Determinants of Physical Aging among Healthy Postmenopausal Women and their Relation with Serum Hormone Levels

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Determinants of Physical Aging among Healthy Postmenopausal Women and their Relation with Serum Hormone Levels

Determinanten van lichamelijke veroudering en de relaties met circulerende hormoon concentraties bij gezonde postmenopauzale vrouwen

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Voor Marine,

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Introduction

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Aim of the thesis

Introduction

Aging is often viewed as an unalterable process of decline and loss associated with development of physical frailty and gradual loss in cognitive function towards the end of life. This view may, however, be too pessimistic. The perceived importance of increasing the number of healthy years with a better preservation of functional and mental capacity grows with the increasing number of older persons in our society. The fulfillment of this need requires the identification of modifiable factors related to aging and explaining differences between individuals. In this thesis, we adopted the definition of successful aging by Rowe and Kahn, which includes avoidance of disease and disability, maintenance of high physical and cognitive function, and sustained engagement in social and productive activities [1].

Endocrine system changes occurring with aging in women

In the fourth decade, prior to a decline in ovarian function, the function of the somatotrophic axis begins to decrease. This decrease in function is further accelerated in parallel with the subsequent decline in ovarian function [2]. As a result, growth hormone secretion, as well as circulating IGF-I and IGFBP-3 levels, decrease significantly with advancing age.

Concentrations of ACTH and TSH remain constant well into the ninth decade. The thyroid gland undergoes progressive fibrosis and concentrations of T_3 decline by 25 to 40%, but most elderly persons still remain euthyroid.

Pancreatic B cell function also undergoes degeneration such that by age 65 years, 50% of subjects have abnormal glucose tolerance tests, and manifest type 2 diabetes occurs in 14% of the population [3].

The function of female endocrine reproductive system fully stops at a (currently) relatively early age.

Menopause, defined as the permanent cessation of menstruation, occurs at a mean age of 51 years in women, as a result of a genetically programmed loss of ovarian follicles further

affected by environmental influences [4-7]. With transition into menopause, estradiol levels decline by ~80%, whereas estrone levels are relatively preserved. The latter reflects peripheral aromatization of adrenal and ovarian androgens. FSH levels increase more than those of luteinizing hormone (LH), presumably because of the loss of inhibin combined with estrogen feedback. Because of the increase in gonadotropin secretion, which stimulates steroidogenesis in ovarian hilar cells or luteinized stromal cells, the ovaries become primarily androgen-producing glands. The postmenopausal ovary produces a larger percentage of testosterone (50%) than does the premenopausal ovary. Still, the overall rate of testosterone production falls in postmenopausal women [8,9], mostly because of a decline in the peripheral and adrenal production of testosterone from androstenedione [10]. From 3 years after the postmenopausal decline, intra-individual endogenous sex steroid serum concentrations remain relatively stable [11-14]. The principal source of estrogens in postmenopausal women is thus peripheral aromatization of androstenedione to estrone in adipose tissue and skin, which is subsequently reduced to estradiol in peripheral tissues. There are several extragonadal estrogen production sites, including adipose tissue, bone, various sites of the brain, and vascular endothelial and smooth muscle cells [15-18]. Postmenopausal estrogen production is thus primarily affected by body weight and not by age.

Dehydroepiandrosterone sulfate (DHEAS) levels decrease in both men and women with aging. Androstenedione is the most important androgen during the reproductive years, and concentrations declines from 5.5 nmol/L to 2.9 nmol/L in postmenopausal women. The postmenopausal ovary contributes only 20% to circulating androstenedione levels.

Given the endocrine changes associated with aging, many symptoms in the aging female may be due to estrogen deficiency or diminished androgen or growth hormone secretion. Disorders that are definitely due to estrogen deprivation include vasomotor symptoms and urogenital atrophy[19,20]. Osteoporosis is thought to be due largely to estrogen deficiency, but this may be exacerbated by the relative decline in growth hormone levels. The same may be said for the hormone-related increase in the prevalence of atherosclerotic cardiovascular disease, changes in body composition, and psychosocial symptoms, including insomnia, fatigue, short-term memory changes, and possibly depression. Both DHEAS and growth hormone may impact on these phenomena as well [2,21-24].

Manifestations of menopause

1- Short term manifestations

In the initial menopausal fall of ovarian function with aging, estradiol levels may be quite variable with chaotic patterns and occasionally very high or very low levels, and abnormal LH pulsatile patterns. This marked variability may lead to a range of symptoms during the perimenopausal years. Hot flashes, a self-limited centrally mediated thermoregulatory dysfunction, with excessive peripheral vasodilatation and increased digital and cutaneous blood flow and perspiration, occurs in 75 percent of women [25]. They are associated with sleeping problems, fatigue, irritability, depression, difficulties with cognitive concentration, and other emotional and psychological symptoms [26]. Epithelial and mucosal atrophy and decreased local blood flow, are the major causes of decreased vaginal lubrication and sexual function in menopausal women [27] and predispose to stress and urge urinary incontinence [28]. In addition to mucosal atrophy, estrogen deficiency can increase vaginal pH and alter the vaginal flora, changes which may predispose to urinary tract infection [29]. Decreased skin and bone collagen content, reversible with estrogen hormone therapy [30], may lead to increased aging and wrinkling of the skin. Increases in collagen matrix in the bone probably result in an increase in flexibility and strength, independent of bone mineral density. Compelling evidence, including data from randomized clinical trials, indicates that estrogen therapy is highly effective for controlling vasomotor and genitourinary symptoms.

2- Long term morbidity related to estrogen deficiency

a- **Cardiovascular disease**

Cardiovascular disease (CVD) is the leading cause of death in women, responsible for more deaths each year than all other causes combined [31]. CVD is unusual in premenopausal women, particularly in the absence of other risk factors [32]. The incidence of myocardial infarction in women, although lower than in men, increases after menopause. Because, due to increased life expectancy, women will now spend more than a third of their lives in the

postmenopausal years, cardiovascular preventive measures are thus important. The rapid increase in cardiovascular risk after menopause provided a strong reason to suspect that cessation of endogenous estrogen production played an important role [33,34]. Consequently, a logical view was that substitution of endogenous hormones by exogenous estrogens would reduce the increased rate of cardiovascular disease in women. A large body of observational evidence supported a protective effect of estrogen replacement therapy on CVD [35,36]. Subsequent randomized trials could, however, not substantiate the cardiovascular preventive benefits of estrogen replacement therapy [37-39]. A number of explanations for this discrepancy between observational and experimental data have been put forward, but as a consequence the enthousiasm about the preventive potential of estrogen replacement has much faded in recent years [40]. The biological plausibility of a protective role of estrogen on cardiovascular disease is supported by the fact that exogenous estrogen lowers plasma low-density lipoprotein (LDL) cholesterol and raises high-density lipoprotein (HDL) cholesterol levels by 10 to 15%. Administration of estrogen also favorably affects lipoprotein(a) levels, LDL oxidation, endothelial vascular function, and fibrinogen and plasminogen activator inhibitor-1. However, estrogen therapy also has unfavorable effects on other biomarkers of cardiovascular risk, it boosts triglyceride levels, promotes coagulation via factor VII, prothrombin fragments 1 and 2, and fibrinopeptide A elevations, and raises levels of the inflammatory marker C-reactive protein.

