The increased risk for CHD may partly be attributable to a proposed preferential accumulation of abdominal fat after repeated weight cycles.[4,5] An enlargement of the visceral abdominal fat depot is associated with CHD, cardiovascular disease and metabolic aberrations such as non-insulin-dependent diabetes mellitus.[6,7] It is still equivocal whether or not changes in fat distribution due to weight cycling occur. Rodin et al.[5] found a positive association between retrospective history of weight cycling and the waist-hip ratio (WHR) in women, but this was not confirmed in a retrospective study including both men and women[8], and in a prospective study in men.[4] In the latter study[4], weight variability was related to an increase in the subscapular-triceps skinfold ratio. Van Dale and Saris[9] observed no significant difference in WHR between obese women with and without a history of weight cycling, and Hainer et al.[10] reported a shift towards a gynoid fat distribution assessed by skinfold thicknesses in a group of weight regainers.

In the majority of studies, effects of self-reported weight cycling histories were investigated retrospectively, and anthropometry was used to assess fat distribution. Anthropometric measures are in general not able to distinguish accurately between the visceral and subcutaneous abdominal fat depots.[11,12] In this study, fat distribution was therefore determined by magnetic resonance imaging (MRI). This method was found to be an appropriate technique for the estimation of separate adipose tissue depots[13,14], and especially for assessing changes in fat depots.[15] The purpose of the present study was to examine prospectively the deposition of fat in the visceral and subcutaneous abdominal depots and in the subcutaneous depot at trochanter level after weight regain, in men and women who had lost body weight previously in a controlled dietary weight loss intervention.

Subjects and methods

Subjects and study design

The study population was a subsample of the ninety-six obese subjects who were recruited for a dietary intervention trial including a period of energy restriction.[15] The subsample (15 women and 17 men) was selected on the basis of their weight regain more than one year after weight loss. Only those subjects who regained more than 30% of their weight loss and who were willing to participate in a third MRI-session are included in the present study. The recruitment of participants for the dietary intervention trial has been described elsewhere.[15] Briefly, ninety-six obese subjects, 48 men and 48 premenopausal women aged between 25 and 51 years, were selected when their body mass index (BMI: in kg/m^2) was between 28 and 38, and when they were apparently healthy according to the results of a medical examination. The subjects signed an informed consent after all procedures were explained to them. The study was approved by the Medical Ethical Committee of the Department of Human Nutrition.

The present subsample of thirty-two subjects did neither significantly differ from the remaining group of selected subjects in baseline characteristics, nor did they differ in changes in body composition and fat distribution due to weight loss treatment (unpaired *t*-tests). The effects of weight reduction on fat distribution have been reported elsewhere.[15] Table 1 lists the baseline characteristics of men and women of the present study.

The study population only had a larger weight regain compared with the remaining group, because they were selected on the basis of their weight regain (11.9 ± 5.1 kg *versus* 8.1 ± 5.8 kg, $P=0.003$).

The study design is illustrated in Figure 1. Information about the diet, dietary compliance, and how energy intake was tailored to meet each person's energy requirement on which the energy restricted diet was based, were reported elsewhere in detail.[15] The weight loss intervention consisted of a 4.2 MJ/day energy deficit diet (42 energy percentage (E%) carbohydrate, 25 E% protein and 33 E% fat) for 13 weeks. After the controlled energy restricted intervention, no other diets or weight-maintenance counselling were provided. Participants were re-examined on average 67 weeks (ranging from 61-77 weeks) after the intervention.

Table 1. Baseline values of body composition and fat distribution variables.

		Women (n=15)		Men (n=17)	
		mean	SD	mean	SD
Age	(years)	40	6	38	7
Body mass index	(kg/m^2)	31.1	2.1	30.8	2.4
Weight	(kg)	84.0	8.1	97.3	8.1
Percentage body fat	(%)	43.0	4.8	33.4	3.6
Fat mass	(kg)	36.4	6.9	32.6	4.7
Waist circumference	(cm)	96.6	6.9	106.5	6.5
Hip circumference	(cm)	112.3	5.3	109.4	3.9
Waist-hip ratio		0.86	0.05	0.97	0.06
Visceral fat	(cm^2)	98	29	140	39
Subcutaneous abdominal fat	(cm^2)	368	64	331	51
Subcutaneous hip fat†	(cm^2)	405	61	271	42
Visceral-subcutaneous fat ratio‡		0.27	0.07	0.44	0.15

† Subcutaneous hip fat was only measured in a subgroup of the population, n=10 women, 14 men; ‡ Abdominal visceral-subcutaneous fat ratio.

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|- 4.2 MJ/day deficit diet|------------ follow-up ------------|
<---------13 weeks ------------><------------ 67 weeks ------------>
↑                         ↑                                     ↑

↑ MRI scanning
  Body composition (densitometry)
  Anthropometry (circumferences)
```

Figure 1. Study design

Body composition

Body weight was measured to the nearest 0.05 kg with a digital scale with the subjects wearing swimming gear. Height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Whole body density was determined by underwater weighing[16] with simultaneous measurement of the residual lung volume by a helium-dilution technique.[17] The measurements were carried out in quadruplicate and the average density was used for the assessment of the percentage body fat, which was calculated according to Siri's formula.[16]

Fat distribution

Body fat distribution was assessed by circumference measures and by magnetic resonance imaging (MRI). Waist circumference was measured midway between the lower rib margin and the iliac crest at the end of a gentle expiration. Hip circumference was measured at the level of the widest circumference over the great trochanters. The circumferences were measured to the nearest 0.1 cm with the subject standing upright. Waist-hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

MRI was performed with a whole-body scanner (GYROSCAN S15, Philips Medical Systems, Best, The Netherlands) with a 1.5 T magnetic field (64 MHz) by using an inversion recovery pulse sequence (inversion time 300 ms, repetition time 820 ms, and echo time 20 ms).[14] Slice thickness was 10 mm. Two anatomic locations similar to the levels where waist and hip circumferences were measured, were chosen for scanning. One transverse scan was taken halfway between the lower rib margin and the iliac crest (abdominal level) with the subjects lying supine. This site was determined by palpation and for most subjects comparable to the umbilicus level and L4-L5 region. Due to logistic reasons, the second scan at the level of the great trochanters (hip level) was only performed in 14 men and 10 women. Baseline characteristics and changes in variables after weight loss and weight regain were essentially equal between those in whom also hip-scans were taken and the remaining group (unpaired *t*-tests). Image analysis to determine the visceral and subcutaneous fat areas was carried out as described by Seidell et al.[14] The visceral-subcutaneous abdominal fat ratio (VSR) was calculated as the visceral fat area divided by the subcutaneous abdominal fat area. Previous studies have shown that the visceral fat area assessed from a single scan taken at L4-L5 level was highly correlated to the volume of visceral fat estimated from multiple scans (*r* coefficients >0.95).[13,18] The reproducibility of the determination of fat areas expressed as coefficient of variation (two-way analysis of variance), was better than 6% for the visceral fat area, and better than 2.5% for the subcutaneous abdominal fat area.[15]

Statistics

Effects of weight loss and weight regain on variables were tested using paired *t*-tests, and differences between the sexes and between groups were tested by unpaired *t*-tests. Mean percentage changes were calculated as the average of individual changes. Pearson's product-moment correlation coefficients and multiple-regression analyses were performed to assess associations between the different fat areas after weight regain and the initial fat areas. Two-tailed P values less than 0.05 were considered to indicate statistical significance. Results are expressed as mean ± SD, if not indicated otherwise. The statistical analyses were performed on the SAS statistical package (SAS Institute, Gary, NC, USA).

