BODY COMPOSITION AND ENERGY METABOLISM IN ELDERLY PEOPLE

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Proefschrift

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STELLINGEN

1. Antropometrie en bio-elektrische impedantie zijn niet bruikbaar om de lichaamssamenstelling van ouderen op individueel niveau te schatten.
   Dit proefschrift

2. Na correctie voor verschillen in lichaamssamenstelling hebben lichamelijk actieve ouderen eenzelfde energieverbruik in rust als inactieve ouderen.
   Dit proefschrift

3. De door voeding geïnduceerde thermogenese is niet direct gerelateerd aan leeftijd.
   Dit proefschrift

4. Roken en een laag activiteitenpatroon zijn geassocieerd met een abdominale vetverdeling.
   O.a. dit proefschrift

5. Voedselconsumptiemethoden onderschatten de energie-inneming van ouderen.
   O.a. dit proefschrift

6. Het bevorderen van een goede voedingsstatus bij ouderen vergt meer dan het produceren en verstrekken van nutriënt-verrijkte voedingsmiddelen.

7. Het stereotiepe beeld van ouderen is aan verjonging toe.

8. De toenemende prevalentie van overgewicht in westerse landen wordt voornamelijk veroorzaakt door een afname van lichamelijke activiteit.

9. Oestrogenen dienen alleen aan vrouwen van middelbare leeftijd te worden voorgeschreven ter behandeling van perimenopauzale klachten.

10. Het automatiseren van meetopstellingen tijdens promotieonderzoek leidt niet altijd tot kostenbesparing en tijdwinst.

11. Aan de Landbouwuniversiteit Wageningen kan het begrijpen van de indeling van het promotiereglement beschouwd worden als een eerste toetsing tot het behalen van de doctorsgraad.

12. Vrouwen met overgewicht en een gluteaal-femorale vetverdeling hebben een verhoogde kans op het dragen van een "legging".

13. Dat vele Nederlanders de inrichting van het huis op ongeïnspireerde wijze laten bepalen door het aanbod van landelijke huishoudwinkelketens blijkt uit het veelvuldig voorkomen van houten ganzen achter de ramen.

Stellingen behorend bij het proefschrift:
Body composition and energy metabolism in elderly people
van Marjolein Visser

Wageningen, 6 september 1995

Aan Jan en Ans Visser

ABSTRACT

BODY COMPOSITION AND ENERGY METABOLISM IN ELDERLY PEOPLE

PhD thesis by Marjolein Visser, Department of Human Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands. September 6, 1995.

This thesis describes several studies related to the three components of energy balance in elderly people: body composition, energy expenditure, and energy intake.

Body composition. The applicability of the body mass index, skinfold thickness method, and multi-frequency bioelectrical impedance was tested in elderly men and women. The first two methods predicted body fat in elderly people on a group level with a mean prediction error of 5%. The impedance method predicted total body water (at 50 kHz) and extra-cellular water (at 5 kHz) with a mean prediction error of 8-9%. Individual predictions should be interpreted carefully. Data from 2341 elderly men and women showed that smoking and physical inactivity were associated with a more abdominal fat distribution in men but not in women.

Energy expenditure. To investigate the possible changes in energy expenditure with aging, energy expenditure at rest (RMR), after a meal of 1.3 MJ (DIT), and during physical activities was measured in elderly men and women and compared with values of young subjects. RMR was lower in elderly subjects, which could not be explained by differences in body composition or fat distribution. No relation between DIT and age 'per se' was observed. The energy costs of several physical activities, performed at a self-selected pace, were similar as compared to reference values based on young subjects.

Energy intake. The reported energy and protein intake of elderly women, obtained by the dietary history method, was validated using measurements of total energy expenditure and urinary nitrogen excretion. An underestimation of 10-12% of the dietary intake was observed.

These studies suggest that the decreasing energy requirement with aging is explained by a decrease in RMR, DIT and possibly a lower physical activity level. The decrease is partly explained by changes in body composition.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

The number of elderly people (65 years of age and older) is increasing rapidly in developed countries as well as in developing countries. In 1930 6.2% of the Dutch population was 65 years and older. This percentage increased to 10.2% in 1970 and is prognosticated to be 14.9% in 2010 [1]. These data reflect the changing age distribution in most western countries.
The demands of health care of an older population will require growing attention by all health care professionals. The prevalence of chronic disease is high in older people and is, like many other health problems, directly or indirectly related to nutritional status. Not only problems concerning the adequacy of micro- and macronutrient intake, but also the adequacy of energy intake need to be considered. The prevalence of low body weight and weight loss is high at older age, especially among institutionalized elderly people [2-5]. On the other hand, an increasing prevalence of overweight can been observed in elderly people. According to the recent Health and Nutrition Survey, 41% of American men and women between the ages 60 and 74 are overweight, as indicated by a body mass index of 28 kg/m^2 or more [6]. In the Netherlands about 9% of the elderly men and about 16% of the elderly women have a body mass index of 30 kg/m^2 or more [7]. Both underweight and overweight are associated with higher morbidity and mortality risk in elderly people [8-11].
Underweight and overweight are the result of a net change in energy balance. Figure 1 shows schematically the three components of energy balance: energy intake, body composition and energy expenditure, which are closely interrelated

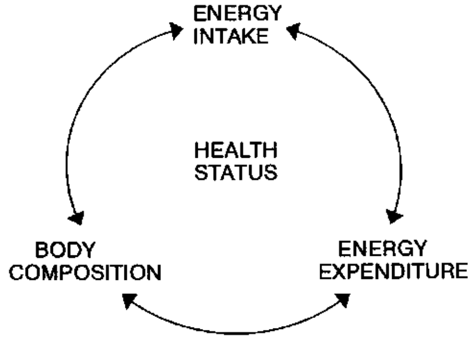

Figure 1. The energy balance equation in schematic form.

and related to health status. An energy intake higher than energy expenditure will generally lead to a positive energy balance and to the accumulation of body fat. A low energy intake compared to energy expenditure will lead to a negative energy balance and to weight loss. In the following paragraphs a brief overview of the three components of energy balance will be given. In addition, changes with increasing age in each of these components will be discussed.

BODY COMPOSITION

Methods for assessing body composition

Direct and indirect methods are used to measure body composition. Direct measurements quantify a compartment of the body directly, e.g., deuterium oxide dilution for total body water. At present there is no direct method to quantify total body fat directly *in vivo*. Several indirect approaches have been developed based on known relations between fat and other directly measured compartments [12,13].

The simplest indirect approach is the two-compartment model in which the body is divided into two major compartments, the fat-free mass and fat mass. Three classic examples of the two-compartment model are body density (underwater weighing) [14], total body water (isotope dilution) [15], and total body potassium (^{40}K counting) [16]. More compartment models have been developed, like the three-component model based on total body water and body density [17]. Recently, four-component models based either on a combination of total body water (isotope dilution), body density (underwater weighing) and total body bone mineral (dual-photon absorptiometry), or based on a combination of total body water (isotope dilution), total body nitrogen and total body calcium (γ neutron-activation analysis) have been developed [18].

An overview of body composition methods is shown in Table 1. The methods vary largely in cost, technical difficulty (and training of the observers), cooperation of the subjects, and accuracy [12]. For use in field studies, methods should be simple and inexpensive. Furthermore, for some populations such as elderly people, methods should be non-invasive and should require minimal cooperation by the subjects.

Table 1. Methods for determining human body fat.*

	cost	technical difficulty	subject cooperation	accuracy % fat
Water Deuterium	2	3	4	3
Potassium	4	4	2	3
Creatinine	2	3	4	1
Densitometry	3	4	5	5
Skinfold thickness	1	2	3	2
Neutron activation	5	5	2	5
Photon absorptiometry	4	4	2	4
Body mass index	1	1	1	2
Bioelectrical impedance	2	1	1	3
Computed tomography	5	5	2	4
Ultrasound	3	3	2	3
Magnetic resonance	5	5	2	4

* Partly based on Lukaski [12], 1 = least and 5 = greatest.

Table 1 shows that measurements of body mass index, skinfolds thickness, and body impedance are relatively low in cost, are technically simple, require little subject cooperation and thus are practical to use in a field situation. These methods could therefore be extremely useful for application in an elderly population in both large epidemiologic studies as well as laboratory studies.

Body mass index. Using body weight as an indicator of body composition is not optimal, since individuals with different body weights can differ in height. Therefore, in 1842 Lambert-Adolf-Jacques Quetelet proposed the Quetelet Index, defined as weight (in kilogram) divided by height (in meters) squared, as a measure of weight corrected for height [19]. This index is nowadays referred to as the body mass index and is highly correlated with body fat mass and percentage body fat [20-22].

Skinfold thickness. Measurements of the fat tissue layer just beneath the skin at several well defined locations of the body using a skinfold calliper are used as an indicator of total body fatness. The skinfold method relies on two assumptions: the thickness of the subcutaneous adipose tissue reflects a constant proportion of the total body fat and the sites selected for measurement represent the average thickness of the subcutaneous adipose [23].

Body impedance. The bio-electrical impedance method relies on the fact that intra- and extracellular body fluids behave as electrical conductors and cell membranes

act as imperfect reactive elements. Two gel electrodes are placed on the hand and two on the foot. The impedance analyzer delivers a current at a frequency of 50 kHz via the electrodes and detects the voltage drop which is a measure of body composition [24,25]. Recently developed bioelectrical impedance equipment is able to measure resistance, impedance, reactance and phase angle at various frequencies, ranging from 1 to 1350 kHz. At low frequencies (1 to 5 kHz) the current mainly passes through the extracellular fluids, while at higher frequencies (100 to 1000 kHz) the current penetrates the cell membranes and thus measures total body water (intra- and extracellular fluids) [26-28].
Prediction equations have been developed to estimate body composition by using measurements of body mass index, skinfold thickness and impedance. These equations have been developed and validated predominantly in young and middle-aged populations using underwater weighing or isotope dilution as a reference method. Due to the changes in body composition with aging, which will be discussed below, the applicability of these prediction equations to an elderly population can be questioned [29,30]. In this thesis the applicability of body mass index, skinfold thickness and impedance was tested in a population of elderly men and women (Chapter 2 and 3).

Changes in body composition with age

Height. Older people are generally shorter than younger people [31]. This is partly due to a secular trend in height, but also due to a shrinkage of the spine with increasing age. This shrinkage with age is due to the loss in vertebral bone, kyphosis and scoliosis [32]. Due to the increased rate of loss of bone mass after menopause in women, the decline in stature is larger in women than men [33].
Body weight. Most studies show an increase in body weight from middle age to old age in healthy elderly subjects [31]. However, at very old age a decrease in body weight is observed [34]. Whether this weight loss is a result of aging *per se* or illness is difficult to separate. Several studies reported a relationship between weight loss and mortality in elderly people [8,9,35].
Fat-free mass. Several longitudinal studies show a decrease in fat-free mass with age [36-38]. A decrease in 24-hour creatinine excretion, an index of muscle mass, has been observed in healthy men followed for 11 years [37]. A slow decrease in lean body mass, as determined by ^{40}K counting, was observed during adult life [34,36,39]. The average rate of loss is about 3 kg per decade [40]. In addition,

many cross-sectional studies show a lower fat-free mass, muscle mass, and body cell mass in older compared with younger subjects [41-43]. Part of the loss in fat-free mass might be caused by the decrease in physical activity level with increasing age [44-47]. Physical activity has been associated with fat-free mass [48] and training studies in elderly people show an increase in fat-free mass [49-51].
Fat mass and fat distribution. Body fat percentage increases with increasing age [52]. Not only the amount of fat, but also the distribution of fat changes with age. Measurements of abdominal fat and fat around and within the muscles of thigh and upper arm made by magnetic resonance imaging or computed tomography show a redistribution of body fat with age. Elderly people have relatively more fat accumulated around the abdomen and less fat at the extremities [52-56]. In women total body fat and abdominal fat may increase especially after menopause, starting in the perimenopausal years [57]. In chapter 4 the relationship between two behavioral factors, smoking and physical activity, and body fat distribution was investigated in elderly men and women.

ENERGY EXPENDITURE

Total energy expenditure consists of three major components. The main component is the resting metabolic rate (RMR) which accounts for about 70% of the total energy expenditure. The resting metabolic rate is the energy expended when the body is at rest totally and is measured under strictly standardized conditions. The RMR is measured when the subject is in the fasting state, physically and mentally at rest (but not asleep), in a supine position, and staying in a thermo-neutral environment. The second compartment of total energy expenditure is the energy expended during physical activity. This part usually accounts for 20-30% of the total energy expenditure and varies largely between individuals [58]. The third part is the diet-induced thermogenesis (DIT) or sometimes called meal- or food-induced thermogenesis. This is the rise in energy expenditure after the consumption of a drink or a meal. It reflects the amount of energy needed for the ingestion, digestion, absorption, processing, and storage of the energy-yielding nutrients. The DIT is about 10% of the total energy expenditure in young adults, depending on the composition of the meal.

Resting metabolic rate

The RMR is largely determined by body composition. The fat-free mass, which contains the metabolic active organs like the brain, heart, liver, and metabolic active tissue like muscle cell mass, is the most important factor influencing RMR [59-61]. Although fat mass is metabolically inactive, it may also contribute to RMR, especially in the obese [60,62-65]. Other factors have been reported to influence RMR, such as physical activity level [66-69], body fat distribution [64,70], menstrual cycle [71], and hormone levels [70,72].

RMR is known to decrease with increasing age [37,42,72-80]. Longitudinal studies show a decrease in RMR ranging from -0.69 to -3.7% per decade [37,77]. Due to a change in body composition with age, especially a decrease in fat-free mass, the RMR is likely to decrease. However, whether the change in body composition fully accounts for the age related change in RMR is still unclear. Data from most longitudinal and cross-sectional studies show that the change in body composition fully accounts for the change in RMR [37,42,76-78,81]. However, in some studies the RMR of elderly subjects was still lower compared to younger subjects even after adjustment for differences in body composition [72,77,79,80,82]. In this thesis RMR is compared between young and elderly men and women. Additionally, the relationship between RMR and body composition, fat distribution and physical activity level was studied (Chapter 5).

Energy expenditure during physical activity

Physical activity level is known to vary greatly among people [58,83]. Since physical activity decreases with age, its contribution to total daily energy expenditure will decrease [44-47]. To quantify the level of physical activity the physical activity ratio (PAR) or physical activity index (PAI) is often used. It is calculated by dividing total daily energy expenditure by the resting metabolic rate. For healthy adults in industrialized countries the mean PAR over a day is about 1.6-1.8. The Food and Agricultural Organization / World Health Organization / United Nations University (FAO/WHO/UNU) reference value for elderly persons is 1.5 [84]. However, for certain groups of healthy, elderly people this value of 1.5 might be too low. Mean PAR values up to 1.8 have been recently reported in the literature [85,86].

Previous studies show a higher PAR for strenuous activities (like walking on a treadmill at a fixed speed) in elderly subjects compared to young and middle-aged subjects [87-89]. These findings suggest that the daily PAR for elderly people may be high due to the high energy costs for physical activities. In chapter 6 of this thesis, energy expenditure during several physical activities was measured in elderly women and compared with the FAO/WHO/UNU reference values for activities.

Diet-induced thermogenesis

The primary determinants of the diet-induced thermogenesis are the energy content and nutrient composition of the meal [82,90-92]. In addition, influences of menstrual cycle [93], hormone levels [94], physical activity level [66,67,95,96], body composition [97-99], and body fat distribution [64,100] on DIT have been reported in the literature.

The results from the different cross-sectional studies investigating the relationship between age and DIT are contradictory. Some studies report a lower DIT in elderly men compared to younger men [82,101-103], while other studies report no significant difference [67,80,104-106]. Data on the relationship between DIT and age in women have never been reported. A study on the DIT in young and elderly men and women is described in chapter 5 of this thesis.

ENERGY INTAKE

Many different methods have been developed to assess dietary intake [107]. An accurate assessment of food intake is necessary to evaluate the adequacy of the diet and may help to estimate energy requirements. Furthermore, the intake of energy and nutrients are studied to investigate their relationship with morbidity and mortality [108].

Several studies investigated the dietary intake of elderly populations in the Netherlands. Table 2 shows the energy intake of healthy elderly women and men from several studies carried out in the Netherlands.

Previous studies have shown that energy intake decreases with increasing age [44,80,111,115]. The results of the Dutch National Food Consumption Survey 1992 [114] also show a lower energy intake in older age groups compared to younger age groups. The observed decrease in energy intake varies widely between longitudinal studies: from -53 to -1898 kJ per decade [11,115,116]. Results from cross-sectional studies suggest a decrease from -25 to -417 kJ per decade [44,80,109,114-117]. Part of these differences are probably caused by differences in study population and dietary assessment methodology.

Assessing dietary intake of elderly subjects reveals some specific problems [118]. The 24-hour recall might be unreliable in elderly people due to a decline in short-term memory with age [119] and a weighed dietary record can be difficult to complete. The dietary history method seems a suitable method for elderly subjects since it requires limited effort by the subjects and does not depend on short term memory [120]. *Validation of the weighed dietary record method against* total energy expenditure showed an underestimation of energy intake by 10-31% in elderly subjects [83,86,121]. Studies investigating the validity of other dietary assessment methods in elderly subjects, using objective reference methods like urinary nitrogen excretion or total energy expenditure, are limited [122]. *A validation study of the dietary history method in elderly women, using* measurements of total energy expenditure as reference method, is described in chapter 7.

Table 2. Energy intake of Dutch elderly women and men.

	Women			Men		
	n	Age (y)	Intake (MJ)	*n*	Age (y)	Intake (MJ)
VCP 1987-88 [109]	261	65+	7.8 ± 2.0	227	65+	10.3 ± 2.6
Löwik *et al.* [110]	269	65-80	7.9 ± 1.9	269	65-80	10.1 ± 2.0
Kromhout *et al.* [111]	-	-	-	315	65-85	9.4 ± 2.1
Moreiras *et al.* [112]	124	70-75	7.8 ± 1.9	114	70-75	10.3 ± 2.4
Voorrips *et al.* [113]	23	60-79	7.4 ± 1.6 *	-	-	-
	25	60-79	6.9 ± 1.4 †	-	-	-
VCP 1992 [114]	263	65+	7.7 ± 1.9	236	65+	9.7 ± 2.4

* sedentary elderly, † active elderly.

OUTLINE OF THE THESIS

The aim of the studies described in this thesis is to validate field methods for the assessment of body composition and dietary intake in elderly men and women. In addition, the relationship of two behavioral factors, smoking and physical activity, on body composition, and the change in energy expenditure with aging was investigated.

The applicability of three commonly used field methods to assess body composition in elderly subjects is investigated and described in chapter 2 and 3. The methods discussed are skinfolds thickness, body mass index and multi-frequency bioelectrical impedance. The possible relationship between two behavioral factors, smoking and physical activity, and body fat distribution is investigated in chapter 4. Data of 2341 elderly respondents from the Longitudinal Aging Study Amsterdam were used to study these relationships. In chapter 5 and 6 of this thesis the three major components of energy expenditure: resting metabolic rate, diet-induced thermogenesis, and energy expenditure during physical activity are measured and compared between young and elderly subjects. Factors that might directly or indirectly influence the possible change in energy expenditure with increasing age are discussed. Chapter 7 describes a validation study of the dietary history method, a method to assess dietary intake, carried out in a group of healthy elderly women. Reported energy intake and protein intake data were compared with measurements of total energy expenditure and 24-hour urinary nitrogen excretion, respectively. Chapter 8 contains a general discussion of the study results presented in this thesis and the overall conclusions.

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CHAPTER 2

PREDICTION EQUATIONS FOR THE ESTIMATION OF BODY COMPOSITION IN THE ELDERLY USING ANTHROPOMETRIC DATA

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ABSTRACT

To study the relationship between health and nutritional status in elderly populations, information about body composition is essential. To collect this information in large epidemiological studies, practical methods based on anthropometric data must be available. In the present study the relationship between body composition, determined by densitometry, and anthropometric data in 204 elderly men and women, aged 60-87 years, was analysed. Existing prediction equations described in the literature, and mainly based on young and middle-aged subjects, generally underestimated percentage body fat in the elderly study population. Therefore, new prediction equations were developed, based on gender and the sum of two (biceps and triceps) or four (biceps, triceps, supra-iliaca and sub-scapula) skinfolds or the body mass index (BMI). Addition of age or body circumferences to the models did not improve the prediction of body density. Internal cross validation and external validation revealed that the formulas are valid for the estimation of body density in elderly subjects. The standard error of estimate of the three models, expressed as percentage body fat, was 5.6, 5.4, and 4.8%, respectively.

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INTRODUCTION

The number of elderly people is increasing in industrialized countries as well as in developing countries. Information about health status, and especially factors influencing health in old age, is therefore needed. Various studies have shown a relationship between health and nutritional status in elderly people. While studying this relationship, information concerning body composition is indispensable [1,2]. Body fat content and distribution in elderly subjects seem to be related to risk factors for cardiovascular disease such as high blood pressure [3,4], lower high-density-lipoprotein cholesterol and higher triglycerides concentrations [5] and diabetes [4,6]. Underweight and undernutrition in old age are found to be related to higher mortality rates [7,8].

To study the effect of body composition on morbidity and mortality, large epidemiological studies are requisite. However, commonly used methods for the assessment of body composition such as densitometry are difficult to perform in elderly subjects and are not suitable for field studies. Apart from that, the densitometric method also has its limitations in elderly subjects as the assumptions underlying this method, i.e. a density of the fat mass of 0.900 kg/L and a density of the fat-free mass of 1.100 kg/L, may be incorrect for elderly subjects [9]. Body composition predicted from relatively simple anthropometric measures is more practical.

Various prediction formulas for percent body fat based on skinfolds [10-12], body mass index (BMI) [12-16], or both [17] have been described in the literature. However, these formulas were mostly developed in young and middle-aged populations [12,14-16] or rather small groups of elderly [10,11,13,17].

Both cross-sectional and longitudinal studies show that body composition changes with age. The amount of fat in the body generally increases and relatively more fat is accumulated internally [18-20]. Stature decreases with increasing age due to senile kyphosis and shortening of the spinal vertebrae [2]. Therefore, prediction formulas developed in young and middle aged subjects based on skinfolds, weight, height or the BMI, are not likely to be valid in elderly subjects.

The aim of the present study was to investigate the relationship between body density and anthropometric measurements in a large group of elderly men and women, aged 60 to 87 years of age.

SUBJECTS AND METHODS

Subjects

The study population was composed of 204 apparently healthy elderly subjects, 128 women and 76 men, aged 60-87 years. The subjects were recruited by advertisements in local newspapers and by visiting homes and clubs for the elderly in the surroundings of Wageningen. All subjects completed a medical questionnaire which was checked by a physician. Subjects taking diuretic drugs that could influence body composition or the state of hydration and heavy smokers (> 10 cigarettes per day) were excluded from the study. The experimental procedures were approved by the Ethical Committee of the Department of Human Nutrition. Characteristics of the subjects are given in Table 1.

For external validation the data of 23 elderly people, aged 62-82 years, were used. These subjects were measured at the Department of Human Biology, University of Limburg, using the same methodology.

Body composition

Anthropometric measurements and the measurement of the body density were performed on the same day. Body weight was measured to the nearest 0.05 kg using a digital scale (ED60-T; Berkel, Rotterdam, the Netherlands). Body height was measured by means of a microtoise to the nearest 0.001 m. BMI was calculated as body weight (kg) divided by height (m) squared.

Body density was determined by underwater weighing to the nearest 0.001 kg (3826MP-81; Sartorius, Göttingen, Germany) with simultaneous determination of the lung volume by a helium dilution technique (Spiro-Junior; Jaeger GmBH, Würtzburg, Germany). The measurement was carried out in duplicate if possible. Body fat content (%) was calculated from density using the Siri's formula [21].

Skinfolds

Skinfolds were measured at the left side of the body to the nearest 0.002 m with a skinfold calliper (Holtain/Tanner-Whitehouse; Holtain Ltd, Crymmych, United Kingdom). The skinfolds were measured in triplicate at the following sites: (1) triceps, halfway between the acromion process and the olecranon process; (2) biceps, at the same level as the triceps skinfold, directly above the centre of the cubital fossa; (3) sub-scapula, about 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body; (4) supra-iliaca, just above the ilia crest, in the axillary line; (5) para-umbilica, at one-third of the distance between the umbilicus and the lateral side of the body; (6) quadriceps, halfway between the ilia crest and the patella in a vertical line; (7) fibula, on the fibula at the level of the greatest circumference. The para-umbilica, quadriceps and fibula were only measured in a subgroup of the population. The average value of the triplicate measurements were used in the statistical analysis. All skinfolds, and the sums of skinfolds, were ^{10}log transformed to correct for a skewed distribution.

Statistical methods

Correlations between body density and other body composition variables were calculated using Pearson's product-moment correlations. Differences between density from underwater weighing and density predicted from skinfolds or BMI equations were tested with paired Student's *t* tests. Stepwise multiple regression analysis was used with density as the dependent variable and gender, age, BMI or (sums of) skinfolds as independent variables. Prediction equations were developed in two groups randomly assigned by a computer program of the total population. Internal cross validation was carried out by testing whether the prediction equation of one group could validly predict density in the other group. The prediction equation based on the total study population was applied to body composition data of another group of Dutch elderly subjects to validate the equation externally. Two-sided *P*-values were considered statistically significant at $P<0.05$. Results are expressed as means with their standard deviation (mean ± SD).

RESULTS

Table 1 shows some characteristics of the subjects. All variables, except age and the supra-iliaca skinfold, were statistically different between gender. Men had a higher body weight and were taller compared with the women. The body density of the women was lower, resulting in a higher proportion of body fat. Nearly all skinfold thicknesses were larger and BMI was higher in women. To investigate whether prediction formulas from the literature were able to predict body fat content in this group of elderly subjects, various formulas were applied to the data (Table 2).

Table 1. Subject characteristics.