Apart from a putative role of falls in endogenous estrogens, there are other risk factors related to menopause that are candidates to affect cardiovascular disease risk [41]. Around menopause many women show an increase in body weight. Obesity is an important coronary risk factor as demonstrated in the prospective cohort Nurses' Health Study [42]. The increase in risk associated with obesity was independent of diabetes, although the two disorders may be closely linked in individual patients [43]. Abdominal or central obesity (waist-hip ratio of above 0.9) is a stronger risk factor than simple body mass index measurements [43,44].

b- **Cognitive function**

Because the elderly are the fastest-growing segment of the population, and cognitive decline is strongly related to aging, the prevalence of dementia will increase markedly in coming years. Although dementia appears not to preferentially affect women, the possible role of estrogen decline in women has also fuelled an interest in a role of estrogen and postmenopausal hormone therapy in cognitive function and dementia. There is ample

biological evidence to support a role for estrogens in cognitive function. Estrogen receptors have been identified throughout the brain, and appear particularly common in the basal forebrain. The latest is the major source of cholinergic innervation to the hippocampus and important for regulation of memory and learning. The hippocampus is the primary region of the brain mediating cognitive function. However, most observational studies have not found a significant effect of endogenous estrogen levels on cognition in women and results from studies with hormone therapy have not supported a protective effect of exogenous estrogens on cognitive functions [45-52]. Studies which addressed the role of the remaining circulating postmenopausal estrogens in cognition were hampered by small sample sizes or incomplete adjustment for potential confounders [53-56]. Two studies reported reduced declines in cognitive function at higher circulating estradiol levels [14,57]. In contrast, however, a recent study reported that higher serum estradiol levels were associated with an increased risk of Alzheimer's disease [58]. Two studies examined the continuous association between plasma estrogen concentration and cognitive function. No relation between estradiol or estrone and cognition or cognitive decline over time could be found [57,59]. However, in one study reduced cognitive decline (measured by MMSE score) was observed in those women with the highest levels of free estradiol and bioavailable estradiol (presumably representing estradiol available to the brain). The findings were, however, based on a very small number of women with evidence of cognitive impairment [57]. More data are clearly needed to conclude with confidence whether or not circulating estrogens affect cognitive function.

c- **Osteoporosis**

The most common skeletal disorder, osteoporosis, is characterized by low bone mass and micro-architectural deterioration of bone tissue. This leads to bone fragility and increased fracture risk. It also leads to considerable morbidity, mortality, and marked health care expense. Nearly half of postmenopausal women from an unselected population showed low bone density (almost 40% had osteopenia, and 7% osteoporosis) [60]. Osteoporosis in postmenopausal women is the end result of years of bone loss due to an imbalance between bone resorption and bone formation. Both increase after menopause, but resorption exceeds formation, resulting in so called high-turnover osteoporosis [61,62]. Soon after the onset of menopause there is a rapid acceleration of bone loss, particularly in trabecular bone. Menopause-related bone loss lasts for about 10 years. After this time, the rate of bone loss decreases [63,64]. The mechanism by which lack of estrogen leads to increased bone loss in

women is not fully understood. Both direct effects of circulating estrogens on osteoclast function and changes in the release of certain cytokines appear to contribute. Whatever the mechanism, estrogen replacement therapy is highly effective in reducing risk of osteoporosis and fractures in postmenopausal women [65]. On estrogen replacement, bone turnover and resorption rates are reduced and bone loss attenuated. More than 50 randomized trials have demonstrated that postmenopausal estrogen therapy, with or without a progestin, rapidly increases bone mineral density at the spine by 4 to 6% and at the hip by 2 to 3%, and maintains those increases during treatment [66].

d- **Changes in body mass and composition**

Menopause, by the combination of aging and declined ovarian hormone secretion, is associated with changes in body composition. Loss of skeletal mass, loss of lean body mass, and increase in abdominal and visceral fat tissue have been described. [67] In addition, GH reductions lead to reduced protein synthesis, decreased lean body and bone mass, and increased percent body fat [68]. Aging related changes in DHEAS levels have also been associated with alterations in body composition. Understanding the age-associated changes differences in regional fat redistribution and the metabolic correlates and factors that can modify these changes, may point at strategies to improve body composition profiles in the middle-aged and the elderly.

The effects of Gonadotropin Releasing Hormone (GnRH) agonists (drugs inhibiting pituitary gonadotropin secretion, thereby profoundly suppressing ovarian estrogen production) on body composition have been well documented in prospective studies in men: GnRH agonists significantly decrease lean body mass and increase fat mass, most of the fat accumulation is in the subcutaneous tissues [69-71]. The decrease in lean body mass and increase in fat mass appear to be early effects with minimal cumulative changes in body composition beyond 18 months of treatment [72]. Treatment-related changes in body composition are accompanied by important metabolic changes including reduced insulin sensitivity [73] and an increase in serum lipids and lipoproteins [71,74]. Reduced testosterone levels in men are probably responsible for the loss of lean body mass, increased body fat, and decreased muscle strength [70-72,75-77]. Similar effects have been found in studies among women [78-80].

Aim of the thesis

The aim of this study in healthy postmenopausal women was to gain further insight into the associations between endogenous estrogens and long term manifestations of menopause: cardiovascular aging (approximated by arterial stiffness), cognitive function (approximated by a global test), bone mineral density, body composition, physical functions and quality of life. This insight may yield answers about the potential for hormonal substitution therapy or other interventions which may improve the quality of life of women at older age and postpone morbidity.

In **Chapter 2,** a study on determinants of large artery stiffness measured using aortic pulse wave velocity is presented (Chapter 2.1), followed by a study on the relations between circulating endogenous hormones and arterial stiffness (Chapter 2.2). **Chapter 3** contains a study on the relations between endogenous estrogen and cognition. **Chapter 4** focuses on the contribution of determinants of bone mineral density (fat mass, lean mass and endogenous hormones) at various locations. Body composition is studied in **Chapter 5** by assessing the relationships between body composition, physical function and disability. **Chapter 6** concerns relations between endogenous hormones, body composition and physical performance.

Finally, body composition, functional and hormonal parameters and quality of life were studied in **Chapter 7.**

In **Chapter 8,** the results described in this thesis are summarized, put in a broader perspective and views on further research are put forward.

Chapter 9 provides a summary of this thesis in Dutch.

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Chapter 2

Arterial Stiffness

2.1 Arterial stiffness in postmenopausal women: determinants of pulse wave velocity

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Abstract

Objective : To investigate the degree and potential cardiovascular determinants of arterial stiffness, assessed by aortic pulse wave velocity (PWV) measurements, and to relate arterial stiffness to absolute 10-12-year risks of stroke, coronary heart disease and death, as estimated by available risk functions, in postmenopausal women.

Method : We performed a cross-sectional study among 385 postmenopausal women, aged 50-74 years, sampled from the general population. Arterial stiffness was assessed non-invasively by measurement of aortic PWV using applanation tonometry. Information on health was obtained by medical history, registration of current medication, and physical examination. Height, weight, waist and hip circumferences, fasting glucose, total and high-density lipoprotein (HDL) cholesterol, triglycerides, resting blood pressure, and heart rate were measured. Three risk scores were used to estimate, for each individual, the absolute risk of stroke, coronary heart disease, and death within 10-12 years as a function of their cardiovascular risk factor profile. The relationship between PWV and these risk scores was subsequently determined.

Results : Significant positive relationships with PWV were found for body mass index, fasting glucose, diabetes mellitus, and triglycerides in analyses adjusted for age, mean arterial blood pressure, and heart rate. Height and HDL cholesterol were inversely related to PWV. The risks of stroke, coronary heart disease, and death increased with increasing PWV in a linear graded manner.