Results

Changes in body composition and fat distribution variables after weight loss and weight regain are given for men and women separately in Table 2. All reductions in variables after weight loss were statistically significant, except for the reduction in VSR in both sexes ($P>0.28$). The increases in variables after weight regain were also statistically significant, but again except for the change in VSR in men and women ($P>0.20$). After weight regain, percentage body fat, waist circumference, and WHR remained below initial level in men and women ($P<0.05$). Despite the non-significant changes in VSR after weight loss and weight regain, the final VSR in both sexes also was slightly lower than before weight loss (men: 0.38 ± 0.17 *versus* 0.44 ± 0.15, $P=0.05$; women: 0.23 ±0.06 *versus* 0.27 ± 0.07, $P=0.04$, respectively).

The relative changes in fat areas expressed as percentage of initial level are illustrated in Figure 2. The results of men and women were combined in Figure 2, because the relative changes in the various fat depots were essentially the same for men and women. The percentage change after weight loss of the visceral fat depot was larger compared with both subcutaneous fat depots, with the smallest decrease in the subcutaneous depot at hip level. These effects were reported previously.[15] After weight regain, the visceral fat depot remained slightly but not significantly below initial level (8%, $P=0.07$). The subcutaneous depots, on the other hand, slightly exceeded baseline level (subcutaneous abdominal fat by 4%, $P=0.10$ and subcutaneous hip fat by 6%, $P=0.04$).

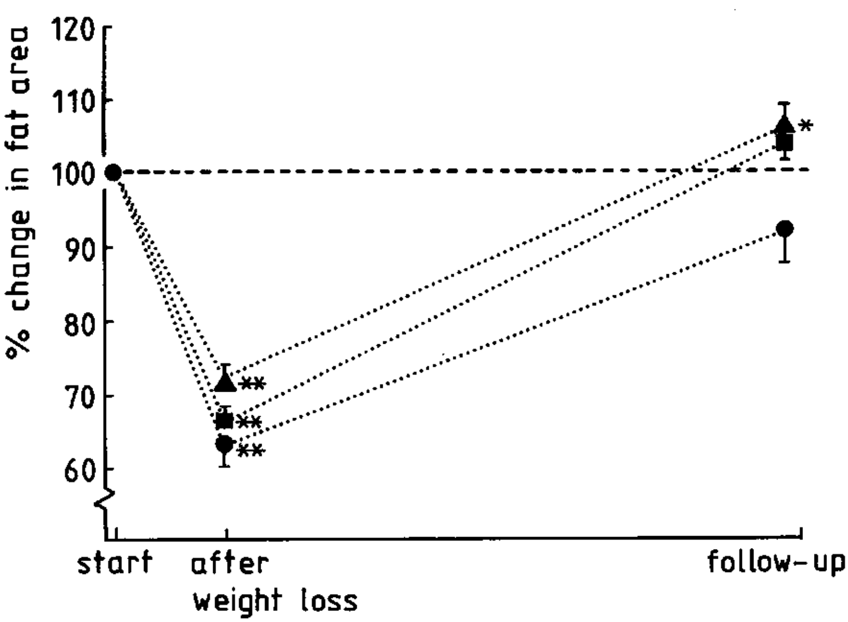

Figure 2. Changes in visceral (•), subcutaneous abdominal (▪) and subcutaneous hip (▴) fat areas after weight loss and weight regain, expressed as percentage change of initial level(=100%, start). Values are mean changes ± SEM. * $P<0.05$, ** $P<0.001$.

Table 2. Changes in body-composition and fat-distribution variables after weight loss and weight regain *.

		Women				Men			
		weight loss		weight regain†		weight loss		weight regain†	
		mean	SD	mean	SD	mean	SD	mean	SD
Weight	(kg)	-12.1	3.8	+11.4	5.8	-13.6	2.7	+12.4	4.6
Percentage body fat	(%)	-8.3	2.4	+6.7	2.7‖	-7.4	1.9	+5.8	2.7‖
Fat mass	(kg)	-11.0	3.0	+9.6	4.4	-10.7	2.2	+8.9	3.8
Waist circumference	(cm)	-11.2	4.4	+8.8	3.9‖	-14.4	3.4	+11.0	3.8‖
Hip circumference	(cm)	-8.5	3.6	+8.0	4.8	-7.6	2.1	+7.4	3.2
Waist-hip ratio		-0.04	0.03	+0.02	0.03‖	-0.07	0.03	+0.04	0.02‖
Visceral fat	(cm^2)	-34	19	+28	16	-54	28	+40	40
Subcutaneous abdominal fat	(cm^2)	-116	44	+136	42	-118	41	+128	40
Subcutaneous hip fat‡	(cm^2)	-105	33	+109	44	-80	24	+105	25**
Visceral-subcutaneous fat ratio§		-0.02	0.06^ns	-0.02	0.06^ns‖	-0.02	0.13^ns	-0.03	0.13^ns‖

* All changes in variables were significant after weight loss ($P<0.001$) and after weight regain ($P<0.05$), except for changes in variables marked with -ns- (= non-significant change); † With respect to body weight achieved after weight loss; ‡ Subcutaneous hip fat was only measured in a subgroup of the population, n = 10 women, 14 men; § Abdominal visceral-subcutaneous fat ratio; ‖ Remained below initial level after weight regain, $P<0.05$; ** Exceeded initial level after weight regain, $P<0.05$.