	Women ($n=128$)	Men ($n=76$)
Age (y)	70.2 ± 5.3	71.0 ± 5.9
Body weight (kg)	68.1 ± 9.5	76.5 ± 9.6 †
Body height (m)	1.616 ± 0.061	1.752 ± 0.071 †
Body mass index (kg/m^2)	26.1 ± 3.6	24.9 ± 2.6 *
Density (kg/L)	1.0037 ± 0.0124	1.0289 ± 0.0119 †
Body fat content (%) ‡	43.3 ± 6.1	31.2 ± 5.6 †
Triceps (mm)	19.8 ± 5.1	12.5 ± 3.3 †
Biceps (mm)	11.8 ± 4.5	6.4 ± 2.2 †
Sub-scapula (mm)	19.8 ± 7.5	17.4 ± 5.5 *
Supra-iliaca (mm)	19.8 ± 8.0	17.9 ± 6.2
Para-umbilica (mm) §	25.7 ± 7.7	20.7 ± 6.2 †
Quadriceps (mm) ‖	32.5 ± 7.1	16.5 ± 6.6 †
Fibula (mm) ¶	15.3 ± 6.0	7.4 ± 2.8 †

* $P<0.01$, † $P<0.001$ significantly different from women; ‡ calculated using Siri's formula; § 125 women and 70 men; ‖ 62 women and 39 men; ¶ 59 women and 33 men.

Nearly all formulas significantly underestimated the percentage body fat in this population. Prediction errors of percentage body fat ranged from -19.8 to -1.2% in women and from -13.9 to -0.5% in men. Because of the large mean differences between predicted and measured body fat contents, new prediction formulas were developed using skinfolds, BMI, gender and age as independent variables.

In Table 3 the correlation coefficients between body density and several skinfolds and the BMI are shown. In women all skinfolds were significantly correlated with body density. In men no correlation was found between body density and the para-umbilica skinfold, the quadriceps skinfold and the fibula skinfold. Generally, both in men and women, the skinfolds on the trunk were more highly correlated with body density than the skinfolds on the extremities, except for the umbilica skinfold in men. The correlation of body density and BMI was higher than any correlation of the body density with skinfolds, in both males and females.

After the total study population was randomly assigned into two groups, prediction equations for body density were developed in each subgroup using multilinear regression techniques.

Group 1 consisted of 109 subjects, 74 women and 35 men, while group 2 consisted of 95 subjects, 54 women and 41 men. The results of three models in

Table 2. Differences between percent body fat predicted from various equations in the literature and estimated by densitometry (difference = predicted - estimated).

	Women	Men
Estimated from densitometry	43.3 ± 6.1	31.2 ± 5.6
Difference skinfolds		
Durnin [10]	-6.0 ± 5.9 ‡	-4.1 ± 5.6 ‡
Noppa [11]	-12.7 ± 5.3 ‡	- -
Heitmann [12]	-19.8 ± 5.8 ‡	-13.9 ± 5.4 ‡
Difference BMI		
Womersley [13]	-8.3 ± 4.8 ‡	-5.2 ± 4.8 ‡
Norgan [14]	-	-9.2 ± 4.8 ‡§
Norgan [14]	-	-0.5 ± 4.5 ‖
Garrow [15]	-10.0 ± 5.0 ‡	-8.8 ± 5.6 ‡
Heitmann [12]	-9.2 ± 5.0 ‡	-8.2 ± 4.9 ‡
Deurenberg [16]	-1.2 ± 5.0 †	-1.2 ± 4.5 *
Svendsen [17]	-14.2 ± 6.4 ‡	-11.8 ± 5.4 ‡

* $P<0.05$, † $P<0.01$, ‡ $P<0.001$ mean predicted value significantly different from estimated value; § equation included BMI only; ‖ equation included BMI and age.

each group are shown in Table 4. The regression model with the highest explained variance (R^2) and the lowest prediction error (standard error of estimate, SEE) in both subgroups contained gender and BMI as independent variables. The best regression model using skinfolds contained the variables gender and the ^{10}log transformed sum of biceps, triceps, sub-scapula and supra-iliaca. However, a model based on gender and the ^{10}log transformed sum of only the triceps and biceps skinfold had only a slightly lower explained variance and a comparable prediction error. Age did not contribute to the explained variance in either subgroup.

Internal cross validation revealed that the prediction equation developed in one subgroup could validly predict body density in the other subgroup (Table 5). Therefore, the data from the groups could be combined (Table 4). The correlation coefficients between measured and predicted densities in group 1 and group 2 were 0.73 and 0.74, respectively, using model 1, 0.76 and 0.76, respectively, using model 2, and 0.82 and 0.81, respectively, using model 3 (all $P<0.0001$). The SEE in body density of the models varied from 0.0100 to 0.0117 kg/L, resulting in a prediction error of about 5% body fat at a body density of 1.0030 kg/L.

Table 3. Pearson's product-moment correlation coefficients between body density and (the sum of) skinfolds (mm) or the body mass index (kg/m²) in elderly men and women.

	Women	Men
Triceps	-0.28 †	-0.26 *
Biceps	-0.27 †	-0.29 *
Sub-scapula	-0.39 ‡	-0.33 †
Supra-iliaca	-0.43 ‡	-0.37 ‡
Para-umbilica §	-0.42 ‡	-0.07
Quadriceps ‖	-0.40 †	-0.19
Fibula ¶	-0.28 *	-0.18
Sum of 2	-0.29 †	-0.31 †
Sum of 4	-0.40 ‡	-0.38 ‡
Body mass index	-0.61 ‡	-0.52 ‡

* $P<0.05$, † $P<0.01$, ‡ $P<0.001$; § 125 women and 70 men; ‖ 62 women and 39 men; ¶ 59 women and 33 men; sum of 2 = $^{10}\log$ (biceps + triceps); sum of 4 = $^{10}\log$ (biceps + triceps + sub-scapula + supra-iliaca); skinfolds are $^{10}\log$ transformed.

External validation of the developed prediction formulas was carried out using the body composition data from an independent sample of 23 elderly subjects. The main characteristics of this population are presented in Table 6. The supra-iliaca skinfold and the sub-scapula skinfold were not measured in this population. Therefore, prediction model 2, using the sum of four skinfolds, could not be validated. Body density of these elderly subjects, predicted by model 1 and model 3, was not statistically different from the measured value (Table 6). The differences were -0.0072 and -0.0054 kg/L for the two models respectively in women, and -0.0011 and -0.0055 kg/L respectively in men. When these values were expressed as percentage body fat the differences were $+3.5 \pm 7.3\%$ and $+2.5 \pm 5.9\%$ in women, and $+0.5 \pm 5.3\%$ and $+2.5 \pm 5.2\%$ in men.

DISCUSSION

The subjects in the present study were volunteers recruited in the surroundings of Wageningen. The sample is therefore not representative of the elderly in the Netherlands. However, comparison of weight and height of the study sample with data from the Central Bureau of Statistics revealed that the study sample was not very different from the general elderly population (aged 60 years or more) in the

Table 4. Multiple linear regression of body density against gender, skinfold thickness and body mass index in the two random groups and in the total population.

	Group 1	Group 2	Total
Model 1			
Gender *	0.0183 ± 0.0033†	0.0189 ± 0.0031	0.0186 ± 0.0023
^{10}Log(sum of 2)	-0.0359 ± 0.0103	-0.0248 ± 0.0092	-0.0300 ± 0.0069
Intercept	1.0567 ± 0.0151	1.0406 ± 0.0137	1.0481 ± 0.0102
R^2	0.54	0.56	0.55
SEE	0.0121	0.0114	0.0117
Model 2			
Gender	0.0213 ± 0.0025	0.0213 ± 0.0025	0.0212 ± 0.0018
^{10}Log(sum of 4)	-0.0440 ± 0.0087	-0.0281 ± 0.0080	-0.0356 ± 0.0059
Intercept	1.0842 ± 0.0160	1.0551 ± 0.0149	1.0688 ± 0.0109
R^2	0.58	0.58	0.58
SEE	0.0115	0.0111	0.0113
Model 3			
Gender	0.0223 ± 0.0021	0.0226 ± 0.0021	0.0226 ± 0.0015
Body mass index	-0.0026 ± 0.0003	-0.0018 ± 0.0003	-0.0022 ± 0.0002
Intercept	1.0704 ± 0.0084	1.0517 ± 0.0079	1.0605 ± 0.0057
R^2	0.68	0.66	0.67
SEE	0.0101	0.0099	0.0100

* all independent variables contribute significantly to the models ($P<0.01$); † regression coefficient ± standard error; gender = 0 for women and 1 for men; sum of 2 = ^{10}log (biceps + triceps); sum of 4 = ^{10}log (biceps + triceps + sub-scapula + supra-iliaca); R^2 = explained variance; SEE = standard error of estimate.

Netherlands (males: 76.6 kg, 1.747 m, females: 68.3 kg, 1.644 m) [22]. The BMI and body composition data are also comparable with data from other studies carried out in elderly subjects in the Netherlands [23,24].

When prediction equations from the literature were applied to the study sample, nearly all equations underestimated body fat content. The equations including age as an independent continuous variable showed the most accurate prediction of percentage body fat [14,16]. The other equations from the literature which were generally developed in young to middle aged subjects largely underestimated body fat content. Of these equations the equation of Durnin and Womersley [10], based on skinfold thickness, showed the best result. When comparing the body fat content of the subjects in the studies of Noppa *et al.* [11] and Svendsen *et al.* [17], it is remarkable that they are much lower compared with those observed in the present study, the study of Durnin and Womersley [10] and the study of Womersley and Durnin [13]. The BMI of the subjects of the study of Svendsen *et al.* [17] is, however, comparable with the present study but body fat, determined by dual-energy x-ray absorptiometry, is much lower. These authors suggested that population specificity may have caused the large differences. It seems unlikely that differences between the Danish population and the Dutch population are the basis for these large differences. Differences in body fat content between populations are more likely to be caused by age differences and/or the different methods used to estimate body fat [25-27].

The skinfold thickness that was best correlated with body density in both elderly men and women was the supra-iliaca. A prediction model for body density based on this single skinfold and gender had an explained variance of 59% and a SEE of 0.0112 kg/L. Despite the fact that this model was comparable with the model using gender and the sum of four skinfolds, this model was not evaluated further. A prediction formula based on only one skinfold will lead to considerable errors when the skinfold is measured inaccurately or when the subjects have an unusual subcutaneous fat distribution. Therefore, only models based on more than one

Table 5. Internal cross validation of the three equations developed for the prediction of body density and body fat content in the two random groups (difference = measured - predicted).

	Group 1 (n=109)	Group 2 (n=95)
Density (kg/l)		
Densitometry	1.0120 ± 0.0176	1.0143 ± 0.0169
Predicted using model 1	1.0117 ± 0.0117	1.0147 ± 0.0139
Difference	0.0003 ± 0.0121 ns	-0.0005 ± 0.0114 ns
Predicted using model 2	1.0114 ± 0.0120	1.0152 ± 0.0145
Difference	0.0006 ± 0.0115 ns	-0.0009 ± 0.0113 ns
Predicted using model 3	1.0122 ± 0.0131	1.0139 ± 0.0153
Difference	-0.0002 ± 0.0102 ns	0.0004 ± 0.0102 ns
Body fat content (%)		
Densitometry	39.27 ± 8.47	38.17 ± 8.09
Predicted using model 1	39.28 ± 5.87	38.03 ± 6.33
Difference	-0.01 ± 5.80 ns	0.14 ± 5.42 ns
Predicted using model 2	39.37 ± 6.05	37.94 ± 6.50
Difference	-0.10 ± 5.51 ns	0.23 ± 5.32 ns
Predicted using model 3	39.17 ± 6.61	38.20 ± 6.97
Difference	0.11 ± 4.84 ns	-0.03 ± 4.76 ns

ns not significant, predicted versus estimated by densitometry.

skinfold were investigated. Models based on gender and the sum of skinfolds had higher explained variances and lower SEE than gender-specific models or models based on separate skinfolds (results not shown). Since there was no interaction between gender and the sum of skinfolds (or gender and BMI) in the elderly population, only one single prediction model was developed for both sexes combined, adding gender as a dummy variable. The model with gender and the sum of triceps, biceps, sub-scapula, and supra-iliaca as independent variables predicted body density slightly better ($R^2=0.58$, SEE=0.0113 kg/L) compared to a model using gender and the sum of the triceps and biceps skinfold ($R^2=0.55$, SEE=0.0117 kg/L). A model based on these two skinfolds has several practical advantages. The biceps and triceps skinfolds are relatively easy to measure, even when the elderly subject is confined to a wheelchair or is bedridden. Furthermore, subjects do not need to undress which is especially practical in field studies.

Table 6. External validation: population characteristics, difference (difference = measured - predicted) and the Pearson's correlation coefficients (r) between predicted and measured body density and body fat content.

	Women ($n=11$)	men ($n=12$)
Age (y)	73.3 ± 5.7	68.8 ± 4.3
Body weight (kg)	66.7 ± 6.0	79.8 ± 7.2
Height (m)	1.561 ± 0.052	1.709 ± 0.059
Body mass index (kg/m^2)	27.4 ± 1.9	27.3 ± 2.0
Sum of 2 (mm)	44.4 ± 11.1	20.2 ± 5.3
Body density (kg/L)		
Measured	1.0063 ± 0.0147	1.0291 ± 0.0120
Difference using model 1	-0.0072 ± 0.0151^{ns}	-0.0011 ± 0.0114^{ns}
r	0.01^{ns}	0.33^{ns}
Difference using model 3	-0.0054 ± 0.0121^{ns}	-0.0055 ± 0.0111^{ns}
r	0.71*	0.39^{ns}
Body fat content (%)		
Measured	42.00 ± 7.18	31.05 ± 5.63
Difference using model 1	3.48 ± 7.34^{ns}	0.46 ± 5.34^{ns}
r	0.01^{ns}	0.33^{ns}
Difference using model 3	2.54 ± 5.91^{ns}	2.51 ± 5.20^{ns}
r	0.71*	0.39^{ns}

^{ns} not significant, predicted versus measured by densitometry; * $P<0.05$.

The best prediction of body density was obtained using gender and the BMI as independent variables (R^2=0.67, SEE=0.0100 kg/L). The SEE, expressed as percentage body fat, of the models based on gender and two skinfolds, gender and four skinfolds, and gender and BMI was 5.6, 5.4, and 4.8%, respectively. These errors are comparable with reported SEE in the prediction of body fat in the literature, which range from 3.5-5.5% in old age groups.

Stature is known to decrease with age due to kyphosis and a shrinkage of the spinal vertebrae [2]. This will effect the BMI, and thus the prediction of body density from the BMI. Shrinkage of the spinal vertebrae and kyphosis was of course also present in the present population, thus this effect is already partly corrected for. To evaluate the effect of an underestimation of real stature with 0.05 m, the difference in predicted density from BMI was calculated. An underestimation of the stature by 0.05 m results in an overestimation of body fat content of only 1.9±0.3% in women and 1.5±0.2% in men. Thus, a quite large error in the measurement of stature results only in small errors in predicted body density.

In all models age was not included. Dividing the total study population into subgroups of 5-year intervals revealed no statistically significant differences between predicted and estimated density in any of the age groups. Besides, in the models containing skinfolds age was not correlated with the residual error. Thus, it seems that the relationship between body density and skinfolds is not dependent on age between 60 to 87 years. The residual error of model 3 was slightly but significantly correlated with age (r=-0.15, P=0.03). Age significantly contributed to this model which contains gender and BMI. However, including age as independent variable in this model increased the explained variance by only 1% and decreased the SEE by 0.0001 kg/L. Because of the minor decrease in prediction error and the fact that no difference between predicted and estimated body density was found in any of the 5-year age groups, age was left out of the equation.

The use of body circumferences for the prediction of body fatness in the elderly has frequently been suggested by several authors [24,28,29]. In the present study circumferences of mid-upper arm, waist, hip and thigh were also measured and related to body density (results not shown). In women the hip circumference (r = -0.56, P=0.0001), and in men the waist circumference (r=-0.58, P=0.0001) was best correlated with body density. Since there were significant interactions between all circumferences and gender, we investigated whether gender-specific equations containing circumferences were better at predicting body density compared with the models shown in Table 4. The explained variance for the best model containing one or more circumferences was 36% in women (hip and waist circumference) and 28% in men (waist circumference). In the gender-specific models some circumferences contributed significantly to models which already contained the sum of two or four skinfolds. However, the SEE of these models, which ranged from 0.0098 to 0.0116 kg/L, were comparable with the SEE of the three models in Table 4, and thus did not really improve the prediction of body density. Durnin & Womersley also reported that complex equations, including skinfolds and several limb circumferences, resulted in only minimal increases in accuracy compared with equations based on skinfolds only [10]. It was concluded that prediction equations using circumferences (together with other anthropometric measures) have no advantages over equations using only skinfolds or BMI. Difficulties in the measurement of circumferences due to a reduced elasticity of the skin and the abdominal wall in elderly subjects may be responsible.

As a reference method in the present study, hydrodensitometry was used. Despite the fact that Siri's formula is generally used to calculate body fat content in elderly subjects, it is questionable whether this is correct [21]. With increasing age the density of the fat-free mass may decrease due to demineralization of the bone and changes in body water. Using a two-compartment model with the assumption that the density of the fat-free mass is 1.100 kg/L could result in a systematic overestimation of body fat content in elderly subjects by 1-2% [9]. Moreover, with increasing fatness the relative amounts of minerals and protein in the fat-free mass may decrease [30]. Therefore, in elderly people calculation of body fat content from body density needs some care. Baumgartner *et al.* [26] investigated the difference in estimated body fat content of elderly men and women between a two- and a four-compartment model. The mean difference between the two methods was about 1.7%, and was correlated with the aqueous fraction of the fat-free mass but not with age or with the mineral fraction of the fat-free mass. This suggests that the error in percent body fat made by using a two-compartment model depends predominantly on the hydration of the fat-free mass. Based on the calculations of Deurenberg *et al.* [9,30] adjustments can be made to the Siri's formula. Therefore, body density, used as dependent variable in the present study, can be used in the adjusted or unadjusted Siri's formula to calculate body fat content. It remains that at an individual level, even after any adjustment of the

Siri's formula, an error of about 3% body fat is possible using the hydrodensitometric method [31,32].

The prediction formulas developed in the present study, based on the BMI or the sum of two or four skinfolds, were internally cross validated and also externally validated in an independent sample. These procedures revealed that the formulas are valid for the estimation of body fat content in groups of elderly subjects. As with all prediction equations, one should always be cautious when applying the formula to elderly populations that are substantially different from the present study. Individual values should always be interpreted cautiously.

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CHAPTER 3

MULTI-FREQUENCY BIOELECTRICAL IMPEDANCE FOR ASSESSING TOTAL BODY WATER AND EXTRACELLULAR WATER IN ELDERLY SUBJECTS

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ABSTRACT

The objective of this study was to investigate the utility of multi-frequency bioelectrical impedance for the estimation of total body water (TBW) and extracellular water (ECW) in an elderly population. Eighty-one women and 36 men, aged 63-87 years, volunteered as subjects. Body impedance at eight frequencies (1-1350 kHz) was measured in duplicate within one week. TBW and ECW were determined using deuterium oxide and potassium bromide dilution, respectively. Application of prediction equations from the literature, which are mostly based on impedance measurements in young and middle-aged subjects, resulted in large prediction errors of TBW and ECW which were related to the water distribution of the body. New gender-specific prediction equations for the estimation of TBW and ECW were developed for the elderly population and internally validated in random subgroups. TBW and ECW were best predicted using impedance at frequencies of 5 and 50 kHz, respectively, and by using body weight. Prediction errors for TBW were 3.1 kg (7.3%) and 2.7 kg (8.5%), and for ECW 2.2 kg (12.3%) and 1.0 kg (7.4%) for men and women, respectively. Multi-frequency impedance measurements are useful to assess TBW and ECW in groups of elderly subjects. However, the prediction errors are larger compared to young and middle-aged subjects, and are related to body water distribution. Individual errors are sometimes unacceptably large.

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INTRODUCTION

Bioelectrical impedance has become a common method to assess body composition. Several cross validation studies, carried out in young and middle-aged subjects, show that the amount of total body water (TBW) can be predicted from impedance or resistance at a frequency of 50 kHz [1-3]. The standard error of estimates in these studies range from 4.1 to 9.7%. Recent studies validated the multi-frequency impedance method for the assessment of extracellular water (ECW) and TBW from resistance or impedance at low frequencies (1 or 5 kHz) and high frequencies (50 or 100 kHz) respectively [4,5]. These studies showed that ECW and TBW could be predicted in healthy, young men and women with reasonable precision using multi-frequency impedance.

Since the bioelectrical impedance method is a simple, noninvasive field method which does not require an active cooperation of the subject, the method would be extremely advantageous for body composition assessment in an elderly population in epidemiological studies but also for clinical application, for example to determine the distribution volume for drugs [6].

Body composition changes with age: the amount of TBW and fat-free mass decreases with advancing age [7-11]. Also the ratio extra/intracellular water changes with age [8]. Since this ratio has an impact on body impedance the relation between impedance and TBW is likely to depend on age [12]. Because of the changes in body water distribution with age, one should be cautious in applying to an elderly population the customary prediction equations from the literature based on impedance measurements in young to middle-aged subjects.

Only a few studies have investigated the bioelectrical impedance method in elderly subjects. The impedance method at 50 kHz was validated against the hydrodensitometric method and deuterium oxide dilution in elderly subjects [13-16]. However, there are at present no studies evaluating multi-frequency impedance to assess TBW and ECW in elderly subjects.

This study investigated the use of multi-frequency impedance in elderly men and women, aged 63-87 years. For reference, isotope dilution methods were used. Equations from the literature were used to predict body water in this population and the predicted values were compared with the reference methods. Equations for the prediction of TBW and ECW in elderly subjects from impedance measurements were developed and validated. The relation between various variables (e.g. fluid distribution) and the residuals of the predicted values were investigated.

SUBJECTS AND METHODS

Subjects

The study was performed in 117 healthy elderly, 81 women and 36 men, aged 63-87 years. The subjects were recruited by advertisements in local newspapers and by visiting elderly homes and clubs in the surroundings of Wageningen. All subjects completed a medical questionnaire which was checked by a physician. Subjects taking diuretic drugs that could influence the state of hydration and subjects with obvious signs of oedema were excluded from the study. The experimental procedures were approved by the Medical Ethical Committee of the Department of Human Nutrition. Some characteristics of the subjects are given in Table 1.

Body composition

All measurements were carried out on the same morning. Subjects arrived at the laboratory after an overnight fast. TBW and ECW were determined by deuterium oxide (D_2O) dilution and potassium bromide (KBr) dilution respectively. At 07:30 h the tracer (a cocktail of D_2O and KBr containing an exactly weighed amount of about 12 g D_2O and 900 mg bromide as potassium bromide) was taken orally by the subjects. After a dilution time of 3 hours, in which the subjects refrained from drinking but consumed a standardised liquid meal of 1.3 MJ (365 g), a venous blood sample was drawn. The deuterium concentration was determined after sublimation of the plasma by infra-red analysis [17]. TBW was calculated from the given dose and the deuterium concentration in the sublimate, corrected for a 5% overestimation of the dilution space [18]. Bromide in plasma was determined after ultra filtration by high-performance liquid chromatography [19]. A correction of 5% was used for the Donnan effect and a correction of 10% for non extracellular dilution [18].

After the blood sampling impedance, body weight and body density were determined. Impedance was measured on the left side of the body. The subjects wore their clothes, but no shoes and socks, and were supine for maximally 10 min on a hospital bed. Four adhesive electrodes with a surface area of 5 cm^2 (Littmann

2330, 3M, St. Paul, USA) were placed on the hand and foot [1]. Impedance, resistance, reactance and phase angle was measured at eight frequencies (1, 5, 50, 100, 250, 500, 1000 and 1350 kHz) using a Xitron 4000 bioimpedance analyzer (Xitron technologies, San Diego, USA). The bioelectrical impedance measurements were repeated within one week following the same protocol. Impedance indices at different frequencies were calculated as height squared (H^2) divided by Z_f, where Z_f is the impedance at frequency f.

Body weight was measured to the nearest 0.05 kg using a digital scale (ED60-T, Berkel, Rotterdam, The Netherlands). Body height was measured by means of a microtoise to the nearest 0.001 m. Body mass index (kg/m^2) was calculated as body weight (kg) divided by height (m) squared.

Body density was determined by underwater weighing to the nearest 0.001 kg (3826MP 81, Sartorius, Göttingen, Germany) with simultaneous determination of the lung volume by a helium dilution technique (Spiro-Junior, Jaeger GmBH, Würtzburg, Germany). The measurement was carried out in quadruplicate if possible. Body fat percentage was calculated from body density using the Siri's formula [20].

Statistical methods

The data were analyzed by using the statistical program SAS (Statistical Analyses System; SAS Institute Inc., Cary, USA). Differences between men and women were tested with the Student's *t* test. The within-person and between-person day-to-day coefficient of variation of the impedance measurements were calculated using ANOVA. Differences between measured and predicted body water were tested with the Student's paired *t* test and were also related to the mean of measured and predicted body water [21]. Correlations between variables are Pearson's product-moment correlations. Stepwise multiple regression analysis was used to investigate the relation between TBW and ECW, and H^2/Z_f, height, body weight, and age as independent variables. Reactance and phase angle, and ratios of low to high frequency impedance or resistance, were also offered as independent variables. Results are shown as mean with standard deviation (mean $\pm$ SD). Two-sided P-values <0.05 were considered significant.

RESULTS

Some characteristics of the elderly men and women are shown in Table 1. Age and the body mass index were not significantly different between the two sexes.

Table 2 shows the body water parameters and the impedance values at eight frequencies. Although there was a gender difference in the absolute values of TBW and ECW, no difference was found in the ratio ECW/TBW. The standard deviation of the impedance at 1 kHz was larger compared to the other frequencies.

The within-person day-to-day coefficients of variation of impedance in women were 8.3, 2.7, 2.3 and 2.2% for the frequencies 1, 5, 50 and 100 kHz, respectively, and 2.3, 2.7, 3.4 and 3.8% for the frequencies 250, 500, 1000 and 1350 kHz, respectively. In men the within-person day-to-day coefficients of variation were 8.6, 3.1, 2.7 and 2.7% for the frequencies 1, 5, 50 and 100 kHz, respectively, and 2.7, 2.7, 2.6 and 2.9% for the frequencies 250, 500, 1000 and 1350 kHz, respectively.

TBW was predicted using several prediction equations published in the literature and compared with TBW as determined with deuterium oxide dilution. The absolute and proportional differences between measured and predicted TBW are shown in Table 3.