Conclusions : This cross-sectional study among postmenopausal women provides evidence that most of the established cardiovascular risk factors are determinants of aortic PWV. Increased PWV marks an increased risk of stroke, coronary heart disease, and death within 10-12 years.

Introduction

Originally, increased arterial stiffness was considered to be intrinsic to the aging process of the artery [1]. However, it has since been demonstrated that factors such as insulin resistance, hypertension, atherosclerosis, and end-stage renal disease (ESRD) [2-9] contribute to the stiffening of the arterial tree.

The clearest consequences of arterial stiffening of large arteries, in particular the aorta, are increased pulsatile blood pressure as a result of a greater systolic (SBP) and a lower diastolic (DBP) pressure, which leads to a increased left ventricular workload in combination with a reduced perfusion of the coronary arteries in diastole [10]. Recent population-based studies have emphasized the increasing interest in markers of arterial stiffness, measurable in a non-invasive manner, each with it own characteristics: brachial pulse pressure, carotid distensibility, and aortic pulse wave velocity (PWV) [10,11].

There have been a number of studies indicating that increased incidences of cardiovascular disease are related to stiffer vessels [2-16]. The most prominent factor is age: with increasing age, arteries become stiffer, apparently in a similar manner for both men and women [17]. In addition, increased blood pressure and indicators of glucose intolerance are strongly related to increased arterial stiffness [18,19]. A number of studies have examined the relationship between arterial stiffness and the presence of atherosclerotic abnormalities elsewhere in the arterial tree [6-8]. An increased aortic PWV showed a linear graded relationship to carotid intima-media thickness, plaques in the carotid arteries, lower extremity arterial disease, and plaques in the abdominal aorta [8]. In a group of patients with hypertension, aortic PWV was positively related to the absolute risk of cardiovascular disease, as estimated by available risk functions [20].

Most of the evidence that relates arterial stiffness to risk factors and the presence of atherosclerosis and prevalent cardiovascular disease comes from studies performed in specific groups of patients. Information on these issues for postmenopausal women is very limited. Some available data stem from studies of the influence of oestrogen; menopause seems to be associated with stiffer vessels [21-23]. However, studies that evaluated whether hormonal therapy (HT) had a direct beneficial effect on arterial stiffness have yielded conflicting findings [24-26]. Nevertheless, studies of HT and endothelial function [27], and of HT and atherosclerosis [28] - both major determinants of arterial stiffness - showed positive results overall.

The aim of this study was to investigate the major determinants of aortic stiffness, measured by aortic PWV, in postmenopausal women. In addition, we related PWV to estimates of individual 10-12-year absolute risk of stroke, coronary heart disease, and death. Three risk scores were used to estimate the individual absolute risk of stroke, coronary heart disease, and death as a function of their cardiovascular risk factor profile [29-31].

Participants and methods

Study population

Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) [32]. In PROSPECT, a total of 17 395 healthy women presenting for breast cancer screening, who were aged 49-70 years and living in Utrecht and the surrounding area, were enrolled between 1993 and 1997. Using the baseline data from PROSPECT, we selected women who had experienced a natural menopause between 8 to 30 years ago. In addition, criteria for inclusion in the study were that the woman should have an intact uterus and at least one intact ovary, and should not have used sex steroids after the reported date of last menstruation. These criteria were used because the primary objective of the present study was to elucidate the role of endogenous sex hormones on several markers of frailty. Of 1803 eligible women, 902 were invited to participate and 553 (61%) agreed to do so. 402 participants were finally included in the study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study centre without assistance. Each participant underwent all tests and assessments during two visits to the study centre. The study was approved by the Institutional Review Board of the University Medical Center Utrecht and written informed consent was obtained from all participants. Data collection took place between September 1999 and March 2000.

Measurements

Information on health was obtained by medical history, registration of current medication and physical examination. A standardized questionnaire on use of oestrogen, alcohol consumption, and smoking was obtained from all women. Individuals were categorized as

current smokers, former smokers, and those who had never smoked. For current and past smokers, the number of pack-years was calculated as the average number of packs of cigarettes smoked per day multiplied by the total years of smoking. Height, weight, and waist and hip circumferences were measured with the woman in a standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in metres.

Blood pressure and heart rate were measured during the first visit using an oscillometric-automated device (Dinamap 8100, Critikon). Measurements were conducted before 1100 h after an overnight fast. After 5 min of rest, blood pressure was taken at the right brachial artery simultaneously with heart rate, twice with the participant lying down and three times with the participant in a standing position, with 1 min between each measurement. SBP and DBP were defined as the average of the two supine measurements. Mean arterial blood pressure (MAP) was calculated as DBP + [(SBP - DBP)/3]. Pulse pressure was defined as SBP minus DBP. Hypertension was defined as SBP at least 160 mmHg, DBP at least 95 mmHg, use of antihypertensive medication, or a combination thereof [33]. Isolated systolic hypertension was defined as SBP at least 160 mmHg and DBP 90 mmHg or less without medication. Fasting venous blood samples were obtained between 0800 and 1100 h. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose and albumen were measured reflectometrically using commercial enzymatic kits with a Vitros 250 (dry chemistry; Johnson & Johnson, Rochester, Minnesota, USA). Glucose was measured by the glucose oxidase/peroxidase method. Total cholesterol was measured by adaptation of the cholesterol oxidase/peroxidase method (fixed time). Triglycerides were measured by the lipase/glycerolkinase/GPO/POD method (fixed time). HDL cholesterol was measured after precipitation with dextran sulphate/Mg2+. The low-density lipoprotein (LDL) cholesterol concentration was estimated using the Friedewald formula [34]. Hypercholesterolaemia was defined as a total cholesterol concentration of 6.5 mmol/l or more, or use of cholesterol-decreasing drug treatment. Diabetes mellitus was defined as fasting serum glucose of 7.0 mmol/l or more, or the use of oral glucose-decreasing drugs or insulin [35].

Arterial stiffness

The Sphygmocor system was used for non-invasive measurement of the stiffness of the aorta [36] (PWV system, PWV Medical, Sydney, Australia). After the woman had rested for 5-10 min in the supine position, aortic PWV was measured by sequential recordings of the arterial pressure waveform at the carotid artery and the femoral artery, using a hand-held micromanometer-tipped probe on the skin at the site of maximal arterial pulsation. Gating the recordings at those two sites to the electrocardiogram (ECG) enabled PWV to be measured. Recordings were taken when a reproducible signal was obtained with high-amplitude excursion - usually 10 consecutive beats, to cover a complete ventilatory cycle. The wave transit time was calculated by the system software, using the R wave of a simultaneously recorded ECG as a reference frame. Distances from the carotid sampling site to the suprasternal notch and from the suprasternal notch to the femoral artery were measured using a compass [37]. The aortic PWV (m/s) was automatically calculated as the distance between the suprasternal notch and the femoral artery minus the distance between the carotid sampling site and the suprasternal notch, divided by the time interval between the systolic R wave and the femoral systolic up-stroke minus the time interval between the systolic R wave and the carotid systolic up-stroke. Aortic PWV was determined as the mean of at least three consecutive beats recorded during 10 s of data acquisition. All measurements were performed by the same observer (C.E.I.L.)