The regain of fat mass was significantly correlated with the increases in both subcutaneous fat depots in men and women (*r* coefficients ranging from 0.66 to 0.79, $P<0.01$). The increase in visceral fat was less strongly related to fat regain (in men: $r=0.43$, $P=0.08$; in women: $r=0.59$, $P=0.02$).

The association between the initial amount of visceral fat and the final amount after weight regain is shown in Figure 3. Multiple-regression analyses revealed that adjustment for the variability in regain of body fat mass significantly improved the associations between initial and final fat areas (Table 3).

None of the intercepts of regression equations of final fat areas on initial fat areas differed significantly from zero, which implies that there were no significant systematic increases or decreases in visceral fat or in both subcutaneous fat depots. Additionally, the slopes of final versus initial fat areas were close to one, suggesting that the amount of fat in each depot returned approximately to its initial level. The results of the statistical tests on intercepts and slopes, however, should be interpreted with caution because of the relative small number of subjects in the analyses.

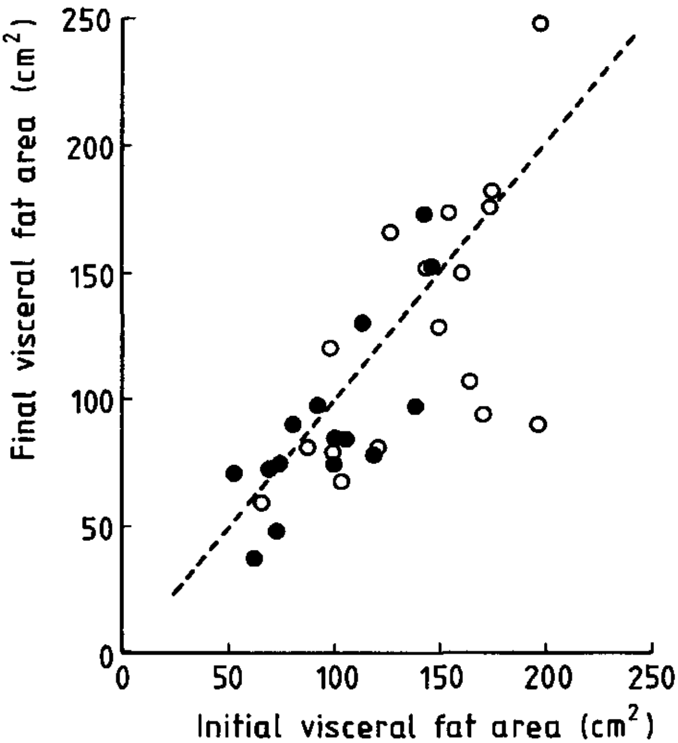

Figure 3. Association between the initial visceral fat area and the final visceral fat area after a weight cycle in men (○, $r=0.64$, $P<0.01$) and women (•, $r=0.79$, $P<0.001$), ---- = line of identity.

Table 3. Partial regression coefficients of the regression of final fat areas on initial fat areas adjusted for the difference in fat mass between before and after the weight cycle, and the explained variances of final fat areas with and without adjustment for difference in fat mass change*.

Dependent variables (cm^2)	Regression coefficients† and explained variance adjusted for ▵ fat mass							not adjusted
			initial level (cm^2)		▵ fat mass (kg)			
	intercept	SE†	ßcoeff.	SE†	ßcoeff.	SE†	r^2	r^2
Women								
Visceral fat	16	17	0.83	0.16	-4.0	1.2‡	0.81	0.63
Subcutaneous abdominal fat	-39	51	1.20	0.14	-9.2	2.2§	0.88	0.70
Subcutaneous hip fat‖	46	54	0.94	0.13	-10.2	1.8§	0.91	0.52
Men								
Visceral fat	5	28	0.94	0.19	-6.1	1.6‡	0.71	0.40
Subcutaneous abdominal fat	-18	55	1.12	0.16	-6.1	1.7‡	0.84	0.70
Subcutaneous hip fat‖	87	44	0.79	0.16	-7.0	1.6§	0.82	0.50

* Regression model: final fat area = intercept + a(initial fat area) + b(▵ fat mass); ß coeff., regression coefficient; SE, standard error; r^2, explained variance of dependent variable (= final fat area); † The regression coefficient for ▵ fat mass and the intercept were tested for difference from zero, and the regression coefficient for initial fat area was tested for difference from one, ‡ $P<0.01$, § $P<0.001$;
‖ Subcutaneous hip fat was only measured in a subgroup of the population, n=10 women, 14 men.

Discussion

This study demonstrates that a moderate weight loss of about twelve kilogram followed by weight regain does not lead to an increase in visceral fat accumulation compared with before weight loss. The amount of visceral fat returned almost to its initial level, whereas, subcutaneous fat areas at the level of the abdomen and hips slightly exceeded the amounts assessed before weight loss. As a consequence, the relative amount of visceral fat expressed as visceral-subcutaneous fat ratio, decreased after the weight cycle in both sexes.