Table 1. Population characteristics.

	Women ($n=81$)	Men ($n=36$)
Age (y)	72 ± 5	72 ± 6
Body weight (kg)	67.5 ± 9.9	78.4 ± 10.6 *
Body height (m)	1.62 ± 0.06	1.76 ± 0.06 *
Body mass index (kg/m^2)	25.9 ± 3.8	25.4 ± 3.3
Body fat content (%) †	41.5 ± 6.2	32.3 ± 5.7 *
Fat-free mass (kg) †	39.1 ± 5.4	52.5 ± 6.5 *

* $P<0.0001$ significantly different from women; † from body density.

The standard deviations of the prediction errors of TBW were between 2.8 and 4.1 kg. In the elderly women the proportional differences ranged from -0.6 to +14.8%, in men from -8.8 to +10.2%. In women the equations of Hewitt *et al.*, Heitmann, and Kushner *et al.* were best in predicting TBW [2-3,16]. However, individual prediction errors using these equations ranged from -42.6 to +29.8% (equation Hewitt *et al.*), -39.2 to +32.3% (equation Heitmann) and -45.3 to +33.4% (equation Kushner *et al.*). In men the prediction equations of Lukaski *et al.*, Hewitt *et al.*, and Heitmann were best [1-2,16]. However, in men the individual prediction errors using these equations were large; -40.9 to +11.7%, -45.3 to +29.5% and -41.0 to +11.7%, respectively. The absolute prediction errors of TBW were not associated with the mean value of measured and predicted TBW. The prediction error of ECW was positively associated with the mean value of measured and predicted ECW ($r=0.27$ and $r=0.35$ for women and men respectively, $P<0.05$) using the equation of Deurenberg *et al.* based on the impedance at 5 kHz [5]. Except for the formula of Hewitt *et al.* [16] in the elderly men, the absolute prediction errors in TBW in men as well as in women were significantly negative correlated with the ECW/TBW ratio (Table 3).

Table 2. Body water and impedance at eight frequencies.

	Women	Men
Total body water (kg)	31.6 ± 3.5	41.9 ± 5.1 †
Extracellular water (kg)	13.7 ± 1.7	17.5 ± 2.7 †
Intracellular water (kg)	17.9 ± 2.9	24.4 ± 4.3 †
ECW/TBW	0.43 ± 0.05	0.42 ± 0.06
Impedance (Ω)		
1 kHz	602 ± 99	557 ± 84 *
5 kHz	649 ± 66	554 ± 62 †
50 kHz	579 ± 59	488 ± 56 †
100 kHz	550 ± 55	463 ± 54 †
250 kHz	512 ± 53	430 ± 51 †
500 kHz	483 ± 51	407 ± 48 †
1000 kHz	443 ± 53	376 ± 45 †
1350 kHz	417 ± 56	356 ± 44 †

* $P<0.05$, † $P<0.001$ significantly different from women.

Table 3. Prediction of total body water (TBW) and extracellular water (ECW) by equations from the literature based on bioelectrical impedance measurements; absolute difference (measured minus predicted), proportional difference (between brackets) and Pearson's correlation coefficients (*r*) between the absolute difference and the ratio of intra- and extracellular water.

	Women			Men		
	mean ± SD	(%)	*r*	mean ± SD	(%)	*r*
Δ TBW (kg)						
Lukaski *et al.* [1]	-	-	-	-0.9 ± 3.4	(-2.5)	-0.48 †
van Loan *et al.* [22]	4.3 ± 3.4 ‡	(13.5)	-0.64 ‡	4.4 ± 3.1 ‡	(10.2)	-0.36 *
Hewitt *et al.* [16]	-0.3 ± 2.8	(-1.3)	-0.73 ‡	-1.1 ± 4.1	(-3.4)	-0.24
Heitmann [2]	0.5 ± 2.8	(0.9)	-0.75 ‡	-0.9 ± 3.2	(-2.8)	-0.42 *
Segal *et al.* [4]	-	-	-	-3.5 ± 3.2 ‡	(-8.8)	-0.45 †
Kushner *et al.* [3]	-0.1 ± 3.1	(-0.6)	-0.73 ‡	-1.6 ± 3.4 †	(-4.1)	-0.46 †
Deurenberg *et al.* [5][a]	4.5 ± 3.0 ‡	(13.8)	-0.72 ‡	3.2 ± 3.1 ‡	(7.4)	-0.43 †
Deurenberg *et al.* [5][b]	4.8 ± 3.0 ‡	(14.8)	-0.72 ‡	3.8 ± 3.1 ‡	(8.7)	-0.43 †
Δ ECW (kg)						
Segal *et al.* [4]	-	-	-	-1.2 ± 2.6 †	(-9.0)	0.69 ‡
Deurenberg *et al.* [5][c]	-0.6 ± 1.9 †	(-5.5)	0.27 *	0.0 ± 2.9	(-2.0)	0.56 ‡
Deurenberg *et al.* [5][d]	0.3 ± 1.1 †	(2.0)	0.58 ‡	0.5 ± 2.2	(0.6)	0.80 ‡

* $P<0.05$, † $P<0.01$, ‡ $P<0.001$; [a-d] frequencies of 50, 100, 1 and 5 kHz, respectively.

Table 4 shows the Pearson's correlation coefficients between TBW and ECW and the impedance index (H^2/Z_f), body weight, height and age. In both men and women the impedance index at 5 kHz was better correlated with body water compared to 1 kHz. No clear differences in correlation coefficients between the impedance indices at 5, 50 or 100 kHz and body water were found. At higher frequencies the correlation of TBW and impedance index was worse compared to 50 and 100 kHz. The prediction of TBW and ECW in the elderly men and women was investigated using multiple linear regression analysis (Table 5). For both TBW and ECW impedance index was first selected in the regression procedure, followed by body weight, except for men in the prediction of ECW. In both men and women the impedance index at 5 kHz was selected for the prediction of ECW, the impedance index at 50 kHz was selected for TBW. Impedance indices at frequencies higher than 100 kHz did not better predict TBW. Reactance and/or phase angle were not selected in the prediction model, nor were ratios of low- to high-frequency impedance or resistance.

Table 4. Pearson's correlation coefficients between total body water (TBW) or extracellular water (ECW) and bioelectrical impedance indices at different frequencies, body weight, height or age.

	Women		Men	
	TBW	ECW	TBW	ECW
H^2/Z_1	0.05	0.21	0.54 †	0.48 †
H^2/Z_5	0.59 ‡	0.76 ‡	0.76 †	0.61 †
H^2/Z_{50}	0.59 ‡	0.76 ‡	0.78 ‡	0.60 ‡
H^2/Z_{100}	0.59 ‡	0.75 ‡	0.77 ‡	0.59 ‡
H^2/Z_{250}	0.58 ‡	0.74 ‡	0.76 ‡	0.58 †
H^2/Z_{500}	0.53 ‡	0.74 ‡	0.74 ‡	0.57 †
H^2/Z_{1000}	0.42 ‡	0.68 ‡	0.66 ‡	0.53 †
H^2/Z_{1350}	0.32 *	0.61 ‡	0.55 †	0.47 *
Body weight	0.55 ‡	0.66 ‡	0.73 ‡	0.42 *
Body height	0.26 *	0.33 *	0.53 †	0.38 *
Age	-0.16	-0.13	-0.30	-0.14

* $P<0.05$, † $P<0.001$, ‡ $P<0.0001$; H^2/Z_f = impedance index = height squared divided by impedance.

The residuals of the developed prediction equations were significantly correlated with the ECW/TBW ratio in both men and women; for TBW r=-0.71, P=0.0001 for women and r=-0.48, P=0.003 for men (Figure 1); for ECW r= +0.59, P=0.0001 for women and r= +0.77, P=0.0001 for men (Figure 2). This relation remained significant after adjustment for the relation between the residuals and TBW (or ECW) or after adjustment for body fatness. The residuals were also related to the mean value of measured and predicted body water. For TBW r=0.51, P=0.0001 (women) and r=0.34, P=0.04 (men), for ECW r=0.34, P=0.002 (women) and r=0.54, P=0.0006 (men). The residuals were not related to age.

DISCUSSION

This is the first study investigating multi-frequency bioelectrical impedance for the assessment of body water compartments in apparently healthy, elderly men and women. ECW and TBW were best predicted by the impedance at frequencies of 5 kHz and 50 kHz respectively, and body weight. This confirmed the results of studies in young subjects that ECW is best predicted by impedance at a low frequency, and TBW at higher frequencies. However, the prediction errors were related to body water distribution and were sometimes very large in individuals. The group of subjects was a non-representative sample of the elderly population in the Netherlands. Only elderly with no signs of oedema and not using drugs that could influence the hydration state of the body participated in the study. Therefore, the results of this study cannot be extrapolated to patients or subjects with a disturbed water distribution.

The within-person day-to-day coefficient of variation of impedance was 2.3% in the elderly women and 2.7% in the men at a frequency of 50 kHz. These figures are comparable with the variation found in younger subjects; 2% [1], 2.8% [23], 2.2% [24], and 2.9% [22]. This suggests that the reproducibility of the bioelectrical impedance method is the same in young and older subjects. The coefficient of variation of impedance at 1 kHz was larger compared to the other frequencies. Since at low frequencies mainly ECW is measured, and since changes in body water (body weight) over a period of 2-7 days mainly reflect a change in ECW, the variation in impedance at low frequencies is likely to be higher compared

Table 5. Multiple linear regression of total body water (TBW) and extracellular water (ECW) as dependent variable against the impedance index (and body weight).

	Women		Men	
	coefficient ± SE	*P*	coefficient ± SE	*P*
TBW				
H^2/Z_{50}	0.2715 ± 0.0714	0.0003	0.3228 ± 0.0930	0.002
Body weight	0.1087 ± 0.0379	0.005	0.1652 ± 0.0718	0.03
Intercept	11.9 ± 2.7	0.0001	8.3 ± 4.3	0.06
R^2	0.41		0.66	
SEE	2.7		3.1	
ECW				
H^2/Z_5	0.1998 ± 0.0286	0.0001	0.2249 ± 0.0498	0.0001
Body weight	0.0571 ± 0.0138	0.0001	- -	-
Intercept	1.7 ± 1.0	0.095	4.8 ± 2.8	0.097
R^2	0.65		0.39	
SEE	1.0		2.2	

H^2/Z_f = impedance index = height squared divided by impedance at 50 or 5 kHz; R^2 = explained variance; SEE = standard error of estimate.

to higher frequencies. Furthermore, at low frequencies the impedance might be influenced by the type of electrodes used and by technical limitations of the instrument [25].

Most studies relate TBW to impedance measured at 50 kHz. Hoffer *et al.* reported a strong correlation between TBW and H^2/Z_{100} [26]. The work of Jenin *et al.*, in which a bipolar, needle electrode instrument was used instead of a tetrapolar, surface electrode device, shows that at a low frequency (1 or 5 kHz) ECW volumes are determined, and at high frequencies (100 kHz or 1 MHz) total water volumes [27]. These results are partly confirmed by Segal *et al.* and Deurenberg *et al.*, who studied the relation between resistance or impedance at various frequencies and body water in young men and women [4,5].

In this study the correlation between H^2/Z_5 and ECW was slightly stronger than between H^2/Z_{50} or H^2/Z_{100} and ECW. The correlation between H^2/Z_1 and ECW was much lower than between H^2/Z_5 and ECW, probably due to the larger within-subject variation of the impedance at 1 kHz. However, after correction for

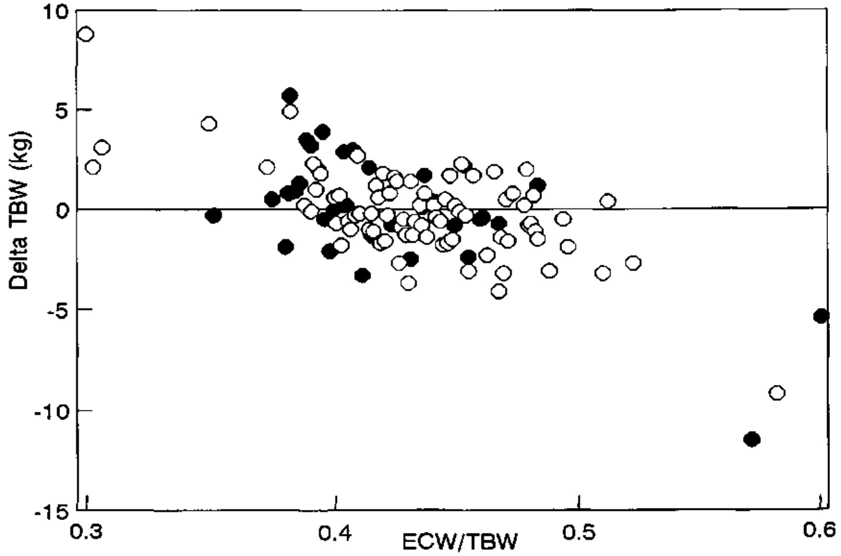

Figure 1. The relation between the prediction error of total body water (TBW) from the developed prediction equation and the ratio of extracellular to total body water (ECW/TBW) for men (●) and women (○).

attenuation the correlation between ECW and H^2/Z_1 changed only from 0.21 to 0.24 for women and from 0.48 to 0.53 for men. The fact that the relation with H^2/Z_5 is better than with H^2/Z_1 may be explained by instrumental limitations, although electrical properties of current at low frequencies may also be responsible [28]. Mathematical modelling techniques to calculate theoretical impedance values at frequency zero and infinitive were not used in the present study. Since the coefficient of variation of the calculated impedances after modelling was shown to be 1-3%, advantages of the technique on the prediction of body water are unlikely [29]. Further, a study of Van Loan *et al.* [30], in which modelling was applied, revealed a SEE of predicted ECW and TBW of 0.95 and 2.28 L respectively, which is comparable with the prediction error from other studies not modelling the data of young subjects.

The strong correlation between ECW and the impedance index at 5 kHz in this population could be caused by the high correlation between ECW and TBW ($r=0.54$, $P=0.0001$ in women; $r=0.52$, $P=0.001$ in men) and between TBW and

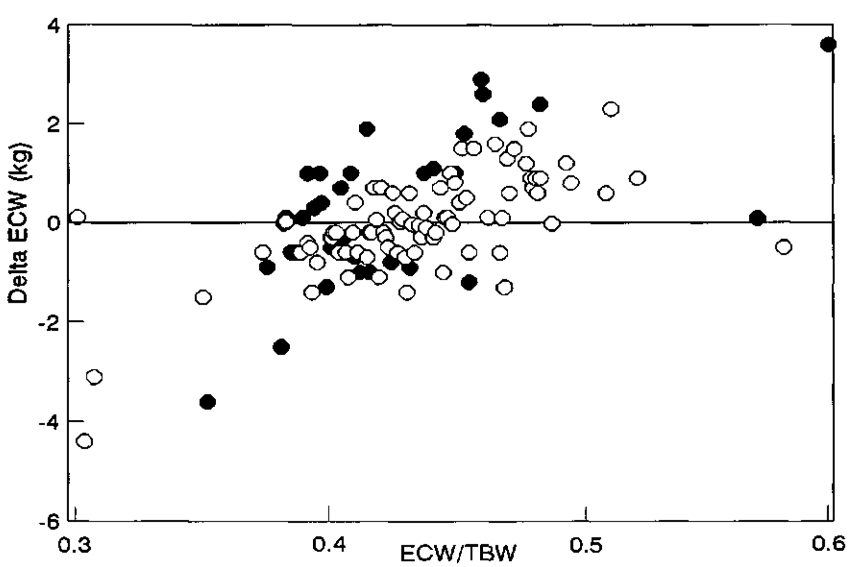

Figure 2. The relation between the prediction error of total body water (TBW) from the developed prediction equation and the ratio of extracellular to total body water (ECW/TBW) for men (●) and women (○).

H^2/Z_5 (Table 4). However, the partial correlation coefficient between H^2/Z_5 and ECW, after controlling for TBW, remained significant and was 0.65 ($P=0.0001$) in women and 0.40 ($P=0.02$) in men. In contradiction to the results of Segal *et al.* [4] and Deurenberg *et al.* [5], the partial correlation between H^2/Z_{50} or H^2/Z_{100} and ECW, after controlling for TBW, also remained significant; $r=0.64$ ($P=0.0001$) in women and $r=0.36$ ($P=0.04$) in men. This suggests that H^2/Z_5 and H^2/Z_{50} do not measure distinctly independent components of body water. This can be explained by the fact that ECW is part of TBW and will therefore contribute to the impedance at high frequencies. This effect is possibly stronger in elderly subjects since the ratio ECW/TBW is likely to increase with age.

When equations from the literature were applied to predict TBW, the prediction errors were large compared to prediction errors of the equations in younger populations and were also correlated with the ECW/TBW ratio. The correlation between the prediction error of TBW and age, after controlling for TBW, was only significant when using the equations of Van Loan *et al.* and Deurenberg *et al.* [5,22]. This implicates that prediction equations based on impedance measurements in young subjects may not be applicable to an elderly population.

Several factors can explain the large prediction error when applying to elderly subjects prediction equations based on impedance measurements in young to middle-aged populations. First, the ECW/TBW ratio increases with increasing age [31,32]. In the present study the impedance index was positively correlated with the ECW/TBW ratio at all frequencies in women. This correlation became even more significant ($r=0.65$, $P=0.0001$ at 50 kHz) after controlling for the relation ($r=-0.38$, $P=0.0005$) between TBW and the ECW/TBW ratio by partial correlation. In men the correlation between the ECW/TBW ratio and the impedance index was significant ($r=0.43$, $P=0.01$ at 50 kHz) after controlling for TBW. This suggests that the impedance index is influenced by the distribution of water in the body. Due to the increase in the ECW/TBW ratio with increasing age, and the dependency of the impedance index on this ratio, the prediction of TBW is biased (overestimated) when using prediction equations based on younger subjects.

Although the observed ECW/TBW ratio in this study is hardly different compared to the ECW/TBW ratio observed in a younger population in our department, the variation of the ECW/TBW ratio in the elderly men and women (14.3 and 11.6%, respectively) in this study was much larger than this variation in younger subjects at our department (5.0 and 4.7% for men and women, respectively [5]). Although

subjects with signs of oedema were excluded from the study, elderly are more likely to have a slightly disturbed water distribution. As the prediction errors are related to the ECW/TBW ratio, a larger variation in ECW/TBW ratio will result in larger prediction errors of TBW and ECW.
When using the bioelectrical impedance method the height of the body is used as a measure for the length of the conductor (arm plus trunk plus leg). However, body height decreases with age, mainly due to a decrease of the length of the trunk which contains most of the body water. The impedance of the extremities, however, largely determine the whole body impedance [33] and their length will change little with age. The relative extremity length thus changes with age, which may influence the relation between the impedance index and body water with increasing age. The prediction of TBW may be biased (underestimated) in elderly subjects when using prediction formulas based on younger age groups.
Finally, since the subjects in this study were not in the fasting state at the moment of the measurement this could have effected the impedance measurements. Deurenberg *et al.* reported no effect of 200 ml tea, but a significant decrease of impedance of 4 Ω was measured after 200 ml beef tea [23]. Drinking 0.7 to 1.8 L fluid increased the resistance at 50 kHz significantly [34,35], although a decrease in resistance would be expected. After 1.25 hours the resistance was back to the baseline level [34]. Since the impedance measurements were carried out 3 hours after the meal was consumed, the discrepancy between measured and predicted body composition in the elderly subjects is not likely to be caused by the consumption of the liquid meal. Furthermore, the liquid consumption will lead to an increase of ECW in the trunk, the body segment which contributes little to impedance compared to the extremities [33].
The above mentioned factors might influence the prediction error when applying prediction equations from the literature to an elderly population. However, the impact of the individual factors remains unclear, and may be different from subject to subject.
By means of stepwise regression analysis predictors of TBW and ECW were determined (Table 5). For both men and women the impedance index at a frequency of 50 kHz was selected for the prediction of TBW while the impedance index at 5 kHz was selected for the prediction of ECW. This confirmed the results of previous studies that ECW is best predicted by impedance at a low frequency, and TBW at higher frequencies [4,5,27]. In the present study impedance at a

frequency of 50 kHz gave a better prediction of TBW compared to a frequency of 100 kHz or higher. This was also reported by Hannan *et al.* who used multi-frequency impedance in surgical patients [36]. Segal, however, reported a sub-optimal prediction of TBW at 50 kHz compared to 100 kHz [37].

The residuals of TBW were negatively correlated with the ECW/TBW ratio. The curvilinear relationship between impedance and frequency does not reach an asymptotic value at a frequency of 1000 kHz. This means that even at that frequency the cell membranes are not completely penetrated by the current. Thus, even at high frequency (> 50 kHz) not all the cell membranes are penetrated by the current and thus not all ICW is measured. This explains the negative correlation of the residuals of TBW with the ratio ECW/TBW. The residuals of ECW were positively related to the ECW/TBW ratio in both men and women. At lower frequencies, for example at 1 or 5 kHz, the curve of the relationship between impedance and frequency is rising steeply. At a frequency of 0 kHz, at which in theory only ECW is measured, the impedance will be higher than measured at 1 kHz. At 1 and 5 kHz already part of the ICW is measured. This explains the positive correlation of the residuals of ECW with the ratio ECW/TBW.

Resistance, phase angle and reactance at different frequencies were not better predictors for body water, nor did they contribute to a model already containing H^2/Z_f (and body weight). The ratios of impedance or resistance at low and high frequencies, of which Jenin *et al.* suggested could indicate the proportionality between ECW and TBW [27], did not contribute to the model. The correlation coefficient between Z_5/Z_{50} and ECW/TBW was -0.13 ($P=0.2$) for women and -0.15 ($P=0.4$) for men. Also Segal *et al.* and Deurenberg *et al.* reported that the ratio of low- to high-frequency impedance or resistance did not provide significantly better predictors of ECW, TBW or the ECW/TBW ratio [4,5].

The standard error of estimate (SEE) for the prediction of TBW using the equation developed in this study was 2.7 kg (8.5%) for women and 3.1 kg (7.3%) for men. The SEE in the elderly was relatively large compared to studies in younger subjects, an observation also described by Boileau *et al.* [14]. The SEE of TBW using the impedance prediction equation from this study is comparable with the SEE from prediction equations based on skinfold measurements or the body mass index in elderly subjects which ranged from 6.7 to 10.0% of the fat-free mass [38]. This suggest that impedance measurements are not preferable to anthropometry when estimating body composition in elderly subjects. However, the use of impedance might have some practical advantage, for example the inter-observer variability of impedance is smaller compared to skinfold measurements [39]. Furthermore, subjects do not need to undress when using bioelectrical impedance.

The developed prediction equations to predict TBW and ECW may be population specific. Furthermore, there may be ethnic or racial differences. Therefore, the equations need to be cross validated before being applied to other elderly populations.

In conclusion, the prediction of TBW and ECW in elderly subjects is biased when using equations from the literature because of the age-specificity of these equations. The standard errors of the prediction were larger compared to younger populations and the prediction errors were related to the ratio of ECW to TBW. Although the impedance index at different frequencies could not clearly discriminate between the different body water volumes, ECW and TBW were best predicted by the impedance at frequencies of 5 and 50 kHz, respectively, and body weight. Standard errors of estimate for the prediction of TBW were 7.3 and 8.5%, and for the prediction of ECW 12.3 and 7.4% for the elderly men and women, respectively. Individual errors were sometimes very large.

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CHAPTER 4

SMOKING AND PHYSICAL ACTIVITY IN RELATION TO BODY FAT DISTRIBUTION IN ELDERLY PEOPLE: THE LONGITUDINAL AGING STUDY AMSTERDAM

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Dorly J.H. Deeg

ABSTRACT

Smoking and physical activity are reported to be related to fat distribution in young and middle-aged populations, however, it is unclear whether these relationships exist in an elderly population. The authors investigated this relationship in a population-based sample of 1,178 men and 1,163 women aged 55-85 years, representative of the Dutch elderly population. Waist/hip-circumference ratio (WHR) was used as an index of fat distribution. Smoking habits and physical activity of the past 2 weeks were obtained by questionnaire. Smoking was positively related to WHR in men only. This relationship remained significant after adjustment for confounding due to age, education level, body mass index, chronic illness, and physical activity. Among men a dose-response relationship between the number of cigarettes smoked per day and WHR was observed. Physically active men had a lower WHR compared to sedentary men, while no association was observed in women. The results of this large scale study show that smoking and activity are associated with fat distribution in elderly men but not in elderly women.

INTRODUCTION

Body fatness and abdominal body fat accumulation increase with increasing age [1,2]. Abdominal obesity at older age is associated with an increased risk for cardiovascular disease and diabetes mellitus [3-10]. Therefore, attention to possible behavioral factors which may be associated with body fat distribution in an elderly population is of major importance. Furthermore, it is yet unknown whether the influence of behavioral factors, such as smoking and physical activity, on health status at older age may be partly explained by their influence on body fat distribution.

Cigarette smoking as well as physical inactivity are reported to be associated with an upper body fat distribution in both cross-sectional [11-19] and longitudinal studies [12,18,20] of young and middle-aged subjects.

Little is known about the relation of smoking and physical activity to body fat distribution in elderly people. Results from two studies show a more central obesity in cigarette smokers than non-smokers [21,22]. However, theses studies did not control for possible confounders such as physical activity level or education level. Training studies carried out in elderly subjects consistently show a decrease in body fatness, waist/hip ratio and intra-abdominal fat after intervention [23-27]. In contrast, cross-sectional studies show no relationship [28] or even a weak, inverse relationship [22] between physical activity and waist/hip ratio.

The purpose of this study is to investigate the relation between two behavioral factors, smoking and physical activity, and body fat distribution in elderly men and women. Possible confounders, such as age, education level, body mass index, and chronic illness were included in the analyses. The data are from the Longitudinal Aging Study Amsterdam, a population-based study of men and women aged 55-85 years.