We performed a reproducibility study among 27 participants, who underwent a second PWV measurement within 2 weeks after the first examination. The mean (SD) PWV was 9.55 (2.36) m/s at the first visit and 9.56 (2.01) m/s at the second visit, with a mean difference of 0.01 (1.0) m/s. The within-class correlation coefficient was 89.6%, indicating that 89.6% of the variance in the PWV measurements was attributable to differences between patients, whereas 10.4% could be attributed to differences between visits (measurement error and intra-individual variability).

Risk functions for stroke, coronary heart disease, and all-cause mortality.

A risk function for an individual estimates the probability of the occurrence of an event within a certain time span as a function of the individual's level of the risk indicators. Risk functions are derived from analyses on data from longitudinal (cohort) studies, in which the

relative and independent contribution of certain risk factors in predicting the occurrence of the event are evaluated.

The risk function for stroke was based on information from 10 years of follow-up of participants in the Framingham Heart Study, who were aged 55-84 years and initially free from stroke, at biannual examinations 9 and 14 [29]. A Cox proportional hazards model was used to estimate the contribution of cardiovascular risk factors to the occurrence of stroke. The report provides detailed information on how an individual's absolute 10-year risk of stroke may be estimated. For women, this is:

Equation $\{1 - 0.9353^{\exp[0.0699(\text{age}) + 0.0161(\text{SBP}) + 0.00026(\text{HRXSBP}) + 0.4404(\text{CVD}) + 0.8055(\text{ECGLVH}) + 0.5219(\text{smoking}) + 1.1173(\text{Atrial fibrillation}) + 0.5604(\text{diabetes}) - 7.5766)]},\}$

where HRXSBP = 0 if systolic pressure 200 mmHg, else HRXSBP is HRX(SBP - 110)(200 - SBP) and HRX = 1 when individual is receiving antihypertensive treatment; CVD, cardiovascular disease present; ECGLVH, left ventricular hypertrophy on ECG.

The risk function predicting 10-year coronary heart disease risk was obtained from data of the Framingham Heart cohort aged 30-74 years and free from cardiovascular disease at baseline [30]. A logistic regression model was used for risk estimation. Risk indicators included in the final model were age, SBP, total : HDL cholesterol ratio, left ventricular hypertrophy (on ECG), smoking, and diabetes mellitus. In the report [30], an extensive and detailed description is given of how to arrive at an estimate of the absolute risk of coronary heart disease within 10 years for an individual, given a certain set of cardiovascular risk factors.

The risk function to predict an individual's probability of dying within 11.5 years as a function of the levels of cardiovascular risk factors was obtained from findings in a follow-up study among 6057 individuals aged 20 years or over, conducted in the Netherlands [31]. A Cox proportional hazards model was used to identify the most relevant cardiovascular factors for death. For each individual, an absolute risk of dying within 11.5 years may be calculated. For women, this is:

$\{1 - 0.99945^{\exp[0.081(\text{age}) + 0.010*(\text{SBP}) + 0.001*(\text{pulse rate}) + 0.119(\text{smoking}) + 0.160(\text{antihypertensive drugs}) + 0.700(\text{diabetes mellitus}) + 0.436(\text{myocardial infarction}) - 0.036(\text{BMI})]}\}$

Data analysis

Data on PWV were not available for 18 women, leaving results from 385 women for analysis. General characteristics of the study population were described by the mean and SD. The association between PWV measurements and cardiovascular risk factors was evaluated using linear regression analysis, and the associations are presented with the linear regression coefficient ([beta]) and its SE. As it is known from the literature that age, mean arterial blood pressure, and heart rate are major determinants of PWV, we adjusted for these factors when evaluating the association with other determinants using multiple linear regression. We evaluated whether the associations differed between women with an early menopause (1969-1979) or a late menopause (1987-1989). Because all interaction terms had a P value > 0.40, no evidence for modification was found. Therefore, overall findings are reported. For each of the 385 individuals, we calculated the absolute risk of stroke, coronary heart disease, and death, using the formulae described above. Then, we evaluated the association between these risk estimates and PWV using linear regression analyses. Statistical analyses were performed using SPSS for Windows (version 9.0).

Results

The general characteristics of the study participants are given in Table 1.

Table 1 General characteristics of the study population ($n = 385$)

Age (years)	66.2 (3.7)
Systolic blood pressure (mmHg)	147 (20.7)
Diastolic blood pressure (mmHg)	76 (13.6)
Mean arterial pressure (mmHg)	99.9 (14.9)
Pulse pressure (mmHg)	71.7 (14.2)
Heart rate (beats/min)	69.1 (10.1)
Hypertension (%)	37.4
Untreated isolated systolic hypertension (%)	12.5
Triglycerides (mmol/l)	1.45 (0.68)
Total cholesterol (mmol/l)	6.3 (1.0)
HDL cholesterol (mmol/l)	1.5 (0.40)
LDL cholesterol (mmol/l)	4.1 (1.0)
Hypercholesterolaemia (%)	52.7 (203)
Serum glucose (mmol/l)	5.2 (1.1)
Diabetes mellitus (%)	7.3
Body mass index (kg/m^2)	25.9 (4.1)
Waist : hip ratio	0.80 (0.06)
Smoking	
Current (%)	13
Past (%)	36.4
Pack-years	14.1 (18.2)
Cardiovascular disease (%)	11.2
Coronary heart disease (%)	9.9
Stroke (%)	1.6
Pulse wave velocity (m/s)	9.2 (2.1)

Values are expressed as mean (SD) in the case of continuous variables, and as percentages in case of categorical variables. HDL, LDL, high- and low-density lipoproteins. For definitions see text.

The 385 participants were aged 56-73 years. The PWV distribution is depicted in Figure 1, showing a mean (SD) PWV value of 9.2(2.2) m/s.

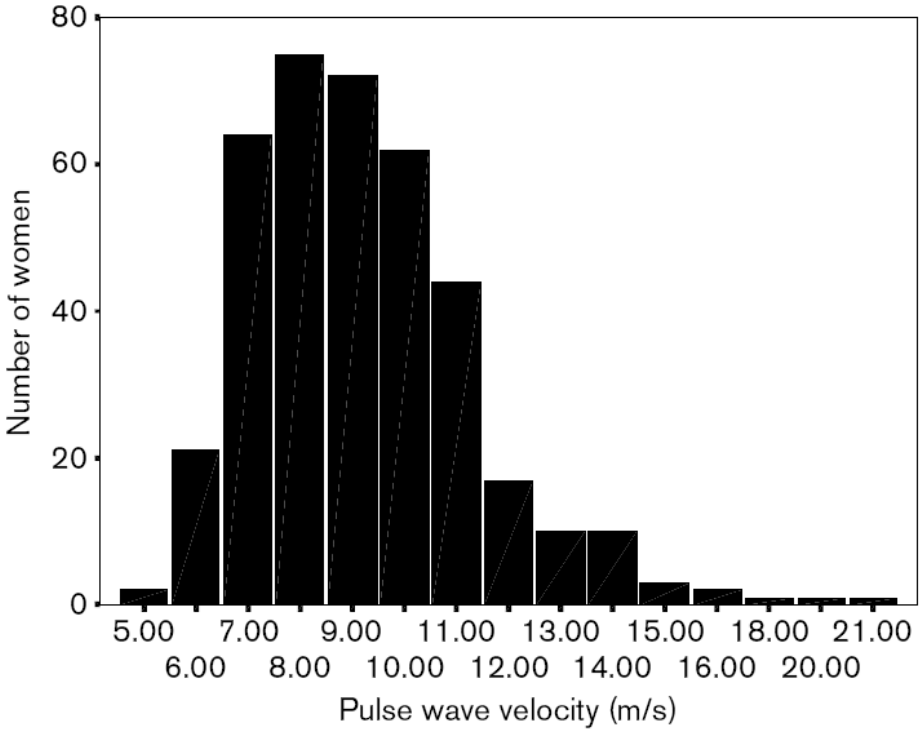


Fig. 1 Distribution of pulse wave velocity in a population of 385 postmenopausal women.