It should be noted that the study design only allowed an prospective examination of one single weight cycle, while other studies examined associations between history of multiple weight cycles and fat distribution.[4,5,8,9,19] The definition of weight cycling varied in those studies (e.g.: counting self-reported weight losses and regains >4.5 kg[5,8,19] or >10 kg[9]; total amount of weight loss (i.e. lifetime weight loss)[8,19]; difference between highest and lowest body weight[8]; or, the root mean square error of individual regression equations of weights on time[4]). In addition, anthropometric measures were used to assess fat distribution. The observed increase in WHR[5] and in the subscapular-triceps skinfold ratio[4] after multiple weight cycles may be due to an expansion of the intra-abdominal fat mass as suggested, but it may also be caused by shifts in the subcutaneous fat patterning. This could not be distinguished by the use of WHR.[15] Hainer et al.[10] reported that weight regainers, who had been subjected to a very-low-calorie diet for one month the year before, changed their subcutaneous fat distribution. Reductions in the abdominal and upper-chest skinfold thicknesses together with a simultaneous rise of thigh skinfold thickness suggested a shift towards a more gynoid fat distribution.[10] In summary, differences in study design, in methods used to assess fat distribution and in definitions of weight cycling, may have caused the different outcomes of studies. Moreover, the present study and some other studies as well[8-10,19] focused on obese subjects, whereas, more heterogeneous populations covering a wider range of body fatness were also studied.[4,5] Effects of weight cycling may be different between obese and non-obese subjects.[8]

The response of overfeeding on fat distribution in identical twins suggested that genetic factors are involved.[20] The fact that in the present study different fat depots almost equalled their initial levels after weight regain might support this view, however, the contribution of heritability seems to be narrow.[21] Behavioral factors such as smoking, alcohol consumption and physical activity appears to have an important impact on fat distribution as well.[21,22] In contrast with the present study, the accumulation of visceral fat in the overfed twins was not related to gain in fat mass.[20] It may be that the rate of weight (i.e. fat) gain plays also a role. The mean rate of weight gain in the twins (8.1 kg in 14 weeks) was higher compared with the rate of weight gain in the 'post'-obese subjects of the present study (11.9 kg in 67 weeks).

With respect to body composition, it has been proposed that regained weight predominately consists of fat, whereas, weight lost due to dieting consists of both fat and fat-free tissue. In other words, 'dieting makes you fat'.[23] Prentice et al.[24] concluded in a review including both animal and human experiments that weight cycling is not associated with increased body fatness. This is in agreement with the present study.

Although an increase in visceral fat and total body fatness after a single weight cycle have not been found, this should not be interpreted as implying that weight cycling is without detrimental consequences. Some investigators[1-3,25], but not all[4,8,10], reported associations between weight cycling and important risk factors, other than abdominal fat distribution, for CHD, and between weight cycling and mortality due to CHD. These associations can not be contradicted by the present results, because this study focused only on fat distribution. Nevertheless, from the present study it can be postulated that an increased risk for CHD due to weight cycling is probably not mediated by a shift towards more visceral fat.

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Chapter 8

General discussion

The effects of a controlled weight loss intervention and subsequent weight regain, on body composition, fat distribution, and resting energy expenditure in moderately obese volunteers are reported in this thesis. Different methods to assess the changes in fat distribution and body composition were used, and the techniques applied did not all result in similar estimates of changes. Weight loss had a beneficial effect on fat distribution as measured by magnetic resonance imaging, namely a reduction in the visceral fat depot that was relatively larger than changes in subcutaneous fat depots. The participants who had successfully lost body weight regained 80% of their weight lost on average, during a follow-up period of more than one year. The reduction in resting metabolic rate observed after weight loss disappeared after weight regain. In addition, percentage body fat and amount of visceral fat also nearly returned to the level similar to that before weight loss. Thus, the observed weight cycle did not lead to a permanently reduced resting metabolic rate that could have implications for further weight loss, nor to a higher body fatness nor to an unfavourable fat distribution compared with the initial levels.

Study population and experimental design

The selection of participants and the experimental design had several consequences for the results and their interpretation. Moderately obese subjects were selected on the basis of their body mass index (between 28 and 38 kg/m^2), premenopausal state, apparent good health, serum lipids and serum glucose levels, and blood pressure had to be between acceptable ranges. None of the subjects used medication known to affect the variables investigated or oral contraceptives, and they had to live near Wageningen. The variability in fat distribution was created as large as possible by selecting subjects at the extremes of the waist-hip ratio distribution of the group of subjects who responded to the call for participants. Both men and women with high and low waist-hip ratios were matched on body mass index and age. This procedure offered the possibility to compare groups with different types of fat distribution, which was especially important for the parallel analyses, described in the thesis of Rianne Leenen, in which fat distribution was related to serum lipid levels, energy expenditure and sex hormones.

The participants were subjected to the same energy deficit diet, which theoretically would lead to similar amounts of weight loss in all subjects. The energy restricted diet and the weight maintenance diets before and after weight loss were supplied to the participants. Only 5% of the daily energy intake was chosen by the subjects themselves according to strict guidelines. Throughout the study trained dieticians checked dietary compliance and supported the participants. During the follow-up, no diets were supplied and participants had no contact with the dieticians or other people involved in the project. It will be clear that the results, although they are not unrealistic for free living subjects, can not be extended to all obese subjects who lose or regain body weight.

As a consequence of the selection criteria, the study population was relatively homogenous with respect to body fatness. It may be expected that the differences between body composition methods would be higher in more severe obese people, because of invalid extrapolation of assumptions of the indirect methods studied (Chapter 2). The poor correlations between changes in fat-free mass and resting metabolic rate may be due to the relatively small and homogenous reductions in both variables as a result of the 4.2 MJ/day energy deficit diet (Chapter 6). Furthermore, changes in body composition and energy expenditure may be different from the present study where obese subjects lose weight more rapidly by using for example very-low-calorie diets. The reductions in fat-free mass and energy expenditure would be larger.[1] Weight loss by increasing physical exercise also led to different results by preventing the reduction in fat-free mass and presumably thereby also the reduction in energy expenditure.[1]

The present study showed that a mild energy deficit diet for 13 weeks is acceptable and effective. Less than 7% of the subjects did not successfully complete the weight loss intervention because of difficulties with adherence to the slimming diet. The magnitude of changes in body composition was in a range normally achieved in comparable weight loss interventions[1] and the methods chosen for comparison were able to detect the relatively small changes in fat-free mass. Therefore, the population sample and study design served well for the comparison of different body composition techniques (Chapter 2).

This study has demonstrated that the reduction in visceral fat after weight loss was considerable (40% in men and 33% in women), and particularly successful in those with high initial amounts of visceral fat (Chapter 4). It should be kept in mind that only fat areas of different fat depots were measured which were assumed to represent the total amount of fat in a specific depot.[2] Measurement errors in fat areas might have affected the outcomes, in particular, those of correlation analyses. Associations between visceral fat areas and alternative fat distribution measures might have been attenuated, especially the associations between changes in the various measurements (Chapter 4 and 5).