SUBJECTS AND METHODS

Subjects

The Longitudinal Aging Study Amsterdam (LASA) cohort was originally recruited for the NESTOR study 'Living arrangements and social networks of older adults'. Registries of 11 municipalities in areas in the West, East, and South of the

Netherlands provided the sampling frame. Each area included one city and two or more rural municipalities. A total random stratified sample of 3,805 persons (response rate 62.3%), weighted according to expected mortality at mid-term (after 5 years) in each group (birth year 1908-12, 1913-17, 1918-22, 1923-27, 1928-32, 1933-37), was obtained for the NESTOR study. The sample reflects the national distribution of urbanization and population density [29].
After 11 months, from September 1992 through September 1993, the participants to this study were approached for the first LASA cycle, including a baseline interview (N=3107, response rate 81.7%), 2 to 6 weeks later followed by a medical interview in their homes or institutions (N=2671, response rate 86.0%). Of the 698 non-responders to the baseline interview, 123 persons (18%) died before the interview, 45 persons (6%) were inaccessible, 134 persons (19%) were not able to be interviewed, and 396 persons (57%) refused to participate in the study. Of the non-responders to the medical interview, 2% died, 19% were inaccessible, 6% were not able to be interviewed, and 73% refused [30]. Non-ambulatory elderly subjects were excluded from the present study, resulting in 2,872 respondents with a completed baseline interview, and 2,524 respondents with a completed medical interview.

Methods

During the baseline interview a questionnaire was administered by trained interviewers living in the same area as the respondents. The questionnaire included questions about socioeconomic status (education level), health status, and physical activity. During the medical interview questions about smoking habits were asked and the anthropometric measurements were carried out by trained and standardized interviewers.

Definition and measurements of variables

Body fat distribution. The waist- to hip-circumference ratio (WHR, waist circumference divided by hip circumference), was used as an index of body fat distribution. Body circumferences were measured to the nearest 0.001 m with the subject standing upright in underwear. The waist circumference was measured midway between the lower rib margin and the iliac crest at the end of a normal expiration. The hip circumference was measured at the level of the widest circumference over the greater trochanter.

Smoking status. Questions about current and former smoking habits were used to classify subjects into the following groups: never smoker, former smoker, pipe or cigar smoker, and cigarette smoker. Cigarette smokers were subdivided into three categories: smoking less than 10 cigarettes per day, smoking 10 to 20 cigarettes per day, and smoking 20 cigarettes or more per day. Two women smoking pipe or cigar were included in the group smoking less than 10 cigarettes per day.
Physical activity level. To assess physical activity level, respondents were asked how often and for how long they were engaged in walking, bicycling, light and heavy household activities, sport activities, and gardening activities in the past 2 weeks. The physical activity questionnaire was based on the questionnaire of Voorrips *et al.* and the Zutphen Study questionnaire [31,32]. A score for regular physical activity (walking, bicycling and household activities) and a score for specific physical activity (gardening and sport activities) was calculated by multiplying the duration, frequency and intensity per activity in the past two weeks and summing this up. The following intensity scores for the activities were used: light housekeeping 0.703, walking and gardening 0.890, heavy housekeeping 1.368 and bicycling 1.890. The intensity scores for the sport activities ranged from 0.297 (e.g. fishing) to 1.890 (e.g. running, soccer) [31,33]. A total physical activity score was calculated by adding the score for regular and specific activities. Questions were asked whether the past 2 weeks were normal compared to the rest of the year.
Education level. Education level was classified in three categories: high (higher vocational, college or university education), middle (lower and intermediate vocational education and general secondary education) and low (completed or not completed elementary education).
Body mass index. The body mass index (BMI, weight standardized for height) was used as an index of body fatness. Height was measured to the nearest 0.001 m using a microtoise. Height of respondents who were not able to stand, who were wearing shoes or with hair disturbing the measurement was set to missing ($n = 17$). Respondents with reported signs of kyphosis, scoliosis or inability to place the head in the Frankfurt plane ($n = 182$) were included in the analysis. Body weight was measured to the nearest 0.1 kg on a bathroom scale. When respondents wore a corset or clothes during the measurement, 1 or 2 kg, respectively were subtracted from their measured body weight.
Health status. Health status was classified into four categories: no reported chronic

illnesses, one, two, and more than two reported chronic illnesses. Respondents were asked whether they had respiratory disease (asthma, chronic bronchitis, lung emphysema) or cardiovascular disease (heart disease, heart attack, arteriosclerosis), diabetes mellitus, stroke or cancer. These diseases are likely to be associated with both body fat distribution and the behavioral factors in this study. Information on diseases of bone and joints, nervous system, stomach, liver, bladder, kidney or skin, gall or kidney stones and chronic back problems were not used in the present study.

Statistical analysis

The data were analyzed using the statistical program SAS version 6.03 (Statistical Analyses System; SAS Institute Inc., Cary, USA). Subjects with missing data on waist- or hip-circumference, body weight, or body height were excluded from the statistical analysis leaving 2,341 respondents (1,163 females and 1,178 males) for the statistical analyses. The analyses were carried out for men and women separately. The relationship between WHR and possible confounders (age group, education level, quartile of BMI, and reported number of chronic illnesses) were tested using analysis of variance. The relationship between possible confounders and the behavioral factors smoking and physical activity was tested by using the chi-square test for smoking habits, and by using linear regression techniques for physical activity.

Differences in WHR between smoking groups or between quartiles of regular or total physical activity level were evaluated by using analysis of covariance. Age, education level, reported number of chronic illnesses, and BMI were adjusted for in the analysis of WHR. This analysis of covariance was repeated in respondents with or without reported chronic illnesses and in respondents <70 or $70+$ years of age. Results are expressed as means with their standard deviation (SD) or standard error (SE). All *P*-values are two-sided.

RESULTS

Baseline characteristics

Body weight, height and WHR were higher in men compared to women (Table 1). BMI was higher in women (27.4 ± 4.5 kg/m^2) than in men (26.0 ± 3.1 kg/m^2).

Among men 9.9% and among women 25.5% of the respondents was overweight (defined by having a BMI larger than 30 kg/m^2). One percent of the men and 1.5% of the women were underweight (BMI lower than 19 kg/m^2). WHR was higher in men (0.98 ± 0.07) than in women (0.91 ± 0.07).

All respondents were engaged in regular physical activities like walking, bicycling, and household activities in the 2 weeks before the baseline interview. Among men 622 respondents (52.8%) and among women 686 respondents (59.0%) participated in sports or gardening activities in the last two weeks. Men spend relatively more time on specific activities than women. The mean intensity score per minute of total activity was 1.09 ± 0.22 for men and 0.94 ± 0.16 for women. The mean intensity score per minute of regular activity was 1.01 ± 0.24 for men and 0.86 ± 0.14 for women. For 72.9% of the women and 80.0% of the men the past 2 weeks were normal compared to the last year. Main reasons for abnormality were illness and holidays. About 33% of the men and 17% of the women were

Table 1. Anthropometry, physical activity, and smoking status of the 1,178 elderly men and 1,163 elderly women with complete measurements of waist/hip ratio.

	Women	Men
Age (y)	69.3 ± 8.5	70.2 ± 8.6
Body weight (kg)	71.4 ± 12.2	78.7 ± 11.3
Height (m)	1.61 ± 0.07	1.74 ± 0.07
Body mass index (kg/m^2)	27.4 ± 4.5	26.0 ± 3.1
Waist circumference (cm)	95.8 ± 11.8	99.4 ± 10.0
Hip circumference (cm)	104.7 ± 8.9	100.9 ± 6.1
Waist/hip ratio	0.91 ± 0.07	0.98 ± 0.07
Regular activity (min/d)*†	194 ± 129	127 ± 133
Specific activity (min/d)‡§	33 ± 41	58 ± 66
Total activity (min/d)*	215 ± 136	165 ± 151
Smoking status (%)		
Never	53.5	7.9
Former	29.8	59.4
Pipe/cigar	0.2	7.5
Cigarettes < 10/d	5.2	8.7
Cigarettes 10-20/d	6.6	8.6
Cigarettes 20+/d	4.6	7.9
Unknown	0.1	0.1

* walking, bicycling and household activities; † 1162 women and 1177 men; ‡ gardening and sport activities; § 668 women and 608 men.

current smokers. The majority of men were former smoker (59%) whereas among women most had never smoked (54%).

In both men and women WHR was related to age (Table 2), older respondents having a more abdominal fat distribution than younger respondents. Respondents with a lower education level had a higher WHR. No chronic illness was reported by 54% of the men and 63% of the women. Less than four percent of the men and women suffered from three or more chronic illnesses. The reported number of chronic illnesses and BMI were both positively associated with WHR.

Smoking was negatively associated with age ($P<0.0001$) in both men and women.

Table 2. Waist/hip ratio by age group, education level, reported number of chronic illnesses, and body mass index among elderly women and men.

	Women			Men		
	n	mean	SD	*n*	mean	SD
Age group						
55-60 y	179	0.89	± 0.07	199	0.97	± 0.07
60-65	214	0.90	± 0.07	225	0.98	± 0.06
65-70	191	0.91	± 0.07	218	0.98	± 0.06
70-75	185	0.92	± 0.06	173	0.98	± 0.08
75-80	223	0.93	± 0.08	183	0.99	± 0.06
80+	186	0.94	± 0.06 †	165	0.99	± 0.07 †
Education level						
Low	357	0.92	± 0.07	587	1.00	± 0.07
Middle	628	0.91	± 0.07	484	0.98	± 0.06
High	193	0.90	± 0.07 †	90	0.96	± 0.07 †
Unknown				2		
Reported number of chronic illnesses						
None	635	0.91	± 0.07	730	0.98	± 0.06
1	382	0.92	± 0.07	292	0.99	± 0.06
2	117	0.92	± 0.07	110	0.99	± 0.08
3+	44	0.95	± 0.08 †	31	1.01	± 0.07 *
Quartile of body mass index						
1 = lean	290	0.88	± 0.07	294	0.94	± 0.05
2	291	0.91	± 0.07	295	0.97	± 0.06
3	291	0.93	± 0.06	294	1.00	± 0.05
4 = overweight	291	0.95	± 0.07 †	295	1.03	± 0.06 †

* for trend $P<0.01$; † $P<0.001$.

The percentage current cigarette smokers was 27% in the youngest women versus 7% in the oldest age group. In men these percentages were 40% and 17%, respectively. Smoking was positively associated with education in women ($P<0.0001$) and negatively associated with education in men ($P=0.003$). Men with no reported chronic illnesses tended to smoke less than men with one or more reported chronic illness ($P=0.06$). Smoking was negatively associated with BMI in both men and women. Never smokers, former smokers, and pipe/cigar smokers had a higher BMI than cigarette smokers ($P=0.0001$ for men, $P=0.02$ for women). No dose-response relation between cigarette smoking and BMI was observed in the present study ($P=0.5$ for men and $P=0.8$ for women). Respondents smoking less then 10 cigarettes per day had similar BMI compared to respondents smoking 20 or more cigarettes per day (25.0 ± 3.2 versus 25.5 ± 3.1 in men; 26.9 ± 4.7 versus 26.8 ± 3.9 in women).

Physical activity was negatively associated with age ($P<0.0001$) and reported number of chronic illnesses ($P<0.0001$ for total activity, $P<0.05$ for regular activity) in both men and women. Physical activity was not associated with education. Total physical activity was negatively associated with BMI in women ($P=0.001$), while regular physical activity was negatively associated with BMI in men ($P=0.01$).

Table 3. Adjusted least-square means of waist/hip ratio by smoking status among elderly men and women.

		SMOKING STATUS				
	n	Never	Former	Pipe/cigar	Cigarette	*P* trend
Women						
Unadjusted	1162	0.92 ± 0.00	0.91 ± 0.00†	-	0.91 ± 0.01†	0.02
Adjusted*	1159	0.92 ± 0.00	0.91 ± 0.00	-	0.92 ± 0.01	0.6
Men						
Unadjusted	1177	0.97 ± 0.01	0.98 ± 0.00	0.99 ± 0.01	0.99 ± 0.00	0.09
Adjusted*	1176	0.97 ± 0.01†	0.98 ± 0.00†	0.98 ± 0.01‡	1.00 ± 0.00	0.0001

* adjusted for age, education level, body mass index, chronic illnesses, and total physical activity level; † significantly different from cigarette smokers $P<0.0001$; ‡ $P<0.05$.

Smoking and WHR

After adjustment for age, education level, BMI, chronic illnesses, and total physical activity, we find that male cigarette smokers had a higher WHR compared to never smokers and former smokers (Table 3). Pipe or cigar smokers had a lower WHR compared to cigarette smokers. Within the group of cigarette smoking men, there was a dose-response relationship between the number of cigarettes smoked per day and WHR ($P=0.0023$). Men smoking 20 or more cigarettes per day had a higher WHR (1.00 ± 0.01) compared to men smoking less than 10 cigarettes per day (0.98 ± 0.01; $P<0.001$) and compared to men smoking 10 to 20 cigarettes per day (0.99 ± 0.01; $P<0.05$). After adjustment for possible confounders, no association between smoking status and WHR was observed in women ($P=0.6$). The findings were similar for young (< 70 years) and older (70 +) men and women. They were also similar for healthy men and for men with one or more reported chronic illness. In women with no reported chronic illnesses no association between smoking and WHR was observed. However, among women with one or more reported illnesses (n = 433) there was a significant association between smoking and WHR. Cigarette smoking women had a higher WHR (0.95 ± 0.01, $P=0.002$) compared to former smokers (0.92 ± 0.01) and never smokers (0.92 ± 0.00).

Table 4. Adjusted least-square means of waist/hip ratio by total physical activity level among elderly men and women.

		TOTAL PHYSICAL ACTIVITY LEVEL				
	n	- 1 - Light	- 2 -	- 3 -	- 4 - Heavy	*P* trend
Women						
Unadjusted	1162	0.92 ± 0.00	0.91 ± 0.00	0.92 ± 0.00	0.91 ± 0.00‡	0.03
Adjusted*	1159	0.91 ± 0.00	0.91 ± 0.00	0.92 ± 0.00	0.91 ± 0.00	0.9
Men						
Unadjusted	1177	1.00 ± 0.00	0.98 ± 0.00‡	0.98 ± 0.00§	0.97 ± 0.00§ ‖	0.0001
Adjusted*	1176	0.99 ± 0.00	0.98 ± 0.00†	0.98 ± 0.00‡	0.98 ± 0.00§	0.001

* adjusted for age, education level, body mass index, chronic illnesses, and smoking; † significantly different from light physical activity level $P<0.05$; ‡ $P<0.01$; § $P<0.001$; ‖ significantly different from level 2, $P<0.05$.

Total physical activity score and WHR

After adjustment for confounders, a negative association between total physical activity and WHR was observed in men ($P = 0.001$) (Table 4). Sedentary men had a higher WHR compared to the more active men. No association between total physical activity score and WHR was observed in women ($P = 0.98$). These findings were not different when the population was divided into healthy respondents and respondents with one or more reported chronic illness, or into young (<70 years) and older (70+) men and women.

Regular physical activity score and WHR

The results for regular physical activity score in men were similar to the results for total physical activity (Table 5). The quartile of men with the lowest regular physical activity scores had a higher WHR compared to the other three quartiles. In women no association between regular physical activity and WHR was observed ($P = 0.5$).

Table 5. Adjusted least-square means of waist/hip ratio by regular physical activity level in elderly men and women not participating in sporting or gardening activities.

		REGULAR PHYSICAL ACTIVITY LEVEL				
	n	- 1 - Light	- 2 -	- 3 -	- 4 - Heavy	*P* trend
Women						
Unadjusted	668	0.91 ± 0.01	0.91 ± 0.01	0.90 ± 0.01	0.91 ± 0.01	0.9
Adjusted*	665	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.5
Men						
Unadjusted	608	1.00 ± 0.01	0.97 ± 0.01‡	0.97 ± 0.01‡	0.97 ± 0.00‡	0.0001
Adjusted*	607	0.99 ± 0.00	0.97 ± 0.00†	0.97 ± 0.00†	0.97 ± 0.00†	0.002

* adjusted for age, education level, body mass index, chronic illnesses, and smoking; † significantly different from light physical activity level $P<0.01$; ‡ $P<0.001$.

DISCUSSION

The results of the present study show that smoking and physical activity are associated with WHR in elderly men but not in elderly women. Smoking in men was associated with a high WHR, while physical activity was associated with low WHR.

Smoking and WHR

A relationship between WHR and smoking was observed in men only. Cigarette smokers had a higher WHR, thus a more abdominal fat distribution, compared to non-smokers. These data confirm results of studies in young and middle-aged populations [12-14,16-19], and the results of two studies carried out in elderly people [21,22]. There was a clear dose-response relation between cigarette smoking and WHR in the elderly men, which was also observed in previous studies [13,19,21,22].

A positive association between smoking status and WHR, after adjustment for age and BMI, has been observed in elderly women [21]. In the present study, however, the association between smoking and WHR disappeared in women after adjustment for possible confounders. These confounders were not taken into account in the study of Barrett-Connor and Khaw [21]. Our study clearly showed an association between education and WHR and between education and smoking. In addition, associations between chronic illness and WHR, and between chronic illness and smoking were observed. These analyses suggest that both education and chronic illnesses might be confounders in the association between smoking and WHR, and should be controlled for in the analyses. Furthermore, an association between physical activity and smoking was observed in the present study. Therefore, in each analysis one should control for the other.

The association between smoking and WHR was not significant in the total population of women nor in the healthy women. The relation was however significant in the women with one or more reported chronic illnesses. Smoking women had a higher WHR compared to non-smoking women. In men the association between smoking and WHR was also stronger in the men with reported chronic illnesses. Since relatively more women reported no chronic illnesses (63%) as compared to men (54%), the association between smoking and WHR may be masked in the elderly women.

Smoking has been associated with higher levels of serum sex hormone and adrenal steroid concentrations in middle-aged and elderly men [34-36]. A dose-response relation between the number of cigarettes smoked per day and androstenedione, estrone and estradiol levels in men has also been reported [34]. Elevated levels of free testosterone have been reported to be associated with visceral fat accumulation [37,38], and could therefore explain the association between smoking and WHR. However, in men negative relationships between testosterone levels and visceral fat has been observed [38,39]. More research is needed to clarify the underlying mechanisms that may explain the relation between smoking and fat distribution, and the gender differences observed in this relation.

Physical activity level and WHR

An association between physical activity level and body fat distribution was present in elderly men. Men with a relatively low physical activity level had a more abdominal fat distribution compared to physically active men. The results did not differ when total physical activity or regular physical activity was used. These results again confirm data in the literature from younger subjects [11,15-17]. They also confirm the results of training studies in elderly men and women showing a decrease in waist circumference [23-25,27], WHR [24,27], sagittal diameter [27], and intra-abdominal fat [24].

Unlike in men, no association between physical activity and WHR was observed in women. The finding that physical activity has a greater effect on abdominal fat in men compared to women has been reported before [40]. Men generally have a more abdominal fat distribution compared to women [41]. Due to a larger amount of visceral fat, they may be more susceptible for (behavioral) influences on visceral fat. For example, an initially large visceral fat depot has been associated with a larger loss of visceral fat during weight loss [42]. Another reason for the observed gender difference may be the difference in intensity of physical activity between men and women. Not physical activity itself but rather the intensity of physical activity has been associated with WHR [15]. Men spend more time in specific activities compared to women. In addition, the intensity of their total and regular physical activities was higher. The higher intensity of activities in men may be partly responsible for the stronger effect of physical activity on WHR in men compared to women.

Although the sample from the LASA study is population based, the population used in the present study may be a relative healthy sample. Respondents with missing values for waist- or hip-circumference, height or body weight were excluded from the present analyses. Furthermore, non-ambulatory respondents were excluded. About 58% of the population in the present study reported no chronic diseases, while this percentage was 55% in the total LASA baseline sample. Therefore, the elderly population used in this study may not reflect the elderly population as a whole. For example, the observed association between smoking and WHR, which may be dependent on the health status of the respondents, could be stronger in the total population of elderly people.

In the present study it was assumed that WHR is an accurate indicator of fat distribution or visceral fat in elderly men and women. Several studies showed that WHR is strongly correlated with visceral fat as determined by computer tomography or magnetic resonance imaging in young and middle-aged men and women [43,44]. However, little is known about the validity of WHR as a measure of visceral fat in elderly people. A training study in elderly men showed small changes in WHR (2%) while much larger changes in intra-abdominal fat (20%) were observed [24]. This suggests that the WHR is a poor predictor of changes in intra-abdominal fat, which was also observed in young populations [44]. Difficulties in measuring the waist and hip circumference of elderly people due to many skinfolds or due to problems in finding the exact locations may cause the WHR to be a somewhat less accurate indicator of abdominal fat as compared with younger populations. These problems are however not likely to be sex-specific in elderly people. Studies investigating the association between WHR and intra-abdominal fat in elderly people are warranted.

Smoking data were self reported in an interviewer-administered questionnaire. Underreporting of smoking, especially in the heavy smokers, can not be excluded. However, a recent meta-analysis observed that this type of questionnaire gives accurate smoking data when compared to a biochemical validation [45].

In summary, the results of this cross-sectional study suggest that behavioral factors like smoking and physical activity are associated with fat distribution in elderly men but not in women. Cigarette smoking men and men with a low physical activity level had the highest waist- to hip ratios indicating a relative abdominal fat distribution. When studying the relationship between behavioral factors and health status in older people, attention should be paid to their possible influence on body fat distribution.

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CHAPTER 5

RESTING METABOLIC RATE AND DIET-INDUCED THERMOGENESIS IN YOUNG AND ELDERLY SUBJECTS: RELATIONSHIP WITH BODY COMPOSITION, FAT DISTRIBUTION AND PHYSICAL ACTIVITY LEVEL

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ABSTRACT

To investigate the relationship between age and energy expenditure, resting metabolic rate (RMR) and diet-induced thermogenesis (DIT, for 180 minutes after a 1.3 MJ meal) were measured by indirect calorimetry in 56 young and 103 elderly subjects. In addition, the influence of body composition, body fat distribution and physical activity level on this relationship was studied. RMR was significantly lower in elderly (3.98±0.46 and 3.33±0.39 kJ/min for men and women, respectively) than in young (5.29±0.53 and 4.08±0.33 kJ/min for men and women, respectively) subjects, which persisted after adjustment for body composition. DIT was significantly lower in older than in younger men (126±27 versus 154±34 kJ/180 min) but not in women (111±26 versus 115±25 kJ/180 min). The difference in men disappeared after adjustment for body composition. No clear relation between physical activity level and RMR or DIT was observed. These results demonstrate a relationship of age *per se* with RMR but not with DIT.

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INTRODUCTION

Knowledge of total energy expenditure (TEE) of elderly subjects is essential for determining the energy needs of an elderly population. TEE mainly consists of three parts: resting metabolic rate (RMR; ≈60-75% of TEE), energy expenditure during physical activity (≈15-30% of TEE) and diet-induced thermogenesis (DIT; ≈10% of TEE), and is likely to decrease with age [1,2]. The RMR is known to decrease with increasing age [3], mainly because of a change in body composition. The fat-free mass decreases with age and because this mass is metabolically active, the RMR is likely to decrease [4]. A lower physical activity level and hormonal changes might also contribute to a lower RMR in older subjects [5,6]. Although interindividual differences between elderly subjects are large, physical activity generally declines with age [1,7] and consequently TEE will decrease. Whether the third factor, DIT, diminishes with age is unclear. The results of studies in which the DIT of elderly men was compared with younger subjects yield contradictory results [5,8-13]. In two of these studies, the difference in DIT between the age groups was explained by differences in body composition [11] and physical activity level [13], suggesting an indirect effect of age on DIT. No data on the DIT of elderly women have been reported.

The main purpose of the present cross-sectional study was to investigate the relationship between age and RMR and DIT. In addition, the influence of body composition, fat distribution and physical activity level on this relationship was extensively studied. The present study included both young and elderly men and women.

SUBJECTS AND METHODS

Subjects

The subjects for this study were recruited by advertisements in local newspapers and posters spread in public buildings. Homes, sports clubs and associations for elderly people were visited to recruit the elderly population. All subjects completed a medical questionnaire that was checked by a physician. Heavy smokers (> 10 cigarettes per day) and subjects using medication known to influence energy expenditure were excluded from the study. The study population was composed of 103 elderly subjects (32 men, 71 women) aged 63-87 years and 56 young subjects (29 men, 27 women) aged 20-33 years. The experimental procedures were approved by the Ethical Committee of the Department of Human Nutrition of the Wageningen Agricultural University. Written informed consent was obtained from all the subjects.

Measurements of energy expenditure

Energy expenditure was measured by indirect calorimetry using an open-circuit ventilated hood system. RMR and DIT were measured on two nonconsecutive days within one week. The young women were measured in the pre-ovulatory phase of the menstrual cycle. The phase of the menstrual cycle was determined by information from the medical questionnaire and by questions on the day of the measurements.

Ventilated hood device. A transparent hood with an air inlet on top and an air outlet at the right side was placed over the head of the subject. Through the hood fresh filtered outside air was drawn by a pump (SCL210, Ocean, Dieren, the Netherlands). The air flow was measured by a thermal mass flow meter (5812N, Brooks, Veenendaal, the Netherlands) and maintained at 40 L/min by a control valve (5837, Brooks). Gas analyses were performed with, respectively, an infrared carbon dioxide analyzer (1410, Servomex, Zoetermeer, the Netherlands) and a paramagnetic oxygen analyzer (1100A, Servomex). Dried standard gases and dried filtered fresh atmospheric air were used to calibrate the analyzers. Before RMR was measured, the span point of the oxygen analyzer was controlled and was recalibrated every 60 minutes. Flow rate, carbon dioxide and oxygen concentrations were integrated over 2-minute intervals. Energy expenditure was calculated by using Weir's equation [14]. Every two weeks alcohol combustion was carried out to test the system. The reproducibility of the ventilated hood measurements was determined by six alcohol combustion tests for each ventilated hood, carried out on separate days over two weeks. Day-to-day coefficients of variation were 2.1% for oxygen consumption, 1.9% for carbon dioxide production, and 1.9% for energy expenditure.

Resting metabolic rate. After an overnight fast the subjects were brought by car to the laboratory at 07:30 h. They were instructed to avoid intensive physical activity on the day before and the morning of the measurements. During the measurements they lay in a half-supine position on a hospital bed in a thermoneutral room (temperature 21 ± 2 °C), and watched nonstressing movies. Subjects were asked to remain awake and motionless during the measurements. RMR was measured continuously for 60 minutes, the mean energy expenditure of the last 45 minutes was used as the RMR. The within-person day-to-day variations of RMR, calculated over the two separate measurement days using analysis of variance, were 4.5 and 5.6% for the younger men and women, respectively, and 7.8 and 6.0% for the elderly men and women, respectively.