Determinants of PWV

The results of the analysis of risk factors and PWV are presented in Table 2.

Increasing age, blood pressure (systolic, diastolic, pulse, and mean arterial), and heart rate were related to increased PWV. In analyses in which age, mean arterial blood pressure, and heart rate were taken into account, graded associations were found for BMI, weight, height, LDL cholesterol, HDL cholesterol, triglycerides, glucose, diabetes mellitus, insulin, and pack-years of smoking. In a multivariate model, the main factors that remained independently related to an increased PWV were mean arterial pressure, pulse pressure, age, heart rate, the number of pack-years of cigarettes smoked, and HDL cholesterol (inverse relationship) (Table 3).

Table 2 **Relationship between cardiovascular risk factors and pulse wave velocity**

	β coefficient
Model I	
Age (years)	0.154 ± 0.028
Heart rate (beats/min)	0.045 ± 0.011
Systolic blood pressure (mmHg)	0.058 ± 0.004
Diastolic blood pressure (mmHg)	0.056 ± 0.007
Pulse pressure (mmHg)	0.070 ± 0.007
Mean arterial pressure (mmHg)	0.069 ± 0.006
Isolated systolic hypertension	1.554 ± 0.311
Model II	
Total cholesterol (mmol/l)	0.097 ± 0.090
LDL cholesterol (mmol/l)	0.156 ± 0.092
HDL cholesterol (mmol/l)	−0.863 ± 0.225
Triglycerides (mmol/l)	0.451 ± 0.135
Hypercholesterolaemia	0.189 ± 0.185
Glucose (mmol/l)*	0.237 ± 0.080
Insulin (mU/l)*	0.033 ± 0.016
Body mass index (kg/m^2)	0.079 ± 0.022
Waist : hip ratio	3.942 ± 1.359
Weight (kg)	0.017 ± 0.008
Height (m)	−3.522 ± 1.550
Smoking class	0.017 ± 0.132
Pack-years	0.018 ± 0.010

Values are mean ± SE. Model I: age, heart rate, blood pressure included in one model. Model II: age, heart rate, blood pressure included and one risk factor in one model. *In analyses with glucose and insulin, individuals with diabetes mellitus were excluded. The β coefficient reflects the change in PWV (m/s) when the risk factors increases by one unit. HDL, LDL, high- and low-density lipoproteins.

Table 3 **Associations of risk factors with pulse wave velocity in a multivariate model including the risk factors indicated**

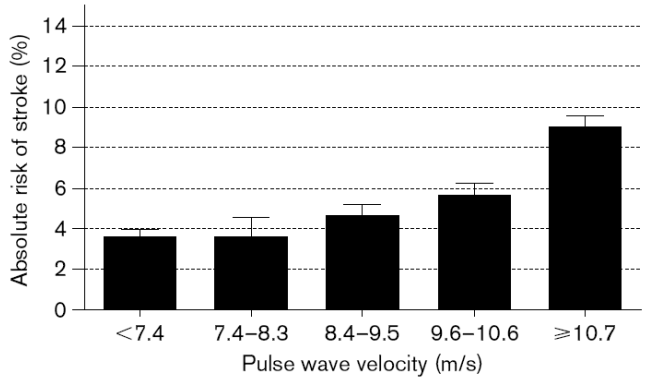
	β coefficient	SE	*P*
Age (years)	0.096	0.024	<0.001
Heart rate (beats/min)	0.025	0.009	0.006
Pulse pressure (mmHg)	0.047	0.007	<0.001
Mean arterial pressure (mmHg)	0.047	0.007	<0.001
Body mass index (kg/m≤)	0.051	0.026	0.053
Waist : hip ratio	0.36	1.572	0.817
Glucose (mmol/l)	0.020	0.084	0.813
Insulin (mU/l)	−0.0038	0.014	0.786
HDL cholesterol (mmol/l)	−0.560	0.237	0.019
Smoking (pack-years)	0.025	0.010	0.015

HDL, high-density lipoprotein.

PWV and absolute risk of disease

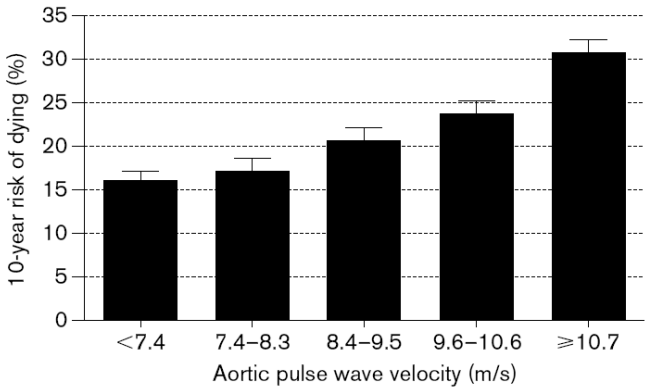
The 10-year absolute risk of stroke increases gradually with increasing aortic PWV (Fig. 2). An increase in PWV of 1 m/s was associated with an increase in absolute risk of stroke of 0.9 [95% confidence interval (CI) 0.7 to 1.1]. The risk of dying within 11.5 years by quintile of aortic PWV is shown in Figure 3. An increase in PWV of 1 m/s was associated with increase in absolute risk of death of 2.2% (95% CI 1.8 to 2.6). The risk of coronary heart disease within 10 years by quintile of aortic PWV is given in Figure 4. A graded positive association was found, with an increase in PWV of 1 m/s associated with a 1.4% (95% CI 0.9 to 1.9) increased risk of coronary heart disease.

Fig. 2



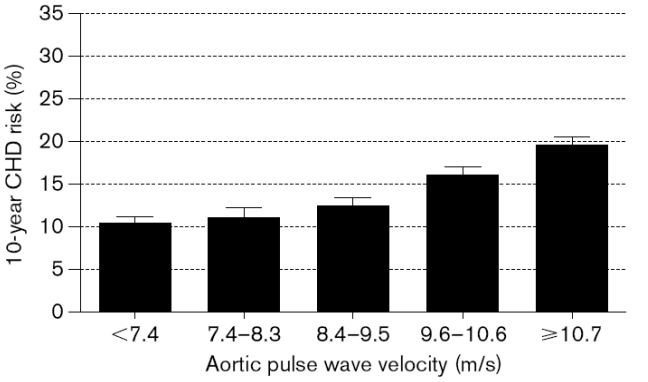
Absolute 10-year risk of stroke by aortic pulse wave velocity (quintiles) (assessed using Framingham Study risk function [29]).

Fig. 3



Absolute 11.5-year risk of death by aortic pulse wave velocity (quintiles) (assessed using a Netherlands follow-up study risk function [31]).

Fig. 4



Absolute 10-year risk of coronary heart disease by aortic pulse wave velocity (quintiles) (assessed using Framingham Study risk function [30]).