The loss of visceral fat was unlike the loss of subcutaneous fat, not related to total weight or fat loss. The visceral fat depot accounts for approximately 5 to 25% of the total amount of body fat, whereas the remaining part mainly consists of subcutaneous fat.[2,3] Reductions in subcutaneous fat might therefore be expected to be more closely related to total weight or fat loss than the loss of visceral fat (Chapter 4).

Due to logistic reasons, it was not possible to observe changes in the rates of fat loss in the different fat depots. Magnetic resonance imaging was not performed during the weight loss intervention. Knowledge about changes in the rate of visceral fat loss may have implications for the treatment of obese subjects with excessive visceral fat deposition. If the main goal of therapy is to lose visceral fat, the duration of treatment may be shortened when the rate of visceral fat loss is relatively fast at the beginning and subsequently slows down.

The effects of weight loss on fat distribution in the premenopausal women studied may not be the same in postmenopausal women. The fat deposition of postmenopausal women changes to a more abdominal type of fat distribution due to changes in hormonal status.[4] Effects of weight loss in postmenopausal women resembled the results found in men of the present study (i.e. larger loss of visceral fat).[5]

In addition, it will be clear that the consequences of the observed weight cycle (Chapter 6 and 7) are also affected by the study population, the controlled weight loss intervention and the length of the follow-up period.

Body composition measurements

Five different indirect methods frequently used in experiments were investigated and, because of the lack of a 'gold standard', a relative validation of methods has been performed (Chapter 2). Densitometry by using hydrostatic weighing had been chosen as the reference method. Despite its limitations, densitometry is often used as a reference and this method corresponded reasonably well with recently developed multiple compartment models in which technically advanced methods were applied in relatively lean subjects.[6]

In multiple compartment models, different components of the body are estimated by separate techniques (e.g. total body bone mass by absorptiometry or neutron activation; body protein by neutron activation; water by isotope dilution; and fat by absorptiometry or total body carbon measurement).[7-10] The advantage of these models is that the limitations of a two-compartment model, in which it is assumed that body components such as water and minerals are fixed proportions of the fat-free mass, could partly be overcome. This is particularly valuable in specific groups like children, pregnant women, athletes, elderly and also severe obese, in which the two-compartment model has been shown to be inadequate.[7,9]

In future research, the validation of readily applicable techniques to measure body composition changes in moderate and severe obese subjects, may benefit from these extended models and advanced measurement techniques.[7,9,11,12] The number of assumptions will be limited and more accurate assessment seems to be possible.

Bioelectrical impedance was found to be inaccurate in the assessments of changes in fat-free mass (Chapter 2). It was hypothesized that this was caused by shifts in the water distribution between intracellular and extracellular compartments of the body during weight loss. Additionally, the apparatus used with a fixed frequency at 50 kHz only partly measures intracellular compartments. In several recent studies[13-16], it was demonstrated that the distribution of water between the extracellular and intracellular compartments, and within the extracellular compartment itself[16], affects the bioelectrical impedance measurements. The changes in water distribution due to weight changes in obese subjects[17] and the way in which this modifies bioelectrical impedance measurements remain to be established. The newly developed multi-frequency bioelectrical impedance technique may be useful for this purpose, because this method has the ability to assess the different water compartments separately.[18-20] Multi-frequency bioelectrical impedance was unfortunately not available at the time of this study.

Forbes et al.[21] has proposed that the equation based on height and resistance, that forms the foundation for the bioelectrical impedance technique: fat-free mass (kg) = $a \times (\text{height}^2\ (m^2)/\text{resistance (ohm)}) + b$, is not appropriate for assessing changes in body composition. Inclusion of body weight in this equation would improve the estimates of fat-free mass. Differentiation of the above mentioned equation extended with body weight reveals that the change in resistance depends on the ratio of change in fat-free mass to change in weight. However, the points raised concerning the water distribution are still interfering in bioelectrical impedance measurements at 50 kHz in such an equation.

The body composition techniques used, all estimated a larger loss of fat-free mass in men compared with women. Densitometry and the deuterium-oxide dilution method showed within a sex, similar estimates of changes in body composition. In addition, the change in resistance measured by bioelectrical impedance in both men and women was not associated with change in fat-free mass as assessed by densitometry (Chapter 2). For the comparison of measurement techniques, it might thus not have been necessary to split the population according to gender.

The differences in loss of fat-free mass between men and women appear to be reflected in the reduction in resting metabolic rate after weight loss, because men showed a greater reduction in resting metabolic rate than women (Chapter 6). Initial body fatness which was higher in women than in men expressed as percentage fat or absolute fat mass but not as body mass index, might have played a role here by protecting the loss of fat-free mass in women.

Fat distribution measurements

Magnetic resonance imaging was used in the present study as the reference method for the assessment of changes in fat distribution. Weight loss resulted in a reduction in visceral fat as measured by magnetic resonance imaging, but anthropometric measurements did not reflect this change adequately (Chapter 4 and 5).

At the group level, waist circumference, waist-hip ratio, both abdominal diameters (sagittal and transverse) and their ratio, reduced after weight loss indicative of a change in fat distribution. However, the changes in those anthropometric measurements were poorly correlated with the changes in visceral fat area. This might partly have been due to the measurement errors of fat areas by magnetic resonance imaging and, more importantly, to the fact that anthropometric measures do not allow distinction between the visceral and subcutaneous abdominal fat depots. Anthropometric methods make therefore only rough assessments of the amount of visceral fat.[22,23] Correction for the thickness of the subcutaneous abdominal fat layer may improve the visceral fat assessments by the waist circumference and the sagittal abdominal diameter. Traditional measurements of the abdominal and dorsal skinfold thicknesses are not accurate for this purpose (Chapter 5), but methods like sonography (ultrasound) may be useful.

Since the establishment of the waist-hip ratio as an independent predictor of metabolic risk factors and mortality[24], it is generally accepted that in cross-sectional studies, waist-hip ratio is a good measure of fat distribution. In the future, more attention should be paid to the development of easily applicable methods for the assessment of changes in fat distribution over time. It should be recognised that with a ratio measure like waist-hip ratio, both the numerator and denominator may change with weight fluctuations and this might disguise any meaningful changes in specific fat depots.[25] Additionally, it should be kept in mind that total body fatness shows strong interrelationships with abdominal fat accumulation and visceral fat deposition.[26] Therefore, changes in the separate fat distribution measurements have to be examined besides the changes in ratios of measures, in groups varying in body fatness.