Diet-induced thermogenesis. Immediately after the measurement of the RMR, the subjects consumed a liquid test meal of 365 g (1.33 MJ, 13.6% of energy from protein, 34.2% of energy from fat, and 52.2% of energy from carbohydrates) using a straw through the hood. The test meal consisted of 60% full cream yoghurt, 30% unsweetened orange juice, 7% white sugar, 1.7% soya oil, and 1.3% Protifar (Nutricia Nederland BV, Zoetermeer, the Netherlands). Protifar consists of milk protein (67% of energy), lactose (31% of energy), and milk fat (2% of energy). The meal was made in five batches and stored at -20°C. The meal was consumed within 5 minutes, after which the postprandial energy expenditure (PEE) was continuously measured for 180 minutes under the same conditions as the RMR. PEE was averaged for every 15 minutes of the measurement. The cumulative postprandial increase above the RMR over 3 hours was defined as the diet-induced thermogenesis (DIT). The DIT was expressed in absolute terms (DIT, kJ/180 min) and as a percentage of the metabolizable energy content of the test meal (DIT%). The within-person day-to-day variation of the DIT was 23.4 and 24.7% for the elderly men and women, respectively, and 31.8 and 21.8% for the younger men and women, respectively.

Anthropometry and body composition

On one of the two days, body composition was determined and the anthropometric measurements were carried out. Body weight was measured to the nearest 0.05 kg by using a digital scale (ED60-T, Berkel, Rotterdam, the Netherlands). Body height was measured with a microtoise to the nearest 0.001 m. Body mass index (BMI, kg/m^2) was calculated as body weight (kg) divided by height (m) squared.

Body composition of the elderly subjects was determined by using hydrodensitometry and isotope dilution; for the young subjects only hydrodensitometry was used. Body density was determined by underwater weighing to the nearest 0.001 kg (3826MP 81, Sartorius, Göttingen, Germany)

with simultaneous determination of the lung volume by a helium dilution technique (Spiro-Junior, Jaeger GmBH, Würtzburg, Germany). The measurement was carried out in duplicate in all young subjects and in the elderly when possible. Body density could not be measured in 24 elderly subjects and was estimated by using a prediction formula [15], based on the BMI. Body fat percentage was calculated from density using Siri's formula [16]. Total body water (TBW) was determined by deuterium oxide (D_2O) dilution only in the elderly subjects. At 07:30 h the tracer (an exactly weighed amount of ≈ 12 g D_2O) was taken orally by the subjects. After a dilution time of 4 hours, in which the subjects refrained from drinking but consumed the standardized test meal, a venous blood sample was drawn. The deuterium concentration was determined after sublimation of the plasma by infrared analysis [17]. TBW was calculated from the given dose and the deuterium concentration in the sublimate, corrected for a 5% overestimation of the dilution space [18]. Fat-free mass was calculated as TBW divided by 0.732, assuming a hydration coefficient of the fat-free mass of 0.732 [19]. Body fat was calculated as body weight minus the fat-free mass. Body fat percentage of the elderly subjects was estimated as the mean value of body fat percentage calculated from body density and from TBW.
Waist circumference was measured midway between the lower rib margin and the iliac crest at the end of a normal expiration. The hip circumference was measured at the level of the widest circumference over the greater trochanter. The circumferences were measured to the nearest 0.001 m with the subject standing upright. The waist/hip ratio (WHR) was calculated as waist circumference divided by hip circumference, and was used as a measure of body fat distribution.

Physical activity level

In the young subjects a score for physical activity was obtained by using a physical activity questionnaire [20]. This questionnaire was validated in young Dutch males and females in the age groups 20-22, 25-27 and 30-32 years. Three separate indices for leisure-time activities, sports activities, and work activities were determined. For the elderly subjects the physical activity questionnaire of Voorrips *et al.* [21], especially developed for and validated in an elderly population, was used to obtain physical activity level. A score for household, sport and leisure-time activities was calculated. A total physical activity score was calculated as the sum of the three indices. The minimum and maximum score is 2.8 and 17.4 for the questionnaire of the young subjects, and 0 and 82.3 for the elderly questionnaire. The main differences between the two questionnaires are the questions about work in the young and questions about housework in the elderly questionnaire. Furthermore, the elderly questionnaire contains questions on walking stairs and shopping, whereas these activities are assumed to contribute little to the total activity score in younger subjects. Because two different questionnaires were used, the physical activity scores could not be compared between the two age groups. The physical activity scores were used to compare RMR and DIT between tertiles of physical activity scores within each gender and age group.

Statistics

Statistical analyses were performed with the statistical program SAS (Statistical Analyses System, SAS Institute Inc., Cary, NC). Differences in age, body composition, body fat distribution, and energy expenditure between the two age groups were evaluated by using Student's unpaired two-sided *t* tests. Analysis of covariance was used to adjust for differences in body composition and body fat distribution when energy expenditure data between the two age groups were compared. Pearson's correlation coefficients were calculated to investigate the relationship between DIT and age, body composition, fat distribution, or physical activity level. Differences in RMR and DIT between tertiles of physical activity level were evaluated by using one-way analysis of variance with Tukey's correction for multiple comparisons. In the elderly women analysis of covariance was used to adjust for differences in body fat percentage and age between the three levels of physical activity. The results are expressed as mean with their standard deviation (mean $\pm$ SD). All *P*-values are two-sided.

RESULTS

Characteristics of the young and elderly men and women are summarized in Table 1. BMI, body fat percentage, and WHR were higher, whereas height and fat-free mass were lower in the elderly subjects than in the younger subjects. The elderly women had a body weight higher than that of the younger women. The physical activity score for the elderly subjects was 19.2 ± 9.9 and 18.5 ± 7.6 for men and women, respectively, and 9.1 ± 1.8 and 8.2 ± 1.3 for the young men and women, respectively. Because two different questionnaires were used, the physical activity levels could not be compared between young and elderly subjects.

Table 2 shows the RMR and DIT in the men and women of both age groups. The RMR was lower in the elderly subjects, also when expressed per kilogram of fat-free mass or body weight. After adjustment of the RMR for fat-free mass and WHR or body weight and WHR, the difference between the age groups persisted ($P=0.0001$ for women and men). The absolute DIT and DIT% were higher in the younger men than in the elderly men ($P<0.001$), whereas no differences were observed between young and elderly women ($P=0.46$ and $P=0.57$, respectively).

The net rise in energy expenditure after consumption of the test meal (PEE minus RMR) is visualized in Figure 1 for the young and elderly men and women separately. After 3 hours the energy expenditure was not completely returned to RMR level. The completeness of the DIT response was evaluated by calculating the difference (expressed as a percentage of the RMR) between the PEE during the last 15 minutes of the measurement and the RMR. These values were not different between the age groups in women (8.6±4.5% and 9.9±5.3% for the young and older subjects, respectively, $P=0.2$) but were slightly different in men (7.4±3.9% and 10.1±6.1% for young and older subjects, respectively, $P=0.03$).

Table 1. Characteristics and body composition of young and elderly men and women.

	Women		Men	
	young ($n=27$)	elderly ($n=71$)	young ($n=29$)	elderly ($n=32$)
Age (y)	23 ± 2	72 ± 5	27 ± 2	73 ± 6
Body weight (kg)	63.4 ± 6.0	66.9 ± 9.4 *	77.2 ± 9.1	78.0 ± 11.1
Body height (m)	1.71 ± 0.05	1.61 ± 0.06 †	1.85 ± 0.07	1.75 ± 0.06 †
Body mass index	21.6 ± 1.9	25.8 ± 3.8 †	22.5 ± 2.3	25.4 ± 3.4 †
Body fat content (%)	27.9 ± 4.3	38.6 ± 6.0 †	13.6 ± 5.8	30.1 ± 5.2 †
Fat-free mass (kg)	45.6 ± 3.2	40.8 ± 4.4 †	66.4 ± 7.0	54.2 ± 6.3 †
Waist/hip ratio	0.80 ± 0.05	0.92 ± 0.07 †	0.82 ± 0.05	0.97 ± 0.05 †

* $P<0.05$, † $P<0.001$ significantly different from younger subjects.

Table 2. Resting energy expenditure (RMR) and diet-induced thermogenesis (DIT) of young and elderly men and women.

	Women		Men	
	young	elderly	young	elderly
RMR				
(kJ/min)	4.08 ± 0.33	3.33 ± 0.39 †	5.29 ± 0.53	3.98 ± 0.46 †
(kJ/h/kg FFM)	5.38 ± 0.36	4.93 ± 0.60 †	4.77 ± 0.29	4.43 ± 0.53 *
(kJ/h/kg BW)	3.87 ± 0.27	3.01 ± 0.37 †	4.14 ± 0.33	3.10 ± 0.46 †
DIT				
(kJ/min)	115 ± 25	111 ± 26	154 ± 34	126 ± 27 †
(% energy of meal)	9 ± 2	8 ± 2	12 ± 3	9 ± 2 †

* $P<0.01$, † $P<0.001$ significantly different from younger subjects; FFM = fat-free mass; BW = body weight.

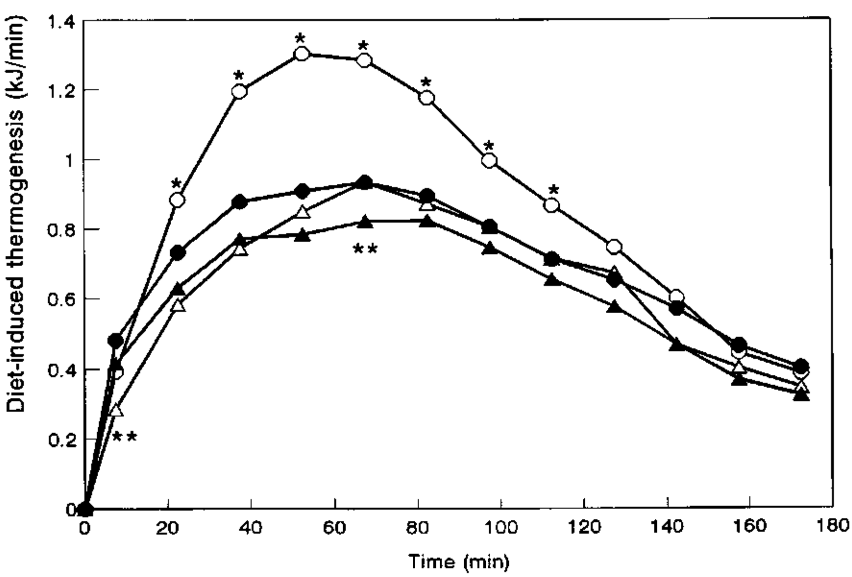

Figure 1. Diet-induced thermogenesis in young (○, $n=29$) and elderly (●, $n=32$) men, * $P<0.05$ young versus elderly subjects, and in young (△, $n=27$) and elderly (▲, $n=71$) women, ** $P<0.05$ young versus elderly subjects. The standard deviation of DIT for the four groups ranged from 0.23 to 0.33 kJ/min at timepoint 60-75 minutes, and from 0.17 to 0.20 kJ/min at timepoint 120-135 minutes.

Table 3. Pearson's correlation coefficients between diet-induced thermogenesis (DIT, kJ/180 min) and age, body composition, body fat distribution, and physical activity score in young and elderly men and women.

	Women		Men	
	young	elderly	young	elderly
Age (y)	0.36	0.18	0.23	-0.07
Fat-free mass (kg)	0.18	0.01	0.32	0.13
Fat mass (kg)	-0.06	-0.12	-0.08	-0.54 *
Body fat content (%)	-0.11	-0.15	-0.21	-0.64 *
Waist/hip ratio	0.06	-0.02	-0.21	-0.42 *
Physical activity score	0.11	0.18	0.19	-0.06

* $P<0.05$.

Table 3 shows the Pearson's correlation coefficients between the DIT and age, body composition, fat distribution, and physical activity score. Only in the elderly men was the DIT negatively associated with body composition (percent body fat and fat mass) and WHR. No relation between age and DIT was observed, probably because of narrow age ranges within each group. DIT was not related to physical activity level.

The difference in DIT between the younger and elderly men disappeared ($P=0.19$) after percent body fat and WHR were controlled for by using analysis of covariance. The age difference in the women remained insignificant ($P=0.84$) after adjustment for these indices. Also, the difference in DIT% between the age groups disappeared after adjustment for percent body fat and WHR ($P=0.19$ and $P=0.74$ for men and women, respectively). Adjustment for fat-free mass and WHR revealed comparable results.

Table 4 shows the RMR and DIT of the young and elderly men and women per tertile of physical activity level. No relationship between physical activity level and RMR or DIT was observed. After adjustment for percent body fat and age, two factors that tended to be related to the physical activity score in the older women, the differences in RMR (3.20 ± 0.08 versus 3.46 ± 0.07 kJ/min, $P=0.02$) as well as the differences in DIT (103 ± 6 versus 121 ± 5 kJ/180 min, $P=0.03$) between the lowest and highest tertile of physical activity, became significant. After adjustment, no differences in RMR or DIT between the lowest and highest tertile of physical activity were observed in the younger women or in men.

Table 4. Resting metabolic rate (RMR) and diet-induced thermogenesis (DIT) in young and elderly men and women, by tertile of physical activity level.

		Sedentary	Middle	Active
RMR (kJ/min)				
Women	young	4.06 ± 0.28	4.23 ± 0.41	3.95 ± 0.27
	elderly	3.25 ± 0.38	3.33 ± 0.37	3.39 ± 0.43
Men	young	5.20 ± 0.60	5.42 ± 0.60	5.25 ± 0.41
	elderly	3.90 ± 0.48	3.91 ± 0.43	4.11 ± 0.47
RMR/FFM (kJ/h/kg)				
Women	young	5.28 ± 0.33	5.47 ± 0.47	5.37 ± 0.25
	elderly	4.90 ± 0.55	4.99 ± 0.63	4.89 ± 0.64
Men	young	4.79 ± 0.29	4.75 ± 0.34	4.77 ± 0.28
	elderly	4.52 ± 0.78	4.39 ± 0.46	4.39 ± 0.32
DIT (kJ/180 min)				
Women	young	107 ± 19	120 ± 30	119 ± 25
	elderly	104 ± 24	107 ± 26	120 ± 28
Men	young	143 ± 37	166 ± 35	153 ± 30
	elderly	123 ± 21	126 ± 34	130 ± 27

DISCUSSION

This study showed a relationship between age and RMR, independent of body composition, in men and women. Only in men DIT was lower in the elderly subjects than in younger subjects. However, the difference in DIT or DIT% between the young and elderly men disappeared after adjustment for differences in body composition and body fat distribution. No relationship between physical activity level and absolute RMR or DIT was observed. After adjustment for differences in body composition and age between active and sedentary elderly women, the RMR and DIT were slightly higher in the active elderly women.

The day-to-day within-person coefficient of variation in the young and elderly men and women ranged from 4.4 to 7.8% for RMR, and 23.3 to 31.8% for DIT. These figures are comparable with values reported in other studies using young subjects [22-25]. The large variation in DIT emphasizes the use of large sample sizes and duplicate measurements when between-groups differences in DIT are studied. However, all previous studies investigating the relation between age and DIT used single measurements and only limited numbers of elderly subjects ($n = 7$-13). In the present study, using duplicate measurements, a difference in DIT of 20% between the two age groups could be detected with a probability of 80%.

A lower DIT in elderly subjects than in younger subjects was reported previously [5,9,10,12]. Only one study reported no effect of age on DIT [8]. Bloesch *et al.* [11] and Poehlman *et al.* [13] reported a lower DIT in elderly men than in younger men; however, the difference disappeared after adjustment for fat-free mass and physical activity level, respectively. A lower DIT in elderly subjects might be the result of age *per se*, or might be caused by other age-related factors, e.g., body composition, the distribution of body fat, or the level of physical activity. Therefore, in the present study particular attention was focused on these factors. Whether body composition is related to DIT is unclear. Some studies have reported a lower DIT in obese subjects than in lean subjects [22,26], whereas others found no relationship between DIT and body composition [13,27-29]. A lower DIT in obese subjects might be caused by their insulin resistance, through which the rate of glucose storage is decreased and therefore postprandial energy expenditure is also [22,30]. The young and elderly subjects in this study clearly differed in body composition; the elderly subjects had a higher percent body fat and a lower amount of fat-free mass. After adjustment for this difference, the age difference in DIT in men dissipated. Only in the elderly men were fat mass and percent body fat related to DIT. In the young men and women no associations were observed between body composition and DIT, probably because of the homogeneity of the young age groups.

Body composition of the elderly subjects was determined by using a combination of hydrodensitometry and isotope dilution. Because the assumptions underlying the hydrodensitometric method may be violated in elderly subjects (resulting in a possible underestimation of fat-free mass and overestimation of fat mass), a second body composition method was included for the elderly subjects. The fat-free mass calculated from the TBW data was 57.3 ± 6.7 kg in men and 42.9 ± 4.8 kg in women. The mean fat-free mass estimated from TBW was significantly higher compared with the fat-free mass calculated from the underwater weighing ($P < 0.0001$). The Pearson's correlation coefficient between estimated fat-free mass from the two methods was 0.84 ($P = 0.0001$) and 0.61 ($P = 0.0001$) for the elderly men and women, respectively. Analyses of the energy expenditure data by using the hydrodensitometric data only did, however, not change the results (data not shown).

Vansant *et al.* [31] reported a significantly higher glucose-induced thermogenesis in abdominal obese women than in gluteal-femoral obese women. Also, in the study by Leenen *et al.* [23] an accumulation of visceral fat in women was associated with a higher DIT in response to a mixed meal. Thus, the DIT might be related to the distribution of fat in the body. Because relatively more fat is accumulated internally with increasing age [32,33], the change in fat distribution could also contribute to a change in DIT with age. The WHR was significantly higher in the elderly than in the young subjects. Only in the elderly men was the DIT related to the WHR, however, in contradiction to earlier studies [23,31], this relationship was negative. In the literature, an abdominal fat distribution is often associated with an increased glucose intolerance and insulin resistance. Because DIT is also related to insulin resistance [22,30], an abdominal fat distribution might be the reason for a lower DIT in elderly subjects. The difference in DIT between the young and elderly men disappeared after adjustment for BMI and WHR, or body fat percentage and WHR, or WHR alone.

The possible influence of physical activity level on DIT has been frequently discussed. Segal *et al.* [22] and Burke *et al.* [34] reported no relation between maximal aerobic fitness and DIT, whereas Poehlman *et al.* [35] reported a lower DIT in highly trained males than in untrained males, which persisted when groups were matched for fat-free mass and body fat. Tremblay *et al.* also reported a lower DIT after a mixed meal in exercise-trained men than in nontrained men [27]. A higher DIT in active compared with sedentary young and elderly males has also been reported, independent of fat-free mass and percent body fat [13]. Lundholm *et al.* also showed a higher thermogenesis in well-trained compared with untrained elderly men [28]. Because physical activity is known to decline with age [1,7], this might influence the DIT at older ages. In this study the physical activity scores were obtained by questionnaires that were especially developed for young and elderly subjects [20,21]. Not only sports activities were asked for, but also leisure-time activities and activities during work or housework, thus an overall activity score was obtained. The activity questionnaire for the elderly provides a reliable and valid method for classifying elderly subjects into categories of high, medium and low physical activity [21]. Because the physical activity scores were obtained by different questionnaires in the young and elderly subjects, the DIT could not be adjusted for physical activity level when young and elderly subjects were compared. However, the effect of physical activity level on DIT was extensively investigated within each gender and age group.

To avoid an effect of the last exercise bout on RMR or DIT, intensive physical activity on the day and the morning before the energy expenditure measurements was forbidden. In the present study no associations between physical activity level and RMR or DIT were observed, nor were differences in absolute DIT or RMR observed between the tertiles of physical activity level. In the present study a large group ($n = 71$) of elderly women was included to evaluate differences in DIT between tertiles of physical activity with sufficient power. A large group of women was included because the relationship between physical activity level and DIT has only been investigated in men (elderly) before. Theoretically, a difference of ≈20% between tertiles could be detected with an 80% probability. No absolute differences in DIT or RMR between the tertiles of elderly women were observed. Because age ($P = 0.13$) and percent body fat ($P = 0.06$) tended to be inversely related to the level of physical activity in the elderly women, a statistical correction for these factors was carried out. After this correction the RMR and DIT were significantly higher in the active group than in the sedentary group. Because the present study shows no clear influence of physical activity level on the absolute DIT or RMR, it is unlikely that the difference in DIT between the young and elderly men could have been caused by a possible difference in physical activity level. Using the score for sport activities in stead of the total physical activity score did not change the results.

It is unclear why a difference in absolute DIT was found between young and elderly men but not in women. Because the present study is the first study reporting data on the DIT of elderly women, comparison with other studies is not possible. An additional analysis was carried out to compare the DIT of the elderly women with the DIT of a group of young women (age 29 ± 4 years) collected during another study at the same department [36]. The same ventilated hood equipment and study protocol were used in that study. The absolute DIT of these young women was 117 ± 19 kJ/180 min and not significantly different from the elderly women (111 ± 26 kJ/180 min). The macronutrient content of the test meal consumed by these young women was different (1.33 MJ, 15% of energy from protein, 30% of energy from fat, and 55% of energy from carbohydrates). Despite the higher protein content of the meal compared with the test meal used in the elderly population in the present study, no difference in DIT was observed between the

young and elderly women.
A possible biochemical background for the relationship between age and DIT might be a change in glucose homeostasis or a change in the sympathetic nervous system (SNS) activity with increasing age. Golay *et al.* reported a lower glucose-induced thermogenesis and a lower glucose oxidation in elderly men than in young men [37]. The response in blood glucose concentrations was higher in the elderly subjects. In the study of Thörne and Wahren, higher blood glucose concentrations at the end of the study period were observed in the elderly men [12]. The differences in blood glucose response in elderly subjects compared with younger subjects might be related to the differences in body composition between the two age groups [22,30]. For this reason DIT might be lower in elderly subjects. The results from the present study, in which the difference in DIT between young and elderly men disappeared after adjustment for body composition, support this hypothesis. However, whether the thermic effect of feeding is related [12,30,37] or unrelated [5] to either glucose or insulin concentrations remains unclear. A change in SNS with increasing age might be another factor influencing DIT. Thörne and Wahren reported higher noradrenaline concentrations from 60 minutes after a mixed meal in elderly men, suggesting a higher SNS activity [12]. Despite a similar appearance rate of noradrenaline, in the study of Schwartz *et al.* the elderly had lesser increments in DIT, suggesting that they were resistant to SNS stimulation [5]. These studies suggest that a blunted responsiveness to β-sympathetic stimulation in elderly subjects may account for the differences in DIT between elderly subjects and young control subjects [38,39].
In summary, the results of this study demonstrate no relationship between age *per se* and DIT. The RMR was lower in the elderly subjects than in the younger subjects, even after adjustment for differences in body composition. No clear relationship between physical activity level and RMR or DIT was observed in either young or elderly men and women.

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CHAPTER 6

ENERGY COST OF PHYSICAL ACTIVITIES IN HEALTHY ELDERLY WOMEN

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ABSTRACT

In recent studies, daily physical activity ratios (PARs) higher than the Food and Agricultural Organization / World health Organization / United Nations University (FAO/WHO/UNU) reference value 1.5 have been reported for elderly men and women. The purpose of this study was to investigate whether a high PAR in elderly subjects can be explained by a higher energy cost of physical activities (EEact). To this end, 12 elderly women aged 69-82 years, completed physical activity diaries during a two-day stay in a respiration chamber. From these diaries, total daily energy expenditure (TEE) in the calorimeter was estimated (TEEfac) using FAO/WHO/UNU PARs for physical activities and measured resting metabolic rate (RMR). TEEfac was 7.0 ± 0.9 MJ/d (PAR = 1.35 ± 0.06). TEE was also measured in the chamber (TEEcal) and was 8.3 ± 1.3 MJ/d (PAR = 1.60 ± 0.16). TEEfac was 14.8 ± 8.1% lower compared to TEEcal. To investigate whether the underestimation of TEEcal was due to a higher EEact in the elderly women as compared with the FAO/WHO/UNU references, EEact of six specific activities ranging from sitting at rest to walking on a treadmill at self-chosen speed was measured with a ventilated hood system. Individually measured PARs of the six activities were similar to FAO/WHO/UNU reference PARs. This study suggest that in elderly women a high TEEcal is not explained by EEact during nonstandardized physical activities performed at self-chosen speeds. Whether these results can be extrapolated to the free-living environment needs to be investigated further.

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INTRODUCTION

According to the Food and Agricultural Organization / World health Organization / United Nations University (FAO/WHO/UNU), the reference value for total daily energy expenditure (TEE) of elderly people is 1.5 times the resting metabolic rate (RMR) [1]. Estimates of energy requirements of elderly people are often based on this reference value. Some studies confirmed a physical activity ratio ([PAR] TEE / RMR) of 1.5 in healthy elderly subjects [2].

However, recent publications suggest that the daily PAR for healthy elderly people might be higher than the FAO/WHO/UNU reference value of 1.5. Values as high as 1.75 and 1.81 were recently reported for healthy elderly men and women, respectively [3,4]. Although the PAR is likely to be variable in elderly subjects due to different levels of physical activity [2], the results of these studies suggest that the reference value of 1.5 might not be correct for certain groups of healthy elderly people.

A possible explanation for these high PARs might be a higher energy cost of physical activities (EEact) in elderly subjects as compared with younger age groups. This was already described by Durnin and Mikulicic [5], who reported a higher energy expenditure during treadmill walking in elderly subjects as compared with young subjects. In a recent study, Voorrips *et al.* [6] reported a higher energy expenditure during treadmill walking in elderly women as compared with middle-aged subjects.

A high TEE in healthy elderly people due to a higher EEact has important implications for nutritional requirements, guidelines, and recommendations. The food and energy intake of elderly subjects should be high enough to prevent weight loss and micronutrient deficiencies but should also preclude obesity. Therefore, more data on the energy expenditure of healthy elderly are indispensable.