Discussion

This cross-sectional study provides evidence that, among postmenopausal women, increased arterial stiffness assessed non-invasively by PWV is significantly and positively associated with age, mean arterial pressure, pulse pressure, heart rate, and pack-years of cigarette

smoking. Height and HDL cholesterol were found to be inversely related to PWV. In addition, this study suggests that PWV is a clear marker of an increased absolute risk of stroke, coronary heart disease, and death within 10-12 years.

Before these results can be accepted, some aspects of this study need to be considered. The measurement of aortic PWV - distance divided by time – allows only an estimate of the actual distance travelled by the pulse, and not the exact distance. The latter can be assessed only with invasive procedures. We tried to approach the true distance by subtracting the distance between the suprasternal notch and the carotid location from the distance between the suprasternal notch and the femoral sampling site, because the pulse travels in the opposite direction [10]. This might underestimate PWV, because arteries become longer and more tortuous with age. However, we consider the error in the distance to be relatively small and therefore not to affect our results seriously. If anything, it might bias our findings towards an underestimation of the associations.

Our findings confirm those from other studies indicating that age, blood pressure, and heart rate are related to increased aortic stiffness. The age-related increase in PWV may be explained by physiological degeneration of elastic fibres, with parallel increases in collagen and mucopolysaccharides within the media, and accumulation of vascular smooth muscle cells in the arterial wall. Those changes are known to be exacerbated by hypertension, arterial degeneration, and cardiac and arterial disease, and are most marked in central arteries such as the aorta [10,16,20,38,39]. The relationship to heart rate may be partly the result of an adrenergic influence on the aortic wall, increasing the smooth muscle tone and leading to increased stiffness of the arterial wall [40]. However, it has been shown that, in elastic arteries, the stiffening effect of tachycardia is exerted independently of sympathetic modulation of the vessel wall properties and that, in muscular arteries, removal of sympathetic influences unmasks the stiffening effect of tachycardia [41-43]. In addition to these three factors, an inverse relationship between body height and PWV has been reported in other studies. Short stature is associated with early systolic arrival of reflected waves, which stiffens the aorta and increases the pulsatile effort of the left ventricle [8,44,45]. Finally, in our analyses, indicators of the insulin resistance syndrome appeared to be related to an increased aortic stiffness - an observation in agreement with the findings of other studies [2,3]. Recently, attention has been focused on the effects of HT in directly affecting vascular characteristics such as endothelial function and atherosclerosis, in an attempt to reduce the risk of future cardiovascular disease [27,28]. By influencing endothelial function and atherosclerosis, HT may also affect arterial stiffness, as these are the two main determinants of arterial stiffness. At present, however, the

advantages and disadvantages of HT in reducing cardiovascular disease events in primary and secondary prevention continue to be strongly debated [27,46,47]. Our results points towards traditional risk factors as the main determinants of arterial stiffness at menopause, and provide a rationale for developing treatment strategies.

The present analyses suggest that an increased PWV is associated with an increased risk of stroke, coronary heart disease, and all-cause mortality, as estimated by available risk functions. This finding of an association of increased PWV and absolute risk of cardiovascular disease estimated using risk functions agrees with findings from a similar study performed in hypertensive individuals [16]. Although both studies suggest that PWV is a clear marker of risk, they had a cross-sectional design, which does not allow inferences as to cause and consequence. Furthermore, the contribution of measurement of arterial stiffness in predicting future disease, relative to that of other cardiovascular risk factors, cannot be determined using studies of that design, but needs to be addressed in a prospective follow-up study.

Direct evidence of the predictive value of measurements of arterial stiffness comes from a small number of studies, mostly among patients with ESRD. In a cohort of 241 patients with ESRD after a mean follow-up of 72 months, increased aortic PWV was associated with all-cause and cardiovascular mortality [15,48,49]. The importance of the prognostic value of aortic PWV measurements in patients with ESRD was further demonstrated in a cohort of 150 such patients [48]. In a recent study among 1980 patients with essential hypertension, who were followed for an average of 4.2 years [49], an increase in PWV of 5 m/s was associated with an increased risk of death of 2.14 (95% CI 1.71 to 2.67) and an increased risk of cardiovascular mortality of 2.35 (95% CI 1.76 to 3.14). These relationships remained statistically significant after adjustments for age, previous symptomatic cardiovascular disease, and diabetes mellitus. Information on the relation of arterial stiffness and risk of cardiovascular disease among other high-risk groups or for the population at large is still lacking. The present study had a cross-sectional design and thus does not allow conclusions to be drawn that are based on longitudinal data. However, we believe that our conclusion that 'PWV is a marker of risk' may be substantiated by two observations. First, in our study, an increased PWV was related to increased risk factors. Thus, if an increased PWV is found in a patient, this patient is likely to have increased risk factors and therefore is at increased risk of cardiovascular disease. This reasoning has been further substantiated by several follow-up studies relating an increased PWV to incident cardiovascular disease events. Second, in our study an increased PWV related to increased absolute risk of cardiovascular disease as assessed using the Framingham Heart

Study risk function. This observation also indicates that, when an increased PWV is found in a patient, this patient is likely to be at increased risk of future cardiovascular disease events.

In conclusion, this cross-sectional study among postmenopausal women provides evidence that PWV of the aorta is associated with increases in most of the established cardiovascular risk factors. In addition, it supports the importance of assessment of arterial stiffness as a marker for cardiovascular disease risk that may be of use as an endpoint in observational and intervention studies.

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2.2 Arterial stiffness is not related to remaining endogenous hormone in healthy postmenopausal women

Abstract

Objective: There is ample data to suggest that endogenous estrogen exposure may protect against cardiovascular disease. Consequently, CVD risk is expected to increase with decreasing estradiol levels. The aim of this study was to investigate whether postmenopausal levels of sex hormones, sex hormone-binding globulin, and a number of other hormones which have been implicated in the aging process are associated with arterial stiffness, a proxy for cardiovascular disease risk, among healthy postmenopausal women.

Method: We performed a cross-sectional study among 384 women aged 56-73 years, 8 to 30 years postmenopausal not using exogenous hormones. Serum concentration of estradiol, estrone, and sex hormone-binding globulin (SHBG), testosterone, cortisol, androstenedione, DHEA and DHEAS, Insulin-like Growth Factor (IGF-1), its binding proteins (IGFBP –1 and –3) and insulin, were all determined by radio immuno assay. Height, weight, waist and hip circumferences, fasting glucose, total and HDL cholesterol, triglycerides, resting blood pressure and heart rate were measured. Arterial stiffness was non-invasively assessed by pulse wave velocity measurement of the aorta using applanation tonometry.

Results: Circulating estradiol, estrone and SHBG levels were not related to pulse wave velocity. None of the other circulating hormone levels measured was associated to pulse wave velocity either.

Conclusions: This cross-sectional study does not support the hypothesis that lower circulating estrogens levels are associated with stiffer arteries in healthy women 8 to 30 years postmenopausal.

Introduction

Cardiovascular disease is currently the most common cause of death and a significant cause of disability among postmenopausal women in Western societies. Because the aging of the population in most Western countries is more pronounced in women than in men, cardiovascular disease has become a major health issue in women. Several lines of evidence have suggested that endogenous estrogens may protect against atherosclerosis and subsequent cardiovascular disease (CVD). Early cessation of endogenous estrogen production, as occurs in early menopause or after surgical ovariectomy, has been shown to increase CVD risk [1-3].