Imaging techniques do not discriminate between the different intra-abdominal fat depots.[27] It has been proposed that especially accumulation of 'portal' fat (i.e. omental and mesenteric fat) is associated with adverse health consequences. Free fatty acids released from the omental and mesenteric fat depots drain directly into the portal vein and long-term exposure of the liver to high concentrations of fatty acids may result in metabolic disorders.[28] The retroperitoneal fat depot has been suggested to be a less harmful intra-abdominal fat depot, because it does not drain into the portal vein. In addition, retroperitoneal fat seems to have a reduced lipolytic capacity compared with omental and mesenteric adipocytes.[29] Rössner et al.[27] showed in only two human cadavers, that the percentage of visceral fat as retroperitoneal fat was 41% and 25% respectively. The physiological aspects of adipocytes of the different intra-abdominal depots are difficult to investigate. Up to now, *in vivo* and *in vitro* studies did not always led to compatible conclusions.[30]

Whether or not the change in visceral fat after weight loss results in beneficial health consequences, is thus also difficult to examine since 'portal' fat can not be measured separately. In the parallel analyses, it could not be clearly established that the changes in the serum lipid profile in men and women separately were due to the reduction in visceral fat.[31] Fukijoka et al.[32] showed that in severely obese Japanese women improvements in only plasma glucose and triglycerides were associated with the reduction in total visceral fat. The changes in total- and high-density-lipoprotein-cholesterol were not associated with visceral fat loss in the Japanese women.[32] For a better understanding of consequences of visceral fat loss, more information about the changes in separate intra-abdominal fat depots seems to be crucial.

Weight cycling

The effects of weight cycling were studied because this phenomenon has been associated with adverse health outcomes.[33,34] In addition, a large number of people experience repeated weight cycles.[35] The suggested increase in body fatness and decrease in energy expenditure due to weight cycling were recently reviewed by Prentice et al.[1,36] who concluded that there is no convincing scientific evidence to support these findings. The present study confirms this conclusion (Chapter 6 and 7).

Consequences on fat distribution have less frequently been investigated and only with the use of anthropometric measurements.[37-39] This study demonstrated that after one single weight cycle there was no increase in visceral fat as measured by magnetic resonance imaging compared with the initial amount (Chapter 7). Thus, an unfavourable effect of a weight cycle on fat distribution also could not be confirmed.

Only a single weight cycle was investigated prospectively in this study, whereas, in the majority of studies weight histories of subjects over several cycles, sometimes self-reported, were analyzed in relation to apparent risk factors for disease.[33,34,37,39,40] An increase in some risk factors may be the consequence of repeated weight cycles and may not be detected following a single cycle. It might be assumed that most of the subjects in the study population had experienced earlier weight cycles, but the exact number and magnitude of these cycles is unfortunately unknown. Longitudinal prospective studies covering several weight cycles should be conducted to confirm the results of this study. Other risk factors for disease as well as the magnitude of weight cycles should thereby be taken into account, because consequences of small (e.g. 1-5 kg) and large (e.g. >5 or 10 kg) weight fluctuations may be different.[41,42]

Modest weight losses have already been shown to improve several cardiovascular risk factors and diabetic control,[43] and relatively small weight reductions might also be easier to maintain. Additional research should focus on how to maintain a reduced body weight, the method of weight loss and whether or not weight reduction is voluntary.[44] In addition, attention should be paid to whether or not morbidity and mortality risks of post-obese reduce after weight loss, in particular since recent studies have suggested that mortality risk, especially of coronary heart disease, increased with increased weight loss.[41,42,44] This is in contrast with the widely held perception that return to 'ideal' weight reduces mortality risk. This unexpected effect of weight loss does not imply that obese individuals should remain obese because obesity itself is a risk factor for mortality and morbidity. In any case, the current findings on mortality risk firmly stress the importance of obesity prevention!

Conclusions

Measurement techniques,

Densitometry and the deuterium-oxide dilution method give similar results in moderately obese people when changes in body composition are evaluated. Bioelectrical impedance measured at a fixed frequency of 50 kHz and skinfold thicknesses are unreliable alternatives. Instead of these techniques, it is better and even much cheaper to use the body mass index.

For the assessment of changes in fat distribution, particularly, the change in visceral fat accumulation, anthropometric measures, such as waist circumference, waist-hip ratio, sagittal and transverse abdominal diameter, and the diameter ratio are not suitable. Imaging techniques are appropriate for this purpose.

Changes in fat distribution with weight loss,

Moderate weight loss of about 12 kilogram produced by a mild energy deficit results in a reduction of the visceral fat area (30-40%). The reduction in subcutaneous fat, at abdominal level and especially at hip level, is relatively less than the change in visceral fat. In other words, fat distribution changes with weight loss. Particularly, those who have a lot of visceral fat initially have the greatest reductions in this fat depot.

Weight loss and subsequent weight regain,

Moderate weight loss followed by weight regain does not lead to a permanent reduction in resting energy expenditure nor to a higher body fatness nor to an increase in visceral fat when compared with the levels before weight loss. Thus, the argument that yo-yo-dieting makes you fat and changes your figure used by obese subjects who stop seeking treatment for overweight, does not seem to be true.

Although no adverse consequences of the observed weight cycle were found in this study, it may not be concluded that weight cycling is harmless. Only one cycle was examined and from the battery of risk factors for disease, only the effects on body fatness, resting energy expenditure, and visceral fat accumulation were studied. In addition, now it has recently been suggested that mortality risk increases with weight loss and frequency of weight cycling, more attention should be paid to obesity prevention and weight maintenance control.

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Summary

Obesity is a common condition in affluent countries and it is related to an increased risk for morbidity and mortality. An abundance of intra-abdominal (i.e. visceral) fat is found to be a stronger predictor of specific metabolic disorders than overall body fatness. Studies on the effects of weight loss on fat distribution, particularly on visceral fat accumulation, have thus far yielded contradictory findings. Furthermore, weight loss is unfortunately very often followed by weight regain. This weight cycling has also been reported to be associated with adverse health consequences, and it seems to increase body fatness, to reduce energy expenditure, and to lead to a more unfavourable fat distribution.