The purpose of this study was to investigate whether a higher PAR in elderly subjects, as compared with the FAO/WHO/UNU reference value of 1.5, could be explained by a higher EEact.

SUBJECTS AND METHODS

Subjects

Twelve women were recruited from a study population of 120 elderly men and women who had participated in a study on body composition and energy expenditure. All subjects were apparently healthy and were living in the surroundings of Wageningen. One woman lived in a residential home, and the other women were noninstitutionalized. Health status was evaluated by means of a medical questionnaire checked by a physician. Subjects who were taking medication that could influence energy expenditure, e.g. β-blockers, and heavy smokers were excluded from the study. Subjects with serious diseases or disabilities that could interfere with the performance of physical activities during the study were excluded. Except for one women (who smoked three to four cigarettes per day), all subjects were nonsmokers. The physical activity scores of the women, as determined by questionnaire [7] suggested that the elderly women were not extremely physically active. However, a large variation in the free-living physical activity level between subjects was apparent. Some characteristics of the women are listed in Table 1. All subjects were familiar with most of the methods used in this study. Other methods were thoroughly explained. All subjects provided written informed consent. The study protocol was approved by the Ethics Committee of the Department of Human Nutrition of the Wageningen Agricultural University.

Study protocol

TEE was measured on 2 consecutive days in a respiration chamber. During their stay in the chamber, subjects completed a physical activity diary. One to 4 days after these measurements, body composition, RMR, and EEact during various activities were determined. The mean time between the measurements in the respiration chamber and measurements of RMR and EEact was 3 ± 2 days.

TEE

TEE was measured using whole-body indirect calorimetry (TEEcal) [8]. Air was drawn from each chamber using an air-suction pump, and the flow was measured by a dry gas meter. Air speed was 31-53 L/min depending on body weight of the subject. Dry gas meters were calibrated before and after each calorimetric session using a Blakeslee piston pump with mercury seals. Twenty-four-hour composite air samples of room air (in duplicate) and of outside air were collected in glass tubes over mercury and analyzed volumetrically for oxygen (O_2) and carbon dioxide (CO_2) with a Sonden apparatus [9]. At the start and at the end of the measurement air samples of room air were drawn and analyzed to correct for changes in O_2 and CO_2 concentration of the calorimeter over 24 hours. Recoveries of combusted alcohol were $100.2 \pm 0.8\%$ for oxygen and $98.7 \pm 0.8\%$ for carbon dioxide. Weir's equation was used to calculate TEEcal from the amount of consumed O_2 and produced CO_2 over 24 hours [10]. TEEcal was measured on 2 consecutive days. Subjects entered the calorimeter in the evening (23:00 h), 8-9 hours before the start of the measurements (07:30 h the next morning). Immediately at the end of the first 24-hour period, the measurement of the following 24-hour period started. The ambient temperature was set at 21-22 °C for daytime and at 18-19 °C during the night, but was adjusted when the subject felt uncomfortable. Mean temperature over 24 hours was 20.7 ± 1.0 °C, and relative humidity was between 60 and 70%. The room was fully equipped with a small kitchen and water-tap, writing desk, television, radio, telephone, bed, toilet chair, comfortable chair, and cycle ergometer (type RH; Lode BV, Groningen, The Netherlands). Two airlocks served as an inlet for food and as an outlet for faeces and urine. The three meals, snacks, and drinks consumed during the stay in the calorimeter were provided, and the amount was based on the results of a dietary history [11]. Subjects kept their regular meal pattern and consumed similar foodstuffs as in the free-living situation. The activity pattern in the room was partly standardized, which consisted of 8 hours of lying down in bed and cycling five times for 15 minutes on a cycle ergometer (20 W at 40 rpm). Two subjects only cycled two to three times per day. The remaining time was spent engaged in sitting and standing activities including dressing, preparing meals, making tea or coffee, and washing dishes, which simulate light housekeeping activities. Performance of strenuous physical exercise was not allowed.

Physical activity diary

Physical activity diaries were used to estimate daily energy expenditure (TEEfac) during the stay in the respiration chamber and to compare this value with TEEcal. Daily activities were grouped into seven categories of energy expenditure to facilitate registration of activities by 5-minute intervals. The categories were as follows: (1) lying down, (2) sitting quietly or very light sitting activity, (3) light to moderate sitting activity, (4) standing or light standing activity, (5) standing activity or walking around, (6) walking activity or cycling, and (7) recreational activity [12]. Use of the diaries was extensively explained to the subjects. Subjects with difficulties understanding the method were asked to complete a test diary for 1 day at home before the TEEcal measurements. This diary was discussed afterwards to make the subjects familiar with the method. On the first page of each diary, examples of activities of each category were given to facilitate classification of daily activities. Activities difficult to classify were noted by the subjects on a separate page in the diary and discussed afterwards with the investigators. TEEfac was calculated by multiplying the sum of the minutes spent on each activity category with the PAR of each activity category and with measured RMR values. For this purpose, both FAO/WHO/UNU [1] factors and measured PARs derived from actual EEact measurements during specific activities were used (to follow). The PAR of the highest activity category, sports, could not be measured. For this category, the FAO/WHO/UNU factor was used only.

RMR and EEact

Using indirect calorimetry, RMR and EEact were measured with an open-circuit, ventilated hood system to calculate PAR factors for each individual.

Through the hood, fresh filtered outside air was drawn by a pump (SCL210; Ocean, Dieren, The Netherlands). Airflow was measured by a thermal mass flow meter (5812N; Brooks, Veenendaal, The Netherlands) and maintained by a control valve (Brooks 5837). Gas analyses were performed with an infrared CO_2 analyzer (1410; Servomex, Zoetermeer, The Netherlands) and a paramagnetic O_2 analyzer (Servomex 1100A). The analyzers were calibrated using dried standard gas mixtures and dried filtered fresh outside air. Before all metabolic measurements, the span of the oxygen analyzer was controlled. Flow rate during the RMR measurement was 40 L/min, during sitting and standing activities 80 L/min, and during bicycling and walking 120 L/min. Flow rate, and CO_2 and O_2 concentrations were integrated over 1-minute intervals. Energy expenditure was calculated using Weir's equation [10]. On a biweekly basis, alcohol combustion resting at the three different flow rates was performed to test the system. Reproducibility of the ventilated hood measurements was determined by six alcohol combustion tests for each ventilated hood, performed on separate days within a period of 2 weeks. Day-

to-day coefficients of variation were 2.1% for O_2 consumption, 1.9% for CO_2 production, and 1.9% for energy expenditure.
RMR was measured after an overnight fast. Subjects were brought by car to the laboratory at 08:00 h. They were instructed to avoid intensive physical activity on the day and the morning before the measurements. During the measurements, they were in a semisupine position on a hospital bed in a thermoneutral room (temperature 22 ± 1 °C) watching video movies. RMR was measured continuously for 60 minutes, and the results of the last 45 minutes were used. All subjects were familiar with the ventilated hood measurements.
After measurement of RMR, subjects were offered a small breakfast consisting of a cup of tea (with sugar) and one cracker with margarine and jam (about 500 kJ). Then energy expenditures during six activities were measured: (1) sitting while watching video films; (2) sitting with light arm movements, ie, at a table completing a jigsaw puzzle; (3) standing with light arm movements, ie, at a table completing a jigsaw puzzle; (4) standing with heavy arm movements, ie, moving wooden blocks (350 g) from one side of the table to the other side, thereby passing the blocks from hand to hand at eyelevel; (5) bicycling on a cycling ergometer with a load of 20 W and a pedalling frequency of 40 rpm; and (6) walking on a treadmill (Enraf Nonius, Delft, The Netherlands). To approximate the normal daily situation, all activities were performed at a self-chosen speed or frequency, except for the cycling activity. The walking activity was first practised for 5 minutes without using the ventilated hood to familiarize the subjects with walking on a treadmill and to adjust to a normal walking speed for the subject. Walking speed ranged from 0.6 to 4.5 km/h, with a mean value of 2.2 ± 1.0 km/h. Each activity was performed for 10-12 minutes. The mean energy expenditure of the last 5 minutes of each activity was used. Mean temperature of the room during the activity measurements was 20°C.

Body composition and anthropometry

All anthropometric measurements were made after voiding, with the subjects wearing swimming suit or underwear. Body weight was measured to the nearest 0.05 kg using a digital scale (ED-60-T; Berkel, Rotterdam, The Netherlands). Height was measured to the nearest 0.001 m using a wall-mounted stadiometer. Body mass index was calculated as weight in kilograms divided by height in meters squared.

Body composition was calculated from total body water measured by isotope dilution. An exactly weighed amount of deuterium oxide (about 14-15 g) was given orally to the subjects. After a dilution time of 3 hours in which subjects refrained from eating and drinking, a venous blood sample was drawn. After sublimation of the plasma, deuterium concentration was determined in the sublimate by infrared analysis [13]. Total body water was estimated from the deuterium oxide concentration in the plasma and corrected for a 5% overestimation of the dilution space [14]. Fat-free mass was calculated as total body water divided by 0.732, assuming a hydration coefficient of the fat-free mass of 0.732 [15]. Body fat was calculated as body weight minus the fat-free mass.

Statistical methods

Data were analyzed using the statistical program SAS (SAS Institute, Cary, NC). Differences between TEEcal and TEEfac were tested with paired Student's *t* tests. Correlations between variables are Pearson's product-moment correlations. Results are presented as the mean ± SD. Two-sided *P*-values less than 0.05 were considered significant.

RESULTS

Mean age and anthropometric characteristics of the 12 women are listed in Table 1. Table 2 lists results for TEE as measured in the respiration chamber. The mean PAR value of the elderly women in the respiration chamber was 1.60 ± 0.16. TEEfac, derived from the activity diaries completed in the calorimeter, showed a PAR of 1.35 ± 0.06, which underestimated TEEcal by 14.8 ± 8.1% when

Table 1. Characteristics of the subjects ($n = 12$).

Age (y)	74 ± 3
Body weight (kg)	65.4 ± 11.6
Body height (m)	1.60 ± 0.05
Body mass index (kg/m^2)	25.6 ± 4.7
Body fat content (%)	37.9 ± 9.6
Fat-free mass (kg)	39.6 ± 3.5

FAO/WHO/UNU PARs were used. The difference between TEEfac and TEEcal was positively correlated with TEEcal ($r=0.78$, $P=0.003$ using FAO/WHO/UNU PAR factors). Thus, the deviation from TEEcal was larger when energy expenditure in the chamber was higher, ie, when more physical activities were performed in the chamber. The prediction error was not related to body weight ($r=0.24$, $P=0.5$), percent body fat ($r=0.06$, $P=0.8$), RMR ($r=0.26$, $P=0.4$) or age ($r=0.43$, $P=0.16$). The physical activity pattern, as reported in the physical activity diaries by the elderly women, is listed in Table 3.

The underestimation of TEEcal using the factorial method might be caused by use of the FAO/WHO/UNU PARs. This was evaluated further by comparing PARs derived from measurements of energy expenditure at rest and during six specific physical activities with PARs for specific activities according to the FAO/WHO/UNU (Table 4). Except for the walking PAR, other values derived from actual energy expenditure measurements were less than FAO/WHO/UNU PARs. The PAR of walking activity was not different from the FAO/WHO/UNU reference value. Energy expenditure during sitting with arm activity was slightly higher as compared with the reference value.

Estimated TEEfac using these individually measured PARs was 6442 ± 707 kJ/d (PAR 1.24 ± 0.06), which is 21.6 ± 7.8% ($P<0.0001$) lower as compared with TEEcal.

Table 2. Comparison of total daily energy expenditure and derived physical activity ratio values (PAR) during the calorimetric sessions of elderly women using indirect calorimetry and FAO/WHO/UNU PAR factors for physical activities in the factorial method.

Indirect calorimetry	
TEEcal (kJ/d)	8299 ± 1294
PAR	1.60 ± 0.16
Factorial method using FAO/WHO/UNU factors and RMR	
TEEfac (kJ/d)	7015 ± 857
PAR	1.35 ± 0.06
Difference from measured TEE (%)	14.8 ± 8.1 *

* $P<0.001$, difference = (TEEcal-TEEfac / TEEcal) x 100%.

Table 3. Activity pattern in the respiration chamber according to the physical activity diary.

Activity category (min/d)	
Lying	525 ± 36
Sitting quietly or very light sitting activity	694 ± 84
Light to moderate sitting activity	0 ± 0
Standing or light standing activity	127 ± 92
Standing activity or walking around	19 ± 26
Walking activity or cycling	66 ± 21
Recreational activity	2 ± 5

DISCUSSION

In the present study, TEE of healthy elderly women was measured in a respiration chamber. Physical activity diaries, in combination with FAO/WHO/UNU PARs and RMR, underestimated TEE as measured in a respiration chamber. This underestimation could not be explained by higher EEact in these elderly women as compared with FAO/WHO/UNU reference PARs.

The study population consisted of 12 healthy elderly women, most of whom were living independently. Age, body weight, height, and BMI were comparable to values of elderly women who participated in an earlier study at our department [16]. In the previous study, mean values for these variables were 70 ± 5 years, 68 ± 10 kg,

Table 4. Energy expenditure (EEact) and physical activity ratios (PAR) at rest and during six activities of 12 elderly women.

	Measured		FAO/WHO/UNU reference
	EE (kJ/min)	PAR *	PAR
Resting	3.59 ± 0.34	- -	1.0
Sitting quietly	4.29 ± 0.37	1.20 ± 0.06 †	1.3
Moderate sitting activity	5.62 ± 0.63	1.57 ± 0.11 ‡	1.5
Light standing activity	5.64 ± 0.75	1.57 ± 0.13 †	1.8
Standing activity	7.47 ± 1.09	2.07 ± 0.20 †	2.7
Cycling	10.61 ± 1.12	2.96 ± 0.30 †	3.5
Walking	12.54 ± 2.53	3.49 ± 0.62	3.5

* PAR = EEact / measured RMR; † P=0.0001, ‡ P=0.06 different from FAO/WHO/UNU PARs.

1.62 ± 0.06 m, and 26.1 ± 3.6, respectively. Body weight was also comparable to that of elderly women of the same age living in The Netherlands: 69.8 kg for women between ages 60-69 years and 67.1 kg for women ≥70 years [17].
The PAR for elderly women in the respiration chamber was 1.60 ± 0.16. In a recent study, the PAR value of a group of younger women (mean age 28 years) in a respiration chamber was found to be 1.48 [18], which is considerably less than the PAR found in the present study group. In this study, the same equipment was used and subjects followed the same standardized activity pattern. Therefore, differences are not likely to be caused by differences in physical activity pattern. The data of Vaughan *et al.* [19] suggest a PAR of 1.39 in elderly subjects and 1.23 in younger subjects in a respiration chamber. Furthermore, in that study the PAR of the younger group was less than the PAR of older subjects, despite the same level of spontaneous physical activity (which was measured by radar units) of the two age groups. The PAR of the elderly subjects in the study reported by Vaughan *et al.* [19] is less than in the present study, which may be due to the fact that subjects in that study did not perform any strenuous activity (like bicycling). These results suggest that even with standardized activity patterns or the same level of physical activity, the PAR in elderly subjects might be higher than in younger subjects. In contrast, a study reported by Pannemans *et al.* showed similar mean PARs (1.58) during 24-hour energy expenditure measurements in young and elderly men performing nearly identical activity protocols [20]. However, in the latter investigation the intensity of the stepping exercise was less in elderly men versus younger men.
Our elderly subjects completed a physical activity diary while in the respiration chamber. When using the FAO/WHO/UNU PAR factors [1,12] and measured RMR for calculating TEEfac, TEEcal was underestimated by 14.8 ± 8.1% at group level, with individual errors between -23.4 and +7.3%. Two other studies comparing a factorial method with continuous respirometry in young men and women reported a high agreement between the two methods (within 1-2% for group results) [21,22]. Individual differences in these studies were between -11.6 and +15.1% [21] and -17 and +25% [22].
The apparent underestimation of TEEcal in the group of elderly women by the factorial method could have been caused by several factors. First, TEEcal may have been too high due to measurement errors or stress of the subjects. However, respiration equipment was calibrated regularly by alcohol burning during 24 hours,

and recoveries were 100.2 and 98.7% for O_2 and CO_2 respectively. Second, in the present study, the within-person, day-to-day variation coefficient for TEE was 3.3%, which is comparable to the values from other studies (4.6% [21] and 2.6% [23]). No significant day effect ($P=0.93$) was found between the two measurement days, and all subjects reported feeling at ease after the first 9 hours after which the actual measurements started. It would therefore appear unlikely that these factors contributed significantly to the large discrepancy observed between TEEcal and TEEfac.

Furthermore, errors may have been made in recording physical activities in the diary. However, the method was extensively explained and all subjects were able to complete the activity diary independently. Any problems were discussed immediately with the investigators. There is no reason to assume that this group of highly motivated women was less capable of completing the activity diaries than younger age groups. However, a recording bias can not be excluded in the present study.

An earlier study performed in 80 elderly women at our department showed that the within-person, day-to-day variation coefficient for RMR was 6.0% [24]. Therefore, it seems improbable that the use of a single RMR measurement could have been the cause of the large difference observed between the predicted and measured TEE, specifically since the elderly subjects were familiar with the ventilated hood equipment and the procedures used to measure RMR.

Another explanation for the discrepancy between TEEcal and TEEfac light be a large difference between RMR (as measured by the ventilated hood system) and sleeping metabolic rate ([SMR] as measured in the respiration chamber). SMR could not be measured in the present study, but a previous study in which the same equipment was used showed good agreement between RMR (4.0 ± 0.4 kJ/min) and SMR (3.8 ± 0.4 kJ/min) in young women [18]. Therefore, the difference between TEEcal and TEEfac seems unlikely to be caused by large differences between RMR and SMR.

The FAO/WHO/UNU PARs used to calculate TEEfac are based on studies in younger women and may have been too low for elderly subjects. Two studies have reported a higher energy expenditure for elderly people as compared with younger subjects during a standardized walking activity on a treadmill, even after adjustment for body weight [5,6]. In the first study, energy expenditure during treadmill walking at two different fixed speeds was compared between young and elderly subjects. Walking activity was standardized at two speed levels: 5.9 and 6.9 km/h. Energy expenditure of elderly subjects was higher, even after adjustment for differences in body weight between the two age groups [5]. The second study showed a higher energy expenditure during treadmill walking in elderly women as compared with middle-aged women [6]. In the latter study, a fixed pace was used (3 km/h). In the present study, the PAR value for walking was not different from the FAO/WHO/UNU value of 3.5. A large difference between the two above-mentioned studies and the present study is the speed of walking. In the present study, the speed of the treadmill was individually adjusted to make the energy expenditure during walking comparable to the free-living situation. Although all women were physically healthy and treadmill walking was practised until the subjects felt comfortable, the mean walking speed in our study was slower, 2.2 ± 1.0 km/h. Durnin and Mikulicic have suggested that walking, which requires use of large muscle masses, movement of many joints, and readjustment of posture, may be less regulated in elderly subjects, therefore increasing their energy expenditure [5]. Furthermore, since body weight increases and the fat-free mass decreases with increasing age, a higher body weight must be moved with relatively less muscle mass, thereby possibly increasing energy expenditure. At a speed higher than the usual pace of elderly subjects, these effects might be even stronger. This is the most probable explanation for the high energy expenditure of the elderly subjects in studies reported by Durnin and Mikulicic [5] and Voorrips *et al.* [6]. Results of the present study suggests that when activities are performed at a normal, self-chosen pace (*ie*, with a lower intensity than in younger subjects [25]), PARs of elderly subjects are equal to the FAO/WHO/UNU reference values. This was also reported by Didier *et al.* [26], in whose study elderly subjects needed a longer time to get up from and lay down on the floor or a bed as compared with young subjects, but energy expenditure was the same [26].

Another reason for the discrepancy between TEEcal and TEEfac might be the food intake of the subjects. Overfeeding the subjects during the stay in the respiration chamber may have led to an increased energy expenditure [8]. Food intake in the chamber was based on the results of a dietary history [11]. The dietary history indicated a mean energy intake of 7.2 ± 1.5 MJ/d. TEE measurements in the present study indicated a TEE of 8.3 ± 1.3 MJ/d. Although actual energy intake during the stay in the respiration chamber was not measured, these results suggest that the subjects were probably underfed (by about 12 percent) while staying in the respiration chamber. therefore, the difference between TEEcal and TEEfac seems unlikely to be caused by overfeeding the subjects. In several studies, the diet-induced thermogenesis of elderly subjects is reported to be the same as or less than that of younger subjects. Therefore, an unexpected high diet-induced thermogenesis seems unlikely to account for the difference between TEEcal and TEEfac. Furthermore, assuming that the diet-induced thermogenesis of the elderly women was 20% of TEE (or 1630 kJ in the present study) instead of the normal 10% of TEE (or 830 kJ), this difference of 830 kJ can not explain the difference between TEEcal and TEEfac (which was 1284 kJ).

As previously reported, energy requirements of elderly people are likely to vary greatly due to large variations in physical activity levels [2]. Furthermore, recent studies suggest that the PAR might be higher than 1.5 for healthy elderly subjects [3,4]. The estimation of energy requirements seems more complex than using a simple constant and multiplying this by (estimated) RMR. As a consequence, the FAO/WHO/UNU reference value of 1.5 can only be used to make a rough estimate of energy requirements.

The present study demonstrated that energy expenditure during nonstandardized physical activities in healthy elderly women was not higher as compared with FAO/WHO/UNU reference values for physical activities. Thus, the difference between measured TEE (using a respiration chamber) and estimated TEE (using a factorial method) could not be explained by higher EEact during non-standardized physical activities in elderly people. The data imply that physical activity in the respiration chamber was probably underrecorded by the elderly subjects, causing an underestimated TEE from the factorial method. Whether these results can be extrapolated to the free-living environment needs to be evaluated further.

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CHAPTER 7

VALIDATION OF DIETARY HISTORY METHOD IN A GROUP OF ELDERLY WOMEN USING MEASUREMENTS OF TOTAL ENERGY EXPENDITURE

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ABSTRACT

The objective of the study was to validate energy intake data, obtained by dietary history, in twelve elderly women 69 to 82 years of age. Energy and protein intake were obtained using the dietary history method with a reference period of 30 days. Reported energy intake was compared with total energy expenditure (TEE) measured on two consecutive days in a respiration chamber. Reported protein intake was compared with mean nitrogen excretion from four 24-hour urine collections. Mean reported energy intake was 7.2 ± 1.5 MJ/d which was lower compared to TEE ($P=0.059$). Reported protein intake was 64 ± 13 g/d and lower compared to estimated protein intake ($P=0.053$). The percentage of underestimation was not related to body weight or percent body fat. Subjects with a relatively high TEE or a relatively high estimated protein intake underestimated their energy intake to a greater extent. The discrepancy between reported energy intake and TEE was positively associated with the discrepancy between reported and estimated protein intake. The results of this study show an underestimation of energy intake of about 12% when using the dietary history method. Physical activity diaries completed in the chamber and during four days at home, as well as pedometer counts indicated a higher level of physical activity in the free-living situation compared to the chamber situation. This suggests that the actual underestimation of energy intake may be even higher in this group of elderly women. These results have implications for the use of the dietary history method in e.g. epidemiology studies carried out in elderly subjects.

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INTRODUCTION

In many epidemiological surveys dietary intake data are collected to study the intake of energy and nutrients and to investigate their relation with morbidity and mortality [1]. Assessing dietary intake of elderly subjects reveals some specific problems [2]. The 24-hour recall might be unreliable in elderly people due to a decline in short-term memory with age [3] and a weighed record can be difficult to complete. The dietary history method seems a suitable method for elderly subjects since it requires limited effort by the subjects and does not depend on short term memory [4].
Many studies validated the dietary history method in young subjects, however, few validation studies have been carried out in elderly subjects [5-9]. In most of these studies weighed records were used as reference method. However, the weighed record is sensitive to systematic bias [10]. Only one study in elderly subjects used a more objective reference method, the urinary excretion of nitrogen [5].
Another approach for evaluating energy intake data is the measurement of energy expenditure [11]. Under the assumption that the body is in energy balance, energy intake should equal energy expenditure. Measuring total, daily energy expenditure and comparing this with estimated energy intake data, can therefore give information about the validity of energy intake data. In elderly subjects only the diet record method has been compared with total energy expenditure [12-14] or with an estimation of total energy expenditure [15]. These studies showed an underestimation of the dietary intake date, ranging from 10 to 31%. A validation of the dietary history method in elderly subjects using measurements of total energy expenditure has not been reported before.

The purpose of this study was to evaluate the validity of energy intake data, obtained by dietary history, in healthy elderly women, aged 69-82 years. As a reference method twenty-four hour energy expenditure was used. In addition, urinary nitrogen excretion was measured to validate the reported protein intake and thereby energy intake.

SUBJECTS AND METHODS

Subjects

From a study population of 120 elderly men and women who had participated in an earlier study on body composition [16], twelve women were recruited. All subjects were apparently healthy, weight stable, and were living in the surroundings of Wageningen. One woman lived in a residential home, the other women were non-institutionalized. All subjects were non-smokers, except for one woman (smoking three to four cigarettes a day), and did not use any medication known to influence energy expenditure. The physical activity score of the women, as determined by means of a questionnaire [17] suggested that the elderly women were not extremely physical active and suggested a large variation in free-living physical activity level between the subjects. Some characteristics of the women are described in Table 1. The study protocol was approved by the Ethical Committee of the department of Human Nutrition of the Wageningen Agricultural University and all subjects signed an informed consent.

Study protocol

During a home visit the methods of the study were thoroughly explained. During a second home visit a dietary history was obtained. In the following week 24-hour energy expenditure (TEE) was measured on two consecutive days in a respiration chamber. On the days between the dietary history interview and TEE measurements two 24-hour samples of urine were collected at home and two 24-hour samples were collected during the calorimetric sessions to evaluate the validity of the protein intake data. To compare physical activity level in the chamber with physical activity level in the free-living situation physical activity diaries were completed during the stay in the respiration chamber and during 4 days at home. As a more objective method, number of steps were counted by a pedometer on the same days as the activity diaries were completed. Body composition was determined after the TEE measurements.