Since atherosclerosis and arterial stiffness are present long before the development of symptomatic coronary heart disease, pulse wave velocity measurements might be useful to detect cardiovascular risk at an early stage. A stiffer aorta has been associated with unfavorable levels of cardiovascular risk factors, with presence of coronary heart disease, atherosclerosis elsewhere and future CVD risk [4-6]. As such, aortic stiffness is an appropriate marker of cardiovascular risk. This simple, reliable, non-invasive method provides a way to study vascular characteristics in populations at large.

Previous studies do not support associations between postmenopausal remaining circulating estrogens levels and symptomatic cardiovascular disease [7-9]. In an attempt to provide further insight into the relationships between postmenopausal sex steroids and CVD, we have examined the associations between remaining estrogen levels measured with new more sensitive methods and aorta stiffness measured by aortic pulse wave velocity. In addition, associations between a number of other hormones that have been implicated in the aging process and aorta stiffness were studied.

Subject and methods

Study population and measurements

We used for this study the same population and measurements as previously described [10]. See chapter 2.1.

Complementary biochemical assessments

Serum concentrations of estradiol (E2, nmol/l), estrone (E1, nmol/l), DHEA (nmol/l) and DHEAS (µmol/l) and total testosterone (T, nmol/l) were estimated using ultra-sensitive double-antibody radioimmunoassay commercial kits purchased from Diagnostic Systems Laboratories (Webster, TX, USA). Sex hormone binding globulin (SHBG, nmol/l) was measured using a chemoluminescence-based immunometric assay on the Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, CA, USA). Total IGF-1 was determined by a IGFBP-blocked RIA in the presence of large excess of IGF-II as described (Blum WF, 1994 Growth regul. 4:11-9). IGFBP-1 and IGFBP-3 were measured with in-house RIAs as described previously. Insulin was measured by a commercially available RIA (Medgenix Diagnostics)

Data analysis

PWV data were not available for 18 women, leaving 384 women for analyses. Results are expressed as the mean and SD, unless otherwise stated. The relationships between circulating hormones levels and the arterial stiffness measures and other variables were assessed using linear regression models and are given as the linear regression coefficient (ß) and its standard error (SE). Multiple linear regression analysis was used to adjust for age, heart rate, blood pressure and body mass index. Using multiplicative interaction terms, we evaluated whether the association of estradiol and PWV differed among smokers and among participants with obesity, hypertension and diabetes mellitus. Statistical analyses were done using SPSS for Windows (version 11.0).

Results

The characteristics of the subjects are shown in Table 1.

Circulating hormone levels were not related to age except for DHEA and DHEAS, which were both significantly lower when women were older (results not shown). Increasing estradiol levels were associated with higher BMI. Higher estradiol levels were associated with higher blood pressure levels independently of BMI. After adjustment for mean arterial pressure, or BMI, estradiol, estrone, testosterone and SHBG were not related to arterial stiffness (Table 2).

TABLE 1. Characteristics of the study population (n = 384).

Characteristic Mean (SD)	Study population N = 384
Age (year)	66.2 (3.8)
Time since menopause (year)	16.9 (6.3)
Age at menopause (year)	49.7 (4.5)
Age at menarche (year)	13.5 (1.6)
Body Mass Index (kg/m²)	26 (4.2)
Waist-to-hip ratio (cm/cm)	0.81 (0.07)
Cardiovascular risk factors	
Systolic blood pressure (mmHg)	147.7 (20.8)
Diastolic blood pressure (mmHg)	76 (13.6)
Mean arterial pressure (mmHg)	100 (14.8)
Pulse pressure (mmHg)	71.8 (14.3)
Heart rate (bpm)	69 (10)
Smoker %(n)	13 (50)
Past smoker %(n)	36.5 (140)
Total cholesterol (mmol/l)	6.3 (1)
HDL-cholesterol (mmol/l)	1.5 (0.4)
LDL-cholesterol (mmol/l)	4.1 (1)
Hypercholesterolemia % (n)	53 (203)
Triglycerides (mmol/l)	1.45 (0.7)
Serum glucose (mmol/l)	5.2 (1.2)
Insuline (mU/l)	11.5 (7.1)
Diabetes mellitus %	7.3 (28)
Hypertension % (n)	37 (143)
Cardiovascular disease % (n)	11 (43)
Hormones	
Estradiol (pmol/l)	20.2 (13.3)
Estrone (pmol/l)	46.5 (36)
SHBG (nmol/l)	59.06 (26.9)
Testosterone (nmol/l)	1.96 (0.8)
Androstenedione (nmol/l)	3.4 (1.5)
DHEA (nmol/l)	13.3 (8.1)
DHEAS (umol/L)	2.3 (1.4)
Cortisol (nmol/L)	463.1 (140)
Insulin (mU/l)	11.8 (7.2)
IGFBP-1 mg/l	34.7 (28)
IGF-1 (nmol/l)	17.9 (7.2)
IGFBP-3 mg/l	3.3 (0.6)
Pulse wave velocity (m/s)	9.2 (2.2)

Values are expressed as mean (standard deviation) in case of continuous variables, and as percentages (number of cases) in case of categorical variables.

The same analyses performed after exclusion of early menopausal participants showed no material changes in the magnitude of above-mentioned associations. No evidence was found for an association of estradiol, and estrone to PWV in different strata of smoking, body mass index, hypertension or glucose intolerance (p values of interactions terms ≥ 0.15). In the same analysis for SHBG, the p value of interaction terms in strata of hypertension and glucose intolerance were significant ($p < 0.05$). SHBG was not associated with PWV in the group with hypertension or with glucose intolerance. None of the other circulating hormone levels measured was associated to PWV (Table 2).

Table 2: Relation of circulating hormone levels with pulse wave velocity, crude and adjusted for potential confounders

	Crude relations		**Adjusted for age and BMI**		**Adjusted for age, BMI, blood pressure and heart rate**	
	ß ± SE	[95% CI]	ß ± SE	[95% CI]	ß ± SE	[95% CI]
Estradiol (10 pmol/l)	0.15 ± 0.08	[-0.01;0.32]	0.04 ± 0.08	[-0.12;0.21]	-0.04 ± 0.07	[-0.18;0.11]
Estrone (10 pmol/l)	0.08 ± 0.03	[0.01;0.14]**	0.03 ± 0.03	[-0.03;0.09]	-0.005 ± 0.03	[-0.06;0.05]
SHBG (10 nmol/l)	-0.03 ± 0.04	[-0.10;0.05]	-0.03 ± 0.04	[-0.11;0.06]	-0.03 ± 0.04	[-0.10;0.05]
Testosterone (10 nmol/l)	2.85 ± 1.41	[0.07;5.62]	1.65 ± 1.34	[-0.98;4.29]	-0.20 ± 1.18	[-2.52;2.13]
Androstenedione (10 nmol/l)	0.20 ± 0.75	[-1.27;1.68]	0.67 ± 0.71	[-0.72;2.07]	0.14 ± 0.62	[-1.08;1.36]
DHEA (10 nmol/l)	-0.26 ± 0.14	[-0.53;0.01]	-0.07 ± 0.13	[-0.33;0.19]	-0.09 ± 0.12	[-0.31;0.14]
DHEAS (10 umol/L)	-1.39 ± 0.81	[-2.98;0.20]	-0.34 ± 0.78	[-1.87;1.19]	-0.32 ± 0.68	[-1.66;1.02]
Cortisol (10 nmol/L)	-0.010 ± 0.008	[-0.03;0.006]	-0.003 ± 0.008	[-0.02;0.01]	-0.013 ± 0.01 *	[-0.03;0.0002]
Insulin (10 mU/l)	0.45 ± 0.16	[0.13;0.77]**	0.18 ± 0.17	[-0.15;0.51]	0.069 ± 0.15	[-0.22;0.36]
IGFBP-1 (10 mg/l)	-0.0003 ± 0.035	[-0.07;0.07]	0.03 ± 0.04	[-0.05;0.11]	-0.0003 ± 0.035	[-0.07;0.07]
IGF-1 (10 nmol/l)	-0.06 ± 0.16	[-0.36;0.25]	0.04 ± 0.15	[-0.24;0.33]	-0.05 ± 0.13	[-0.31;0.20]
IGFBP-3 (10 mg/l)	-1.61 ± 2.02	[-5.60;2.37]	-1.88 ± 1.91	[-5.63;1.88]	-3.22 ± 1.66 *	[-6.49;0.05]