The interpretation of consequences of weight changes, weight loss as well as weight gain, on fat distribution, body composition and energy expenditure may be influenced by the methods used to assess changes in fat distribution and body composition. The use of different measurement techniques in the various experiments may partly explain the discrepancies between previous studies.

The aim of the present study was to examine the effects of a controlled dietary weight loss intervention of thirteen weeks, on body composition, fat distribution, and resting energy expenditure in moderately obese men and women. In addition, the consequences of weight regain during sixty-seven weeks after intervention were studied as well. Different methods to assess the changes in fat distribution and body composition were compared.

In Chapter two, five indirect methods for the assessment of body composition are described, and their estimates of changes in body composition after weight loss (on average 13 kg) were compared. The deuterium oxide dilution method and densitometry (hydrostatic weighing) led to similar results, whereas, skinfold thicknesses, body mass index and bioelectrical impedance equations showed larger reductions in fat-free mass than densitometry. No correlation was found between the loss of fat-free mass measured by densitometry and change in resistance as measured by bioelectrical impedance.

The next three chapters concentrate on measurement techniques for the assessment of fat distribution. A review of methods focusing on principles of techniques, their accuracy and reproducibility, and aspects of costs and safety, with special emphasis on the measurement of visceral fat, is given in Chapter three. Among available methods, imaging techniques seem to be the most appropriate for the estimation of visceral fat accumulation, and also to assess changes in this fat depot.

Magnetic resonance imaging was used in the present study as the reference method for the assessment of fat distribution and it was compared with readily applicable anthropometric measurements such as circumferences and diameters. Waist circumference, waist-hip circumference ratio, sagittal and transverse abdominal diameter, and the diameter ratio revealed to be poor predictors for the assessment of changes in visceral fat after weight reduction. The main disadvantage of these anthropometric measures is that an accurate distinction between subcutaneous and visceral abdominal fat can not be made.

Weight loss had a beneficial effect on fat distribution as measured by magnetic resonance imaging, namely a reduction in the visceral fat depot that was relatively larger than changes in subcutaneous fat depots. The proportional reduction of the visceral fat

area was 40% in men and 33% in women, and the loss of subcutaneous fat was particularly smaller at trochanter level (men 29% and women 26%).

After successful weight loss, the participants regained 80% of their weight lost on average, during a follow-up period of sixty-seven weeks. The reduction in resting metabolic rate observed after weight loss was larger in men than in women, but it disappeared in both sexes after weight regain. In addition, percentage body fat and amount of visceral fat also nearly returned to the levels similar to that before weight loss.

It is concluded that bioelectrical impedance, skinfold thicknesses and the body mass index are not as good as densitometry or the deuterium-oxide dilution method for the evaluation of changes in body composition. Only rough estimates of visceral fat can be achieved by anthropometry. As a consequence, the assessment of changes in visceral fat by anthropometry is limited. Furthermore, fat distribution changes with weight loss. The proportional reduction in visceral fat exceeds those in subcutaneous fat. Finally, a weight cycle as observed in this study does not lead to a permanently reduced resting energy expenditure, nor to a greater body fatness nor to an increase in visceral fat compared with the initial levels.

Although the effects of a single weight cycle as observed in this study are different from the consequences assumed, it should not be concluded that weight cycling is without adverse health consequences. This study focused only on body fatness, resting energy expenditure and visceral fat accumulation, and not on other risk factors for disease and mortality. In addition, up to this moment there is conflicting evidence whether weight loss increases longevity. It has recently been suggested that weight loss as well as weight cycling increases mortality risk. In the future, therefore, more attention should be paid to obesity prevention and weight maintenance control.

Samenvatting

Obesitas oftewel vetzucht is een bekend verschijnsel in welvarende landen. Het verhoogt het risico voor het krijgen van bepaalde ziekten (bijv. hart- en vaatziekten en niet-insuline afhankelijke diabetes) en vroegtijdige sterfte. Deze verhoogde risico's worden niet alleen bepaald door de mate van overgewicht, maar voor een belangrijk deel door de manier waarop de extra kilogrammen lichaamsvet zijn verdeeld over het lichaam. Een overmaat lichaamsvet in de buikholte blijkt een betere voorspeller voor bepaalde metabole afwijkingen, dan de mate van overgewicht alleen. Onderzoek naar de gevolgen van gewichtsverlies op de verdeling van vet over het lichaam en in het bijzonder op de afname van vet in de buikholte, hebben tot nu toe tegenstrijdige resultaten opgeleverd. Verder blijkt gewichtsverlies vaak gevolgd te worden door gewichtstoename. Herhaald afvallen en weer aankomen in gewicht is recentelijk in verband gebracht met negatieve gevolgen voor de gezondheid. Het zou onder andere leiden tot een toename van de hoeveelheid lichaamsvet, een daling van de energie-stofwisseling en een grotere vetstapeling in de buikholte.

De interpretatie van de gevolgen van gewichtsveranderingen op de vetverdeling, de lichaamssamenstelling en de energie-stofwisseling kunnen worden beïnvloed door de technieken die worden gebruikt om deze veranderingen te meten. In het verleden heeft het gebruik van verschillende meettechnieken in diverse onderzoeken waarschijnlijk mede geleid tot niet-eenduidige resultaten.

Het onderzoek, beschreven in dit proefschrift, had als doel het bestuderen van de gevolgen van een energie-beperkte voeding op de energie-stofwisseling tijdens rust, de lichaamssamenstelling en de vetverdeling. Dit werd gedaan bij mannen en vrouwen met een zekere mate van overgewicht. Het energie-beperkte dieet werd gedurende dertien weken verstrekt. Verder zijn ook de gevolgen van gewichtstoename onderzocht door de diverse metingen ruim een jaar na het afslanken te herhalen. Verschillende meettechnieken zijn gebruikt voor het bepalen van de veranderingen in lichaamssamenstelling en vetverdeling. De resultaten van die metingen zijn vervolgens met elkaar vergeleken.