Dietary history

Energy intake was determined using a modified dietary history with a reference period of 30 days [18]. All interviews were carried out by the same trained investigator at the subject's home. Usual portion sizes were weighed to the nearest

1 g on a digital scale (GMBH PT6, Sartorius, Göttingen, Germany) or standardized household measures were used. For the calculation of energy intake and macronutrient intake the Dutch food composition table was used [19].

Total energy expenditure

TEE was measured using whole body indirect calorimetry [20,21]. Recoveries of combusted alcohol were 100.2 ± 0.8% for oxygen (O_2) and 98.7 ± 0.8% for carbon dioxide (CO_2). The equation of Weir was used to calculate TEE from the amount of consumed O_2 and produced CO_2 over 24 hours [22].

TEE was measured on two consecutive days. The subjects entered the calorimeter in the evening (23:00 h), 8-9 hours before the start of the measurements (07:30 h the next morning). Immediately at the end of the first 24-hour period, the measurement of the following 24-hour period started. The ambient temperature was set at 21-22 °C at daytime and at 18-19 °C during the night. Since the temperature was adjusted when the subjects felt uncomfortable it can be assumed that the subjects stayed in a thermoneutral room. Mean 24-hour temperature was 20.7 ± 1.0 °C. Relative humidity was between 60 and 70%. The room was fully equipped with a small kitchen and water-tap, writing desk, television, radio and telephone, bed, toilet chair, comfortable chair and cycle ergometer (type RH, Lode BV, Groningen, the Netherlands). Two airlocks served as an inlet for food and as an outlet for faeces and urine. The three meals, snacks and drinks consumed during the stay in the calorimeter were provided and the amount was based on the results of the preceding dietary history. Subjects kept their own meal pattern and consumed similar foodstuffs as in the free-living situation. The activity pattern in the room was partly standardized. The standardization consisted of 8 hours lying down in bed and cycling five times for 15 minutes on a cycle ergometer (20 W, 40 rpm). Two subjects cycled only two to three times a day. The remainder of the time was spent on sitting activities and standing activities (dressing, preparing meals, making tea or coffee, washing dishes), simulating light house-keeping. The performance of strenuous physical exercise was not allowed.

Urine collection and analysis

Twenty-four hour urine collections were made in plastic 2 litre jugs (containing 5 ml of acetic acid 100% and 15 ml water) on two days at home and while the subjects were in the respiration chamber. Collections commenced after the first void in the morning. Subjects were instructed to report incomplete urine collections to the investigators. In that case an additional set of urine jugs was given to collect 24-hour urine on another day. In the respiration chamber a toilet chair was available, thus the subjects had to collect all the urine. The investigators checked the time of the first and last void. The 24-hour urine samples were stored at -20°C prior to analysis. The urea concentration (mmol/l) was measured (Boehringer Mannheim BV 396346 kit, Almere, the Netherlands) and multiplied with urine weight to obtain the total urea production (g/day). The assumption was made that 85% of urinary nitrogen (U_N) is excreted as urea [23,24]. The protein intake (g) was estimated as $6.25 * (U_N + 2)$ [25]. A mean value of the four separate days was used to compare the estimated protein intake with the reported protein intake and thus to validate indirectly the energy intake.

Physical activity diary

TEE of the elderly women was measured in a confined situation. To compare physical activity level in the respiration chamber with the free-living physical activity level, all subjects completed physical activity diaries during their stay in the respiration chamber and also during 4 days at home (two normal weekend days and two normal weekdays). All physical activities were recorded in 5-minute intervals. To facilitate recording the physical activities were categorized into seven groups of different energy expenditure level, ranging from 'lying' to 'sports activities' [26]. Subjects who had difficulties understanding the method were asked to complete a test-diary for one day. This diary was discussed afterwards to make the subjects familiar with the method. On the first page of each diary, examples of activities of each category were given to facilitate the classification of daily activities. Activities difficult to classify were noted by the subjects on a separate page in the diary and discussed afterwards with the investigators.

Pedometer

On the day the activity diaries were completed, the subjects wore a pedometer (Kasper & Richter, Uttenreuth, Germany) to determine the number of steps a day. The pedometer was attached to a belt around the waist. Every subject used the same pedometer at home and during the stay in the respiration chamber. Step size was adjusted to 0.95 m for all subjects, as step size varies during different activities. The total recorded distance during one day was divided by the adjusted step size to calculate the number of steps per day.

Body composition and anthropometry

All anthropometric measurements were made after voiding with the subjects only wearing swimming suits or underwear. Body weight was measured to the nearest 0.05 kg using a digital scale (ED-60-T, Berkel, Rotterdam, the Netherlands). Height was measured to the nearest 0.001 m using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Body composition was calculated from total body water measured by isotope dilution. An exactly weighed amount of deuterium oxide (about 14-15 g) was given orally to the subjects. After a dilution time of 3 hours, in which the subjects refrained from eating and drinking, a venous blood sample was drawn. After sublimation of the plasma, the deuterium concentration was determined in the sublimate by infrared analysis [27]. Total body water was estimated from the deuterium oxide concentration in the plasma and corrected for a 5% overestimation of the dilution space [28]. Fat-free mass was calculated as total body water divided by 0.732, assuming a hydration coefficient of the fat-free mass of 0.732 [29]. Body fat was calculated as body weight minus the fat-free mass.

Statistical methods

The data were analyzed using the statistical program SAS (Statistical Analyses System; SAS Institute Inc., Cary, USA). Differences between reported energy intake and TEE as well as differences between reported and estimated protein intake were tested with paired Student *t* tests. Correlations between the variables are Pearson's product-moment correlations. All results are presented as mean ± standard deviation (SD). Two-sided P-values <0.05 were considered significant.

RESULTS

Table 1 summarizes some general characteristics of the study population. Table 2 gives the results of the estimated energy intake and measured TEE. Mean energy intake of the women was 7166 ± 1532 kJ/d. Reported energy intake was 11.6 ± 7.4% lower compared to TEE. Only three out of twelve women had a higher energy intake compared with TEE (Figure 1). The percentage underestimation of

Table 1. Characteristics of the 12 women participating in the study.

Age (y)	74 ± 3
Body weight (kg)	65.4 ± 11.6
Body height (m)	1.60 ± 0.05
Body mass index (kg/m²)	25.6 ± 4.7
Body fat content (%)	37.9 ± 9.6
Fat-free mass (kg)	39.6 ± 3.5

energy was not associated with body fat percentage (r=-0.31, P=0.3), BMI (r=-0.35, P=0.3) or body weight (r=-0.44, P=0.2). However, the difference between energy intake and TEE was associated with TEE (r=-0.58, P=0.049) (Figure 2). That is, the dietary history method underestimated energy intake more in subjects with a relatively high TEE.

Protein intake was 64±13 g/d as estimated by the dietary history method (Table 2). Reported protein intake was on average 10.1±7.6% lower compared to the estimated protein intake as calculated from the mean nitrogen excretion from four 24-hour urine collections. The mean weight of the urine collected at home was 2.11±0.46 kg, and not significantly different from the weight of the urine collected in the chamber (1.92±0.85 kg, P=0.25). The protein intake at home, as estimated from two 24-hour urine collections, was similar to the estimated protein intake in the chamber, 74.4±15.0 g and 74.4±14.9 g, respectively (P=1.0). The discrepancy between the estimated and reported protein intake was not related to body fat percentage (r=-0.27, P=0.4), BMI (r=-0.44, P=0.2) or

Table 2. Energy and protein intake, obtained by dietary history, versus total energy expenditure (TEE) and estimated protein intake from 24-hour urine collections in elderly women.

Energy (kJ)		
Intake	7166 ± 1532	
TEE	8299 ± 1294	
Difference	-1132 ± 537	0.059 *
Protein (g)		
Intake	64.4 ± 13.2	
Intake estimated from urine	74.4 ± 14.1	
Difference	-10.0 ± 4.6	0.053

* P value dietary history method versus reference method, difference = dietary history minus reference.

body weight (r=-0.47, P=0.1). The difference between the two methods was associated with the estimated protein intake (r=-0.62, P=0.03) (Figure 3). This suggests that the dietary history method underestimated protein intake more in subjects with a relatively high estimated protein intake. The difference of reported energy intake and TEE was positively related to the difference between reported and estimated protein intake (r=0.72, P=0.009) (Figure 4).

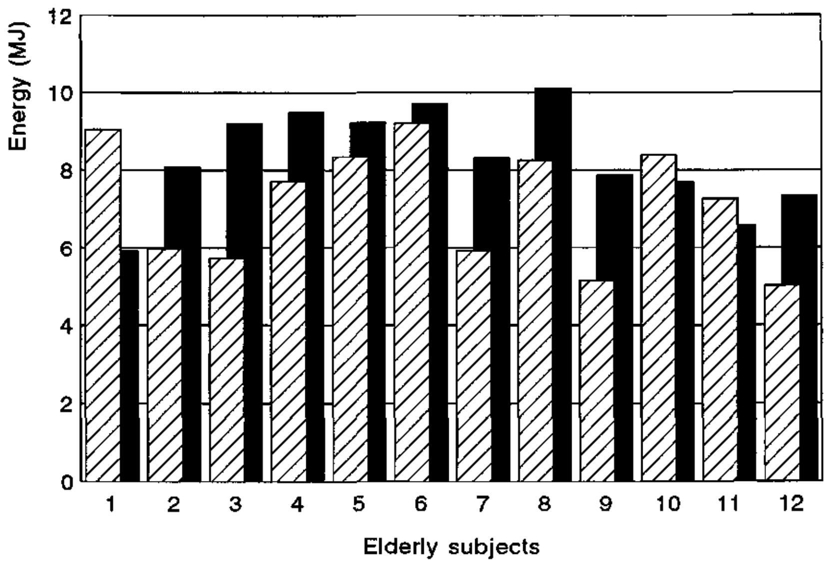

Figure 1. Energy intake (▨), obtained by dietary history, and total energy expenditure (■) of 12 elderly women.

The results of the physical activity diaries are shown in Table 3. Minutes spent per activity category in the chamber versus the free-living situation are reported. The diaries suggested that relatively more time was spend on more strenuous physical activities in the free-living situation compared to the situation in the respiration chamber. Thus, TEE in the respiration chamber might be lower than the normal TEE of the elderly women. To compare physical activity level in the chamber with the free-living situation more objectively, pedometers were used to count the number of steps on the same days as the diaries were completed (Table 3). This method also indicated a much higher physical activity level in the free-living situation.

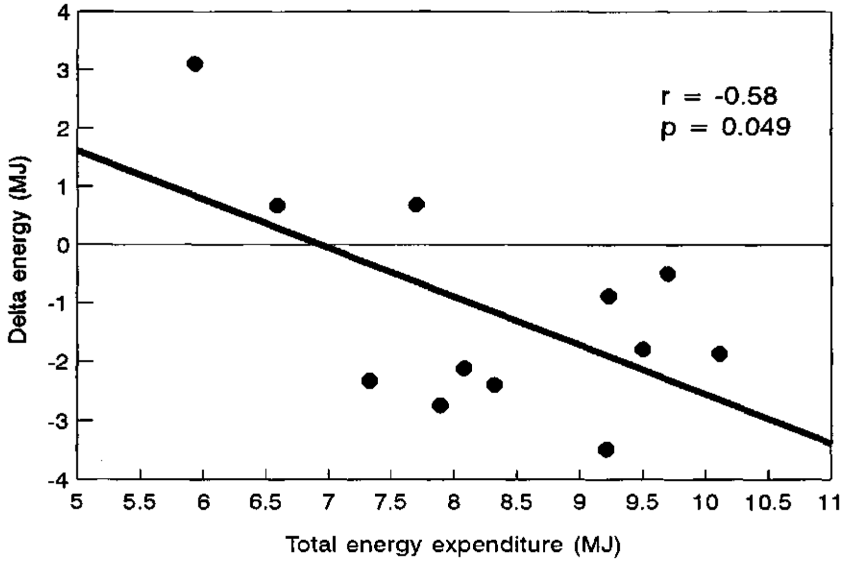

Figure 2. *Total energy expenditure versus the difference between reported energy intake and energy expenditure of 12 elderly women.*

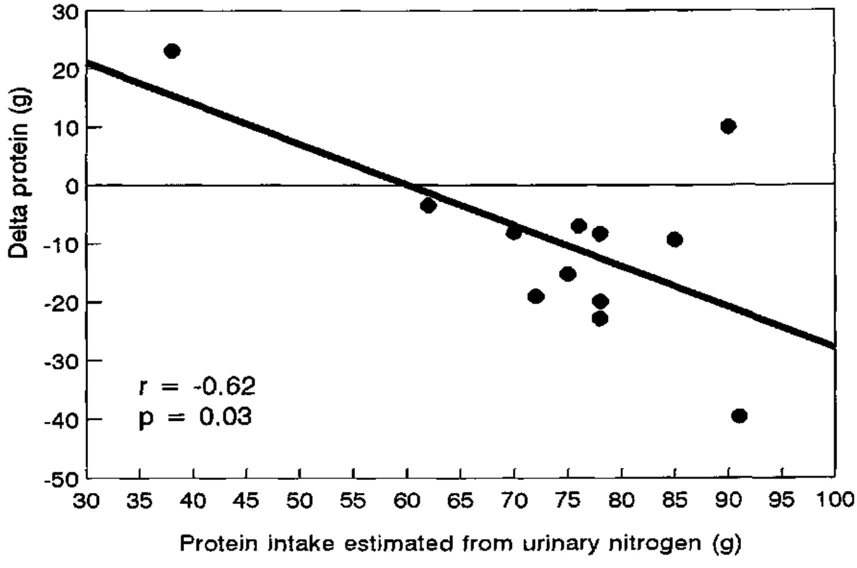

Figure 3. Protein intake estimated from urinary nitrogen versus the difference between reported reported protein intake and estimated protein intake of 12 elderly women.

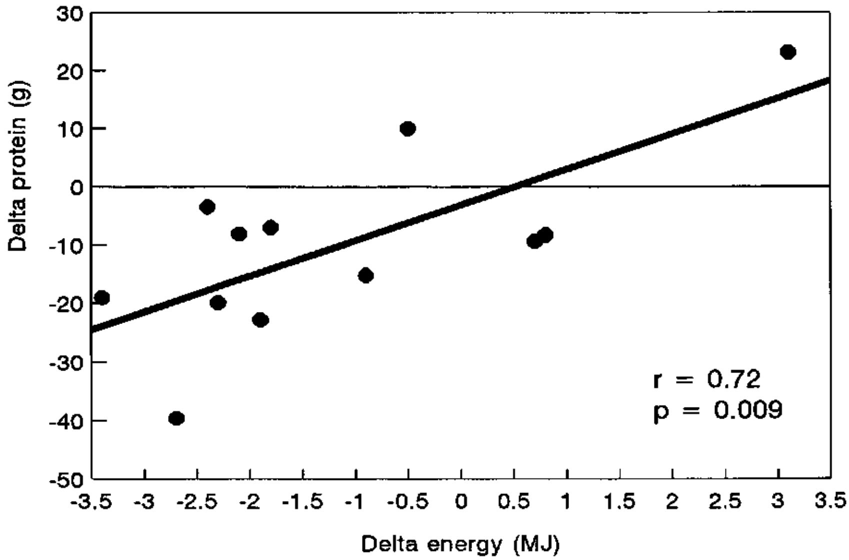

Figure 4. The over- or underestimation of protein versus the over- or underestimation of energy intake of 12 elderly women.

Table 3. Activity pattern and pedometer counts of elderly women in the respiration chamber and in the free-living situation.

	Respiration chamber	Free-living	
Activity category (min/d)			
Lying	525 ± 36	539 ± 89	0.6 *
Sitting quietly or very light sitting activity	694 ± 84	488 ± 98	0.0001
Light-to-moderate sitting activity	0 ± 0	21 ± 24	0.01
Standing or light standing activity	127 ± 92	186 ± 100	0.06
Standing activity or walking around	19 ± 26	110 ± 96	0.002
Walking activity or cycling	66 ± 21	87 ± 58	0.2
Recreational activity	2 ± 5	9 ± 9	0.03
Pedometer			
Steps per day	1749 ± 1276	7757 ± 3350	0.0001

* *P* value respiration chamber versus free-living situation.

DISCUSSION

In this study energy intake data of elderly women, estimated by means of a dietary history, were validated. Both reference methods, 24-hour energy expenditure and 24-hour urinary nitrogen excretion, indicated a considerable and comparable underestimation of reported energy and protein intake in this group of elderly women.

The study population consisted of twelve healthy elderly women, most of them living independently. Body weight, height and body composition were comparable with values of elderly women in earlier studies at the department [16]. Body weight was also comparable with body weight of elderly women of the same age living in the Netherlands: 69.8 kg for women between the ages 60-69 years and 67.1 kg for women at age 70 years and older [30]. Underestimation of energy intake is reported to be more pronounced in obese compared to lean subjects [10]. Since the women in the present study had a normal body composition for their age, and since they were all highly motivated it seems unlikely that body fatness influenced their report of energy intake to a great extent.

If it is assumed that the energy intake data in this study are correct, and that measured TEE in the respiration chamber is a good estimate of daily, free-living energy expenditure of the elderly women, nine of the twelve women would be in a negative energy balance. Since body weight of the women was measured in an earlier study, the value was compared with their present body weight. During the last 11 ± 3 months the mean change in body weight of the group was $+0.50 \pm 1.34$ kg ($P=0.22$). This indicates that the women were on average in energy balance in the last year and that the energy intake data may not reflect actual energy intake.

The mean reported energy intake of 7.2 MJ in the present study was comparable with other studies on energy intakes of apparently healthy elderly women carried out in the Netherlands. In these studies energy intakes of elderly women were 7.9 MJ (Wat eet Nederland, 1988), 7.9 MJ [32], 7.8 MJ [33], and 6.9 and 7.4 MJ for active and sedentary elderly women respectively [34]. These studies used either a two-day 24-hour diet record [31] or a dietary history method [32-34] to collect energy intake data.

In the present study estimated energy intake was only $88 \pm 26\%$ of TEE in the respiration chamber. Several studies report an underestimation of energy intake compared to TEE in elderly women [12-14]. The energy intake data in these studies were respectively 69, 73 and 90% percent of the TEE which was determined using the doubly-labelled water method. These studies used 3-day diet records [12-13] or 4-day diet records [14] to obtain the energy intake data. Recently, Johnson *et al.* [15] showed an underestimation of energy intake by a 3-day diet record by 24% in elderly women. However, these authors did not actually measure TEE but used an estimated TEE based on leisure time activities and resting metabolic rate. This suggest that not only the diet record seems to underestimate energy intake in elderly women, but that the dietary history method, which in general reports higher intakes than the record method [9] might also underestimate energy intake.

It is unlikely that the lower energy intake compared with the measured TEE could have been caused by an overestimation of TEE in the respiration chamber. The respiration equipment was calibrated regularly by alcohol burned during 24 hours, showing a recovery of 100.2 and 98.7% for O_2 and CO_2 respectively. Secondly, no significant day-effect ($P = 0.93$) was found between the two measurement days. All subjects reported to feel at ease after the first 9 hours after which the actual measurements started. The within-person day-to-day variation coefficient of total energy expenditure was 3.3%, which is comparable with the values from other studies (4.6% [35] and 2.6% [36]). Thus, it may be assumed that the TEE data are correct and may be used as a reference method.

The urinary nitrogen excretion method was used to validate reported protein intake and thus indirectly validate energy intake. In the present study a positive association was observed between reported protein and energy intake ($r = 0.58$, $P = 0.049$). A close association between energy and protein intake in elderly people has been reported before. The data of the SENECA study show narrow ranges for the percent of energy from protein in elderly people from 18 towns in 12 countries of Europe (12.4-16.6% in men and 12.9-17.4% in women) [33]. The reported protein intake tended to be lower than the protein output as calculated from the excretion of nitrogen in the urine (U_N). The ratio U_N with dietary N (which was calculated as protein intake / 6.25) was 118±28%, and much higher than the value of 81% as reported by Bingham and Cummings [24]. These results indicate an underestimation of the protein intake by the dietary history method. Incomplete urine collections may have contributed to the error in the difference between reported protein intake and estimated protein intake. However, incomplete urine collections would lead to a lower estimated protein intake. If some incomplete urine collections had been used the underestimation of the dietary history method would in fact be larger than the observed 10-12%. Only one study compared the protein intake data of elderly people with the urinary nitrogen output [5]. In that study the protein intake of elderly females was 63 g and their output (24-hour urinary nitrogen output multiplied by 6.25) was 66 g which was not significantly different. However, in that study the dietary nitrogen was estimated from a single 24-hour urine collection which can be substantially in error [24].

From the physical activity diaries it was concluded that free-living physical activity level was considerably higher compared to the physical activity level in the respiration chamber. In the respiration chamber relatively more minutes were spend on light activities (A-C, Table 3) compared to the free-living situation (1219 ± 100 min and 1048 ± 94 min, respectively, $P = 0.0001$). Furthermore, in the respiration chamber relatively less minutes were spend on more strenuous activities (D-G, Table 3) compared to the free-living situation (214 ± 102 min and 392 ± 94 min, respectively, $P = 0.0001$). Also the amount of steps as measured by a pedometer was much higher in the free-living situation compared to the situation in the respiration chamber. This is probably due to the fact that in the free-living situation significantly more minutes were spent on standing and walking around (110 ± 96 min) compared to the chamber situation (19 ± 26 min, $P = 0.002$). The difference between reported energy intake and TEE was borderline significant. However, since the physical activity level of the elderly women was higher in the free-living situation compared to the situation in the respiration chamber, the deviation between reported energy intake and TEE might even be larger than shown in this study.

The results of this study may have implications for the use of the dietary history method in e.g. epidemiological studies investigating the relationship between energy intake and morbidity. Since subjects with a high TEE level or with a high estimated protein intake underestimated their energy intake to a greater extent, differential misclassification can occur. More studies investigating the problem of underestimation of energy intake in elderly subjects are necessary.

In summary: the results of this study suggest that energy intake, obtained by a dietary history, may be underestimated by 10-12% in healthy, elderly women compared to total energy expenditure as measured in a respiration chamber. Physical activity level of the elderly women participating in this study was higher in the free-living situation compared to their physical activity level in the respiration chamber. Therefore, underestimation of energy intake may be even higher in the free-living situation. The results of the present study point to the need for validation studies of dietary assessments methods in elderly people.

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CHAPTER 8

GENERAL DISCUSSION

ENERGY BALANCE IN ELDERLY PEOPLE

The three major components of energy balance are energy expenditure, energy intake and body composition. Age may directly or indirectly influence these three components. Energy expenditure generally declines with aging. This decline may have several consequences for the energy balance of elderly people.

When energy intake is not adapted to the decline in energy expenditure, the resulting positive energy balance may lead to the accumulation of body fat. As a consequence, obesity may limit physical activity and energy expenditure will further decline [1]. Obesity in elderly people should be avoided since it is associated with increased mortality [2-6] and an increased risk profile for cardiovascular disease [7-9] and diabetes mellitus [10]. However, the optimal weight of elderly people, that is the body weight associated with a minimum morbidity and mortality risk, is still topic of discussion [11,12]. Modest weight gain with increasing age may be protective and desirable [13].

Adjustment of energy intake according to the decline in energy expenditure will keep the body in energy balance and avoid obesity. However, a low energy expenditure may lead to a very low energy intake in elderly subjects. It has been reported before that low energy intakes carry the risk of inadequate nutrient intakes in elderly people [14-18]. In addition, age-related changes in the sensation, perception and preference of taste and odour, oral and dental problems, loss of appetite due to disease or medication, drug-nutrient interactions, eating alone, economic status, depression and institutionalization may further effect appetite and dietary selection [15,19-24]. Furthermore, a low energy intake and weight loss may lower the diet-induced thermogenesis and resting metabolic rate which will lead to an additional decline in total energy expenditure.

These examples show that health status of elderly people is clearly associated with energy balance. Changes in body composition, energy expenditure, and energy intake due to aging or due to factors closely related to aging, may disturb energy balance and lead to an increased health risk. In this thesis several studies are described related to the three components of energy balance. This chapter will summarize the main findings and discuss some methodological issues related to the studies.

BODY COMPOSITION

Assessing body composition in elderly people

In chapter 2 and 3 of this thesis the applicability of three methods for the assessment of body composition was tested in an elderly population. These methods were body mass index, skinfold thicknesses, and multi-frequency impedance. A population of 117-204 elderly men and women, aged 60-87 years, was used.

Chapter 2 showed that the sum of two skinfolds (biceps and triceps), the sum of four skinfolds (biceps, triceps, sub-scapula and supra-iliaca), or body mass index (BMI) can be used to predict body density in elderly men and women. The mean prediction error of the three models varied from 0.0100 to 0.0117 kg/L, which results in a prediction error of about 5% body fat at a body density of 1.0030 kg/L. These prediction errors are comparable with prediction errors based on skinfold thickness or BMI in young populations [25,26], and show that these methods are as good as an indicator of body fatness in elderly subjects as in younger subjects. The individual prediction errors of the equations were sometimes large, which implies that one should always be cautious with the interpretation of individually predicted body composition values.

The developed prediction equations using BMI or skinfold thicknesses were externally validated in an elderly population from the south of the Netherlands. Population specificity of the prediction equation can still not be excluded. The equations should be validated first before being applied to a population that differs greatly from the one upon whom the original method was developed.

The prediction error of the equation based on BMI was smaller as compared to the skinfold thickness equations. Body weight and height are relatively simple measurements with a small measurement error. The inter-observer errors for body weight and height are smaller as compared to skinfolds, especially in older adults [27]. In addition, body weight and height are commonly used measurements in health practice. The study showed little effect of measurement errors in height (e.g. due to kyphosis) on the prediction of body density. This pleads in favour for the use of the prediction equation based on BMI. There are, however, some conditions where skinfold thicknesses are more suitable. When the subject is confined to a wheelchair or bedridden, the measurement of two skinfolds on the arm (biceps and triceps) for the prediction of body fat may be more practical.

The use of multi-frequency impedance for the prediction of body water in elderly subjects is described in chapter 3. The results confirm the findings in younger populations that at a low frequency mainly extracellular water is measured, and at high frequencies total body water [28,29]. In the elderly population the prediction error was larger and related to the body water distribution. However, on a group level the prediction error was comparable with studies in younger populations. The mean prediction error of total body water, using the equation based on bioelectrical impedance at a frequency of 50 kHz, was 7-8%. Individual errors were sometimes much larger which shows the limited value of clinical application of the method for the prediction of distribution volumes for drugs or for the determination of the hydration state of the body.