Vijf indirecte methoden om de lichaamssamenstelling te bepalen werden gebruikt om de veranderingen te meten in de lichaamssamenstelling na gewichtsverlies (gemiddeld 13 kg) (hoofdstuk 2). De deuterium-oxyde verdunningsmethode en densitometrie (onderwater-weging) gaven overeenkomstige resultaten. Voorspellingsformules gebaseerd op huidplooi-dikte-metingen, de Quetelet-index (gewicht (kg)/lengte^2 (m^2)) en de impedantie-techniek resulteerden daarentegen in een grotere schatting van de afname in vet-vrije massa in vergelijking tot densitometrie. Er werd ook geen verband gevonden tussen de afname in vet-vrije massa, gemeten met behulp van densitometrie, en de afname in weerstand zoals die gemeten werd met de impedantie-methode.

De drie volgende hoofdstukken beschrijven technieken om de vetverdeling te meten, met in het bijzonder aandacht voor het bepalen van de hoeveelheid vet in de buikholte. Hoofdstuk 3 is een overzichtsartikel waarin de principes van verschillende meettechnieken worden behandeld. Ook de nauwkeurigheid en reproduceerbaarheid van de verschillende technieken en de kosten en veiligheid komen hier aan bod. Beeldvormende technieken lijken op dit moment het meest geschikt om de hoeveelheid vet in de buikholte te schatten en om veranderingen te bepalen in dit vetdepot.

De beeldvormende techniek gebaseerd op kernspin resonantie werd in deze studie gebruikt als referentie-methode en vergeleken met eenvoudige anthropometrische metingen, zoals omtrekmaten en diameters. De middelomtrek, middel-heup ratio, de sagittale en transversale diameter ter hoogte van de navel en de ratio van deze diameters bleken geen goede voorspellers voor de veranderingen in de hoeveelheid vet in de buikholte. Het belangrijkste nadeel van deze anthropometrische maten is dat ze geen nauwkeurig onderscheid kunnen maken tussen de hoeveelheid onderhuidse vet en de hoeveelheid vet in de buikholte.

Gewichtsverlies had een gunstig effect op de vetverdeling. Met behulp van de bovengenoemde beeldvormende techniek kon worden aangetoond dat er een relatief grotere reductie was in het vet in de buikholte, dan in het daaromheen liggende onderhuidse vet. De afname van het vet in de buikholte was 40% bij de mannen en 33% bij de vrouwen, terwijl het verlies van het onderhuidse vet, met name het onderhuidse vet ter hoogte van de billen, minder was (mannen 29% en vrouwen 26%).

De deelnemers kwamen gemiddeld weer 80% van het gewichtsverlies aan in het jaar volgend op de afslankstudie. De afname in energie-stofwisseling tijdens rust die werd waargenomen na gewichtsverlies, was groter bij mannen dan bij vrouwen, maar deze afname verdween zowel bij mannen als bij vrouwen na gewichtstoename. Het percentage lichaamsvet en de hoeveelheid vet in de buikholte keerden ook bijna terug op het initiële niveau (niveau voor het afslanken).

Het onderzoek heeft geleid tot de volgende conclusies:
1. De impedantie-methode, huidplooi-dikte-metingen en de Quetelet-index zijn minder geschikt voor het meten van veranderingen in lichaamssamenstelling dan densitometrie en de deuterium-oxyde-verdunningsmethode.
2. Met behulp van anthropometrie kunnen alleen ruwe schattingen worden gemaakt van de hoeveelheid vet in de buikholte. Anthropometrie heeft daarom een beperkte waarde voor het schatten van veranderingen in dit vetdepot.
3. De vetverdeling verandert als het gewicht afneemt. Er verdwijnt relatief meer vet in de buikholte dan onderhuids.
4. Gewichtsverlies gevolgd door gewichtstoename heeft niet tot gevolg dat de energie-stofwisseling tijdens rust op een lager niveau wordt gehandhaafd, noch dat de hoeveelheid lichaamsvet en de hoeveelheid vet in de buikholte toenemen ten opzichte van de initiële niveaus.

De bovengenoemde effecten van gewichtsverlies gevolgd door gewichtstoename wijken af van de veronderstelde gevolgen. Uit dit onderzoek kan echter niet worden geconcludeerd dat gewichtsfluctuaties geheel zonder risico zijn voor de gezondheid. In dit onderzoek is immers alleen gekeken naar de effecten van een éénmalige gewichtscyclus op de lichaamssamenstelling, de vetopslag in de buikholte en de energie-stofwisseling tijdens rust. Andere bekende risico-factoren voor ziekte en sterfte zijn niet meegenomen. Tot op heden is het ook nog niet duidelijk of gewichtsverlies leidt tot een verlengde levensduur. Recent is gesuggereerd dat gewichtsverlies, evenals het herhaaldelijk afvallen en weer aankomen, het risico voor vroegtijdige sterfte juist verhoogd. In de toekomst zal daarom meer aandacht moeten worden besteed aan de preventie van overgewicht en aan het handhaven van een succesvolle gewichtsafname.

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Curriculum Vitae

Karin van der Kooy werd geboren op 10 juni 1964 te Rotterdam. In 1982 behaalde zij het diploma Gymnasium-ß aan het Corderius College te Amersfoort. Hierna begon zij de studie Humane Voeding aan de Landbouwuniversiteit te Wageningen. Tijdens haar studie deed zij een afstudeervak Klinische Voeding aan de Rijksuniversiteit Limburg (vakgroep Humane Biologie, Prof.dr. W.H.M. Saris). De praktijktijd werd doorgebracht in Japan (Osaka City University, Osaka University en Sanyo Gakuen College te Okayama). Tevens deed zij een afstudeervak Humane Voeding aan de Landbouwuniversiteit (vakgroep Humane Voeding, Prof.dr. J.G.A.J. Hautvast). In 1988 behaalde zij haar ingenieursdiploma, waarna zij bij de vakgroep Humane Voeding in dienst trad als assistent-in-opleiding. Hier werd het onderzoek uitgevoerd dat beschreven is in dit proefschrift. In 1992 werd haar een tweejarig epidemiologisch fellowship toegekend door de Nederlandse Kankerbestrijding. Vanaf mei 1993 is zij als fellow werkzaam bij de afdeling Epidemiologie van het Nederlands Kanker Instituut te Amsterdam.