Validity of reference methods

In chapter 2 the underwater weighing method was used as a reference method to determine body density. In chapter 3 isotope dilution methods were used as reference methods for the determination of total body water and extracellular water. A few remarks about the validity of these reference methods in an elderly population are necessary.

Validity of underwater weighing method. Although frequently used, the underwater weighing method needs some concern when used in an elderly population. The method relies on the assumption that the body consists of two parts, fat mass and fat-free mass (FFM), and that the densities for these two parts are 0.900 and 1.100 kg/L, respectively [30]. However, due to demineralization of the bones and a possible change in the hydration of the FFM with increasing age, the density of FFM might be lower than 1.100 kg/L in elderly subjects [31]. Equations to adjust body density for these age changes have been developed [32].

Since the density of FFM might be lower in elderly people compared to the assumed 1.100 kg/L, the underwater weighing method may overestimate percent body fat by 1-2% [33]. This overestimation has to be taken into account when using the method as a reference method to validate anthropometric methods for assessing body composition in elderly people. The study in chapter 2 showed an underestimation of 0.5 to 19.8% body fat when prediction equations based on measurements of skinfold thickness or BMI in young populations were applied to the elderly subjects. The overestimation of body fat in elderly people due to the underwater weighing method may be partly responsible for this.

In contrast, a recent analysis carried out with data of the Rosetta Study using an advanced four-compartment model to assess body composition suggested no relationship between the density of FFM and age in white men and women aged 20-94 years. Although changes were observed in both the mineral and water fraction of FFM with age, the mean density of FFM remained 1.100 kg/L [Visser *et al., in preparation*]. This confirms the findings from other studies reporting no association between the density of FFM and age [33,34]. These results implicate that the assumptions underlying the underwater weighing method seem valid in an elderly population. More research to these validity questions of two-compartment models, as used in the underwater weighing method, seems warranted.

Validity of isotope dilution methods. In chapter 3 isotope dilution methods were used for the determination of total body water and extracellular water as a reference method for the impedance method. Since the isotope dilution methods are direct methods for the estimation of body water, these methods seem valid for application in an elderly population. There are no indications that the applied correction factor of 5% for the overestimation of the dilution space changes with age. Nor are there any indications that the correction of 5% (Donnan effect) and 10% (non-extracellular dilution) should be adjusted for elderly people.

To predict fat-free mass from total body water a hydration factor of 73.2% is generally used [35]. Human data do not indicate a change in the average hydration of fat-free mass in normal aging [36]. Baumgartner *et al.* [33] observed no association between water fraction of FFM and age in 108 elderly men and women, and Mazariegos *et al.* [34] observed no difference in the water fraction of FFM between young and elderly women matched on body weight and height. Although the hydration of FFM seems 73.2% in elderly subjects, the variation in the hydration is much larger as compared to younger adults. In a population of 27 healthy men and women aged 63-92 years the hydration varied from 63.6 to 80.4% [37].

Influence of behavioral factors on body composition

In chapter 4 the relationship of two behavioral factors, physical activity and smoking, to body fat distribution was studied in 2,341 elderly men and women aged 55-85 years. This epidemiologic study showed that smoking men and sedentary men had a more abdominal fat distribution, as indicated by a high waist/hip ratio, than non-smoking or physically active men, respectively. Only two studies investigated the relationship between smoking and fat distribution in elderly subjects, showing a higher waist/hip ratio for smokers as compared to nonsmokers [38,39]. Studies on the effect of physical exercise in elderly subjects have shown a change in fat distribution after training [40-44]. Our results confirm these findings. No clear relationship between the two behavioral factors and fat distribution was observed in elderly women. A greater influence of physical activity on intra-abdominal fat in men as compared to women has been reported before [45]. The reason for these gender differences still remains unclear.

Future studies

Anthropometric measurements, like skinfold thicknesses or circumferences, and bioelectrical impedance are currently used in many large scale longitudinal studies. However, knowledge about the validity of these methods to predict body composition or body fat distribution is limited. Our studies showed the methods can be used to predict body composition in elderly people on a group level. In what way these methods can predict changes in body composition or body fat distribution in elderly people is not known. The interpretation of body composition results from epidemiologic studies is therefore hampered. Future studies should pay more attention to the methodological issues of measuring body composition in elderly populations.

ENERGY EXPENDITURE

Resting metabolic rate

The study on energy expenditure at rest (RMR) and after consumption of a meal, presented in chapter 5, showed a lower RMR in elderly men and women as compared with young men and women. Expressing the RMR per kilogram of fat-free mass did not change the results, the RMR remained 7-8% lower in the elderly subjects.

Resting metabolic rate is known to be largely determined by body composition. The fat-free mass is the strongest predictor of RMR [46-48]. Therefore RMR is often expressed per kilogram of FFM or body weight to adjust for differences in body size between study groups. Although still being used, the ratio RMR/FFM may be incorrect since the intercept of the regression line nonequals zero. Statistical adjustment for fat-free mass should be preferred above the use of ratios [49]. When the data on RMR (chapter 5) were re-analyzed adjusting for FFM statistically instead of using ratios the same conclusions were drawn.

Not only the amount, but also the composition of FFM is not constant, which might influence energy expenditure. The mass of individual aerobic tissues and their metabolic rates may vary from person to person. Garby and Lammert showed [50] that about 5% of the between-subject variation in energy expenditure, after correction for differences in fat and fat-free mass comes from the variation of the fat-free mass composition. Changes in the composition of FFM may occur with aging. Body cell mass as a proportion of FFM decreases with age [51]. The proportion of FFM as skeletal muscle mass also decreases with age [52]. It has been proposed to subdivide FFM into organ mass and skeletal muscle mass [47]. Future energy expenditure studies should consider the different components of body composition. Development of methods for assessing the size of various organs [53,54] and skeletal muscle mass [56,57] *in vivo* by magnetic resonance imaging, computed tomography, and dual-energy x-ray absorptiometry is ongoing.

Diet-induced thermogenesis

In chapter 5 the diet-induced thermogenesis (DIT) was compared between young and elderly men and women. Only in men the DIT was lower in elderly as compared with the young subjects. No differences in DIT were observed between the young and older women. DIT was measured on two non-consecutive days within one week. The study showed a within-person day-to-day coefficient of variation of 31.8 and 21.8% for the young men and women, respectively, and 23.4 and 24.7% for the elderly men and women, respectively. This large variation emphasizes the use of large sample sizes and multiple measurements when the DIT is compared between groups. All previous studies used single measurements of DIT and often a limited number of subjects (Table 1). This may explain some of the non-significant findings when young and elderly subjects were compared. The table also shows that adjustment of the DIT for differences in the energy content of the meal, for body composition or for physical activity level may influence the conclusions of the study.

Our study was the first study to investigate possible differences in DIT between age groups in women. Very recently, another study was conducted in women which confirms our results [67]. In that study no difference in DIT was observed between young and elderly women when the measurement was continued until energy expenditure returned to baseline level. When the DIT was calculated over the first three hours only, older women had a higher response than young women. This example shows that the calculation procedures may also influence the results of studies.

Table 1. Characteristics and conclusion of reported studies investigating possible differences in diet-induced thermogenesis between young and elderly subjects.

Reference	gender	*n* young	*n* old	content meal (MJ)	time (h)	suggested change with age absolute	suggested change with age adjusted
Tuttle *et al.* [58]	M	6	8	2.1	4.5	--	
Golay *et al.* [59]	M/F	16	13	1.6	3	↓	↓ [1]
Morgan & York [60]	M	8	8	2/4	3	↓	↓ [1]
Bloesch *et al.* [61]	M/F	12	12	1.2	3	↓	-- [2]
Schwartz *et al.* [62]	M	20	12	3.4	2	↓	↓ [1]
Thörne & Wahren [63]	M	10	7	4.4*	3	↓	↓ [1]
Poehlman *et al.* [64]	M	10	9	2.7*	3	↓	-- [3]
Vaughan *et al.* [65]	M/F	64	38	8.6*	24	--	-- [1]
Pannemans *et al.* [66]	M	13	10	- †*	24	↓	-- [1]
Melanson *et al.* [67]	F	8	8	0-4.2‡	§	--	
Visser *et al.*	**M**	**29**	**32**	**1.3**	**3**	↓	-- [2]
	F	**27**	**71**	**1.3**	**3**	--	-- [2]

* energy content individually adjusted; † based on the calculated basal metabolic rate multiplied by 1.45; ‡ four test meals were used: 0, 1.1, 2.1 and 4.2 MJ; § measurement was continued until return to RMR; [1] adjusted for energy intake; [2] adjusted for body composition; [3] adjusted for physical activity level.

Energy expenditure during physical activity

In twelve elderly women the energy costs of several physical activities ranging from sitting to walking on a treadmill was measured. The women performed these activities on a self-selected speed. The energy costs, expressed as a multiple of the RMR, were not different from reference values in the literature based on younger subjects (FAO/WHO/UNU). These results suggest that the energy costs of physical activities are similar for young and elderly women. However, two previous studies have shown a higher energy expenditure during walking on a treadmill in elderly subjects as compared with young [68] and middle-aged subjects [69]. As discussed in chapter 6, the speed of walking in these studies was fixed (3, 5.9 and 6.9 km/h) and may be much higher than the usual speed of the elderly subjects. The self-selected speed of walking was only 2.2 km/h for the elderly women in our study. This difference in speed may explain some of the discrepancies in the results of the studies.

Total energy expenditure of elderly people

Our studies show a lower RMR in older men and women than in young subjects. A lower absolute DIT in older subjects as compared with young subjects was only observed in men. Energy expenditure during exercise performed at a self-selected pace was not different from the FAO/WHO/UNU reference values for exercise and suggest no change in this component of energy expenditure with age. However, since physical activity usually decreases with age, total energy expenditure will decrease. These changes will result in an overall decrease in total energy expenditure in elderly people.

Up till now only few studies are available in which the free-living total energy expenditure of elderly men and women is directly measured [70-74]. In these studies doubly labelled water was used to estimate total energy expenditure. Values ranged from 10.1 to 11.2 MJ/d for men and from 7.8 to 9.2 MJ/d for women (FAO/WHO/UNU report [75]). The calculated physical activity ratio (PAR) for the older men ranged from 1.51 to 1.75, and for the older women from 1.43 to 1.80, which is higher than the current recommendation of 1.51 for the PAR in older individuals [76]. Our study described in chapter 7 indicated a PAR value of 1.6 in elderly women as measured in a respiration chamber. Although these measurements of total energy expenditure were performed in a confined situation, which may cause an underestimation of the actual free-living PAR, these results again suggest a higher PAR value compared to the FAO/WHO/UNU reference value. These studies indicate that the recommendations of the FAO/WHO/UNU from 1985 may underestimate the usual energy needs of some groups of elderly people [71]. In the above mentioned studies small samples were used of generally healthy subjects. The results may therefore not be representative for the total elderly population. More studies concerning total energy expenditure in elderly populations of varying health status or of different categories of aging are needed to determine the energy requirements of older people.

ENERGY INTAKE

Energy intake elderly people

Chapter 7 shows the results from a validation study of the dietary history method carried out in 12 elderly women. The mean reported energy intake of the women was 7.2 ± 1.5 MJ/day. The mean energy intake of Dutch elderly women from other studies ranged from 6.9 to 7.9 MJ/d [77-81]. These values suggest that the energy intake as reported by the women in our study is comparable to that of other Dutch studies.

Validity of energy intake data in elderly

The results suggested an underestimation of energy intake data obtained by the dietary history method in a group of 12 apparently healthy elderly women. Mean energy expenditure, as measured in a respiration chamber, was 8.3 MJ while the reported energy intake was only 7.2 MJ per day. These results confirm the conclusions of previous studies reporting an underestimation of energy intake data by elderly men and women when compared with free-living total energy expenditure [70,72,82]. Several dietary assessment methods were used in these studies and the underestimation of energy intake ranged from 10 to 31%. Our results suggested an underestimation of about 10-12%, but this is likely to be larger since total energy expenditure as measured in a respiration chamber was used as reference method and not total energy expenditure in the free-living situation.

The reported underestimation of energy intake may have important implications for nutrition research in older people. Studies investigating the adequacy of micro- and macronutrient intake or energy intake may overestimate the number of elderly people at risk for malnutrition. The energy requirements for elderly people are likely to be underestimated when based on energy intake data. Furthermore, the results of our study suggested that subjects with high energy expenditure tended to underestimate energy intake to a greater extent compared to subjects with a low energy expenditure. Differential misclassification can therefore occur when energy intake data of elderly people are used. These examples show that dietary intake data of elderly populations should be interpreted cautiously. More validations studies on dietary assessments methods in elderly people are warranted. Where possible it is recommended to include biological markers of intake.

CONCLUSIONS

- Skinfold thickness measurements and body mass index can be used in an elderly population to predict body fat on a group level with a mean prediction error of 5%.
- Multi-frequency bioelectrical impedance can be applied in elderly men and women to predict total body water (at 50 kHz) and extracellular water (at 5 kHz) with a mean prediction error of 8-9%.
- Smoking and a low physical activity level are associated with an upper body fat distribution in elderly men but not in elderly women.
- Energy expenditure at rest is lower in older adults as compared to young adults which can not be explained by differences in body composition, as measured by underwater weighing in combination with total body water, or by differences in body fat distribution, as measured by the waist/hip ratio.
- Diet-induced thermogenesis is not lower in elderly subjects as compared with younger subjects due to aging *per se*.
- Physical activity level is not associated with resting metabolic rate and diet-induced thermogenesis in young and elderly men and women.
- The energy costs of physical activities performed at a self-selected pace by elderly women are similar to the FAO/WHO/UNU reference values for physical activities.
- The dietary history method underestimates both energy and protein intake in elderly women by 10-12%.

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SUMMARY

The number of elderly people is increasing in developed countries as well as in developing countries. A growing attention for the health status and the quality of living at old age is necessary. Nutrition is closely related to health status. Not only the composition of the diet, such a the amount of macro- and micronutrients, but also the adequacy of energy intake needs to be considered. The prevalence of both underweight and overweight, result of a negative and positive energy balance, respectively, is high in the elderly population. Both underweight and overweight are associated with higher morbidity and mortality risk at old age. In this thesis several studies are described which are closely associated with the three components of energy balance in elderly people: body composition (chapter 2, 3, and 4), energy expenditure (chapter 5 and 6), and energy intake (chapter 7).

Body composition

Body composition changes with aging. It is therefore unclear whether specific methods to estimate body composition in young populations can be applied to an elderly population. The aim of the studies described in chapter 2 and 3 was to evaluate the applicability of three commonly used methods to estimate body composition in elderly people.

The body mass index and skinfold thickness method were evaluated in 204 subjects 60-87 years of age. Prediction equations from the literature which are developed in young populations, underestimated body fat percentage in the elderly subjects by 0.5 to 19.8%. Therefore, new prediction equations were developed and internally as well as externally validated. The body mass index and the sum of two or four skinfold thicknesses can be used to predict body fat in the elderly subjects on a group level. The mean prediction error in body fat was 5%. The equation based on the body mass index had the smallest prediction error. Individual prediction errors were sometimes very large and should be interpreted carefully.

The applicability of the bioelectrical impedance method was tested in 117 elderly men and women. Since this study is the first study in which the multi-frequency method is applied to elderly subjects, prediction equations were developed. In combination with body weight, total body water and extracellular water could be predicted using a frequency of 50 kHz and 5 kHz, respectively. The mean prediction error was 8% for total body water and 9% for extracellular water and

was related to the distribution of body water. Individual prediction errors were sometimes very large which shows the limited clinical use of the method.
Chapter 4 describes an epidemiological study on the association of two behavioral factors, smoking and physical activity, and fat distribution in elderly men and women. Data of 1,178 men and 1,163 women, aged 55 to 85 years, from the Longitudinal Aging Study Amsterdam was used. The waist/hip circumference ratio was used as an index of body fat distribution. A clear association between the behavioral factors and fat distribution was observed in men but not in women. Men who smoked or were physically inactive had a more abdominal fat distribution as compared with nonsmokers or active men.
The results of these studies suggest a valid prediction of body composition on a group level in elderly people using the body mass index, skinfold thickness method or bioelectrical impedance. Smoking and physical activity seem to be associated with body fat distribution in elderly people.

Energy expenditure
To investigate possible changes in energy expenditure with aging, two studies were conducted in which energy expenditure of older people was compared with values of younger subjects or with reference values from the literature (chapter 5 and 6). Energy expenditure at rest and after a meal was measured by indirect calorimetry in 56 young (age 20-33 years) and 103 elderly (age 63-87 years) men and women (chapter 5). Resting metabolic rate was lower in the elderly than in the young subjects. This difference persisted after adjustment for differences in fat-free mass between the two age groups. The rise in energy expenditure after a standardized meal of 1.3 MJ, measured for 180 minutes, was lower in older men than young men. The difference disappeared after adjustment for body composition or body fat distribution. No difference in energy expenditure after a meal was observed between young and elderly women. In this study the relationship between physical activity level, obtained by questionnaire, and energy expenditure was also investigated. However, no association between physical activity level and resting metabolic rate or diet-induced thermogenesis was observed.
Chapter 6 describes a study on the energy costs of physical activities in a group of twelve elderly women. Activities, ranging from sitting to walking on a treadmill, were performed at a self-selected speed. Energy expenditure as measured by indirect calorimetry was similar to the reference values of activities as given by the

FAO/WHO/UNU in 1985.
These studies suggest a decrease in energy expenditure at rest with aging *per se*, but no changes in energy expenditure after a meal or during physical activities.

Energy intake
In chapter 7 a validation study of the dietary assessment technique dietary history is described. This technique is frequently used in elderly people. Reported energy and protein intake of twelve elderly women (69-82 years of age) was compared with total energy expenditure and 24-hour urinary nitrogen excretion, respectively. Mean reported energy intake was 11.6% lower than measured total energy expenditure. Mean reported protein intake was 10.1% lower than the calculated protein intake using the nitrogen excretion data. The results of the study show an underestimation of dietary intake by 10-12% with the dietary history method. This indicates a careful interpretation of dietary intake data of elderly people.

SAMENVATTING

Het aantal ouderen neemt toe in de westerse en niet-westerse wereld. Aandacht voor de gezondheid en de kwaliteit van het leven op oudere leeftijd wordt in de toekomst steeds belangrijker. De voeding speelt hierin, naast vele andere factoren, een grote rol. Niet alleen de samenstelling van de voeding, zoals de hoeveelheid macro- en micronutriënten, maar ook de totale hoeveelheid energie is van belang. De prevalentie van zowel ondergewicht als overgewicht, het resultaat van een negatieve respectievelijk een positieve energiebalans, is hoog bij ouderen. Beide condities zijn positief geassocieerd met het risico op ziekte en sterfte. In dit proefschrift worden enkele studies besproken die nauw in relatie staan met de drie onderdelen van de energiebalans van ouderen: de lichaamssamenstelling (hoofdstuk 2, 3 en 4), het energieverbruik (hoofdstuk 5 en 6) en de energie inneming (hoofdstuk 7).

Lichaamssamenstelling

Bij veroudering treden veranderingen op in de lichaamssamenstelling. Mede daardoor is het onduidelijk of bepaalde methoden die de lichaamssamenstelling kunnen schatten in jonge populaties ook gebruikt kunnen worden in ouderen. Het doel van de in hoofdstuk 2 en 3 beschreven studies was de toepasbaarheid te onderzoeken van drie veelgebruikte methoden om de lichaamssamenstelling bij ouderen te bepalen.

Allereerst werd de Quetelet index en de huidplooi methode onderzocht in 204 personen in de leeftijd 60-87 jaar. Voorspellingsformules uit de literatuur, die ontwikkeld zijn in jonge populaties, bleken allen het vetpercentage bij ouderen te onderschatten. De onderschatting varieerde van 0.5 tot 19.8%. Nieuwe voorspellingsformules werden daarom ontwikkeld en zowel intern als extern gevalideerd. Zowel de Quetelet index als de som van twee of vier huidplooien bleken de lichaamssamenstelling bij ouderen op groepsniveau goed te schatten. De gemiddelde fout in het geschatte lichaamsvetpercentage was 5%. De formule gebaseerd op de Quetelet index was het meest nauwkeurig. Op individueel niveau kan de schattingsfout veel groter zijn en moet de geschatte waarde voorzichtig geïnterpreteerd worden.

De toepasbaarheid van de bio-elektrische impedantie methode werd eveneens bestudeerd in 117 oudere mannen en vrouwen. In deze studie is voor het eerst de

impedantie techniek met verschillende frequenties toepast bij ouderen. Daarom werden specifieke voorspellingsformules voor ouderen ontwikkeld. De methode bleek, in combinatie met lichaamsgewicht, geschikt voor de voorspelling van de hoeveelheid totaal lichaamswater (bij 50 kHz) en extra-cellulair water (bij 5 kHz) op groepsniveau. De schattingsfout was gemiddeld 8% voor totaal water en 9% voor extra-cellulair water. De schattingsfout was echter gerelateerd aan de verdeling van water in het lichaam. Individuele schattingsfouten kunnen groot zijn waardoor een klinische toepassing van de methode niet mogelijk lijkt.
Hoofdstuk 4 beschrijft een epidemiologische studie naar de relatie tussen twee gedragsfactoren, roken en lichamelijke activiteit, en de vetverdeling bij oudere mannen en vrouwen. De gegevens van 1178 mannen en 1163 vrouwen in de leeftijd 55 tot 85 jaar, deelnemers van de 'Longitudinal Aging Study Amsterdam', werden hiervoor gebruikt. De middel/heup omtrek verhouding werd gebruikt als maat voor de vetverdeling. De studie liet een duidelijke associatie tussen de gedragsfactoren en de vetverdeling zien bij mannen maar niet bij vrouwen. Mannen die roken en mannen met een laag activiteiten patroon hadden een hogere middel/heup omtrek verhouding vergeleken met niet-rokende of actieve mannen.
De resultaten van deze studies suggereren een valide voorspelling van de lichaamssamenstelling op groepsniveau bij ouderen met behulp van de Quetelet index, huidplooien of impedantie. Er lijkt tevens een verband te bestaan tussen gedragsfactoren en vetverdeling bij ouderen.

Energieverbruik

Om te onderzoeken of het energieverbruik afneemt bij veroudering, werd in twee studies het energieverbruik van ouderen vergeleken met die van jongeren of met referentie waarden uit de literatuur (hoofdstuk 5 en 6). Het energieverbruik in rust en na een maaltijd werd gemeten met behulp van indirecte calorimetrie bij 56 jonge (leeftijd 20-33 jaar) en 103 oudere (leeftijd 63-87 jaar) mannen en vrouwen (hoofdstuk 5). De studie liet een lagere ruststofwisseling zien van de ouderen, zowel voor als na correctie voor de verschillen in vet-vrije massa tussen jong en oud. De stijging in energieverbruik na een standaard maaltijd van 1300 kJ, gemeten over 180 minuten, was lager in de oudere mannen vergeleken met de jonge mannen. Dit verschil verdween echter na correctie voor de lichaamssamenstelling of vetverdeling. Er werd geen verschil in energieverbruik na de maaltijd waargenomen tussen de jonge en oudere vrouwen. In deze studie werd eveneens

de relatie onderzocht tussen de mate van lichamelijke activiteit, gemeten met een vragenlijst, en het energieverbruik. Er werd echter geen verband tussen lichamelijke activiteit en de ruststofwisseling of het energieverbruik na een maaltijd waargenomen.
Hoofdstuk 6 beschrijft een studie naar het energieverbruik tijdens lichamelijke activiteiten in een groep van twaalf oudere vrouwen. Diverse activiteiten, variërend van rustig zitten tot fietsen of lopen op een tredmolen, werden op eigen snelheid uitgevoerd. Het energieverbruik gemeten met behulp van indirecte calorimetrie was niet hoger dan referentie waarden zoals opgegeven door de FAO/WHO/UNU in 1985.
Deze studies suggereren dat de energiekosten tijdens rust afnemen, en dat de energiekosten na een maaltijd of tijdens lichamelijke inspanning gelijk blijven bij veroudering.

Energie inneming
Hoofdstuk 7 beschrijft een validatie studie van de voedselconsumptie techniek 'dietary history'. Deze techniek wordt veelvuldig toegepast bij ouderen. De gerapporteerde energie- en eiwitinneming van een groep oudere vrouwen (69-82 jaar) werd vergeleken met respectievelijk het gemeten 24-uurs energieverbruik en de stikstof uitscheiding in 24-uurs urine. De gerapporteerde energie inneming was gemiddeld 11.6% lager dan het gemeten energieverbruik. De eiwitinneming was gemiddeld 10.1% lager dan de berekende eiwitinneming op basis van de stikstof uitscheiding in de urine. De studie suggereert een onderschatting van de voedselinneming van 10-12% met de dietary history techniek. Deze uitkomst suggereert dat voedselconsumptiegegevens bij ouderen voorzichtig geïnterpreteerd dienen te worden.

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CURRICULUM VITAE

Marjolein Visser was born in Bodegraven, the Netherlands, on August 17, 1966. She passed secondary school, Athenaeum B, at the Farel College in Amersfoort in 1984. That same year she started the study "Human Nutrition" at the Wageningen Agricultural University. During this study she worked for six months at the Instituto Mexicano de Tecnología del Agua in Mexico. She obtained her M.Sc.-degree in Human Nutrition in 1990 with main topics endocrinology and physiology. From December 1990 until August 1991 she worked as a research assistant at the department of Human Nutrition where she conducted a clinical trial. In September 1991 she was appointed as an associate investigator at the department of Human Nutrition where she started the research described in this thesis. During her Ph.D.-project she attended the Annual New England Epidemiology Summer Program at Tufts University, Boston, USA in the summer of 1993 and was officially registered as 'Epidemioloog A' by the Netherlands Epidemiology Society. In 1994 she worked for five months as a research scholar at the Obesity Research Center, St. Luke's-Roosevelt Hospital, New York, USA. She was selected to participate in the second European Nutrition Leadership Program, Luxembourg, in March 1995. In December 1995 she will start working at the National Institute on Aging, National Institutes of Health, Bethesda, USA.

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Disce, ut semper victurus,
vive, ut cras moriturus