ß coefficient reflect PWV changes in m/s per 10 units circulating hormone levels. For example, PWV decreases 0.041 msl per 10 pmol/l serum estradiol level.
*p ≤ 0.05 **p ≤ 0.01

Discussion

This study investigated the association between remaining endogenous estrogens, testosterone and sex hormone-binding globulin and arterial stiffness among women who had experienced their last menstruation between 8 and 30 years ago. Endogenous estrogens and SHBG levels did not change with age, age at menopause or time since menopause in this population. This is consistent with previously reported data on postmenopausal serum sex steroid concentrations, where a relative stability of intra-individual estrone and estradiol levels was shown from 3 years after the postmenopausal decline [11-13]. No association was found between the remaining endogenous hormones levels measured and arterial stiffness. This may be due to an unexpected association of higher estradiol level with higher blood pressure in our population which might have counteracted a beneficial effect of estrogens on distensibility if present at all. The analysis of the association is complicated by the fact that postmenopausal estrogen production primarily depends on body weight, the latter being a strong determinant of blood pressure and atherosclerosis as well.

Strong evidence supports the hypothesis that a longer exposure to premenopausal levels of endogenous estrogens protects against cardiovascular diseases en that estrogens might be implicated in short-term protective effects on arterial distensibility by interacting with endothelial function as a local modulator, and in long-term effect by influencing structural arterial wall modifications (elastin/collagen ratio) [14-16]. Vascular stiffness arises as a result of age-related and irreversible degenerative arteriosclerotic changes. The age related increase of pulse wave velocity is due to physiological degeneration of elastic fibers with parallel increase in collagen and mucopolysaccharides within the media, and accumulation of vascular smooth muscle cells in the arterial wall [17-19]. A number of studies have shown that the normal decline in endogenous estrogen production during the menopausal transition period is related to increased arterial stiffness [20-23] which can be prevented or delayed by hormonal therapy (HT) [24]. Furthermore, several studies in postmenopausal women have demonstrated that the administration of estrogen has short-term vascular effects, including dilatation of coronary arteries, improvement of the elastic properties of the aorta and reduced wave reflection in the arterial periphery [25,26].

The absence of associations found in our study is not necessarily in contradiction with possible protective effects of estrogens on arterial stiffness. If irreversible structural modifications of the arterial wall occur during exposition to low serum estrogens

concentrations, considering the fact that our participants experienced menopause 8 to 30 years ago, endogenous estradiol levels have probably been too long far below a threshold at which any beneficial arterial effect can be demonstrated. The findings of the WISE study where disruption of ovulatory cycling characterized by hypoestrogenemia of hypothalamic origin appears to be associated with angiographic coronary artery disease among premenopausal women support this view [27]. Hypoestrogenemia was defined as estradiol < 184 pmol/l, whereas in our study mean estradiol levels were 20 pmol/l.

Furthermore, the endothelium-dependent vasodilating effect of estrogens may also be less apparent on large arteries especially if stiffening has already occurred within the media. HT started a long time after menopause seems not to be related to lower arterial stiffness [28,29]. This view is supported by the findings from different studies, in particular the Cardiovascular Health study, the Prevention Atherosclerosis Trial (EPAT), the Estrogen Replacement and Atherosclerotic trial and even in the HERS study, where greater vasodilatation effects of estradiol were found among hormone replacement users without cardiovascular disease, compared to hormone users with established coronary artery disease or non hormone users [30-35]. Neither vasodilating effects of estradiol nor effects on the progression of atherosclerosis have been demonstrated among women with established atherosclerosis, while those effects were present among women younger than 45 year without pre-existent cardiovascular disease [30-35].

Findings in animal studies also suggest that estradiol has little anti-atherosclerotic effects in the presence of established coronary artery disease. Hormone therapy started two years after oophorectomy produced no effect on the extent of coronary artery plaque formation, while similar hormone treatment administered to monkeys immediately after surgery resulted in a 50% reduction in atherosclerotic development [36]. The WHI study results also support the argument that the population was already too old to expect a beneficial effect of estradiol administration. The conflicting results about benefit and risks of HT among postmenopausal women can be explained by the previously discussed possibility that it only prevents onset and progression of atherosclerosis when started before the occurrence of major vascular damage, and may work adversely in the presence of generalized atherosclerosis with occurrence of clinical events [37,38].

The relation observed between SHBG and arterial stiffness is interesting and not fully understood. Synthesized in the liver, SHBG levels regulate plasma free and protein-bound androgens and estrogens. SHBG decreases with increasing visceral fat, triglyceride and insulin concentrations, and increases with higher levels of HDL cholesterol. Therefore, SHBG

seems to be an important and reliable marker of the interrelationships between sex steroid hormones, obesity, and cardiovascular disease risk [39-42]. Recently, SHBG has been shown to mediate extra-cellular steroid hormone actions after binding to specific high affinity SHBG receptors [43]. However, it is not known whether an intrinsic protective effect on the arterial wall works through SHBG-receptors.

In conclusion, in this study among postmenopausal women having experienced last menstruation between 8 and 30 years ago, the remaining endogenous estrogen levels are not associated with aorta stiffness.

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Chapter 3

Endogenous hormones and cognition

Endogenous oestrogens are related to cognition in healthy elderly women

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Abstract

Objective: To investigate whether levels of endogenous hormones, in particular circulating oestrogens and SHBG, are associated with cognition in healthy postmenopausal women.

Design: Cross-sectional study

Patients: 402 healthy postmenopausal women aged 56-73 years between 8 and 30 years after menopause, none taking oestrogen.

Measurements: Serum concentration of oestradiol, oestrone, and sex hormone binding globulin (SHBG) determined by immunoassay. Cognition assessed using the mini-mental state examination questionnaire (MMSE).

Results: In this group, 149 individuals had a MMSE score < 27, while only 89 individuals had a MMSE score < 26, indicating a relatively healthy population with regard to cognitive ability. Cognition decreased with age, time since menopause and blood pressure, and was better with higher age at menopause. Serum oestrogens and SHBG levels were not related to age, age at menopause, or time since menopause, and oestrogen levels were positively associated with blood pressure. After adjustment for mean arterial pressure and SHBG, the frequency of mild cognitive impairment decreased significantly with higher oestradiol and oestrone serum levels [ORs Q5 versus Q1: 0.41 (95% CI 0.20-0.84) and 0.51 (95% CI 0.20-0.99) for oestradiol and oestrone, respectively].

Conclusions: Postmenopausal women with higher remaining circulating oestradiol levels appear less likely to suffer from cognitive impairment. This effect is independent of age at menopause, time since menopause and BMI. These findings support the hypothesis that endogenous oestrogens may protect against cognitive decline with aging.

Introduction

An increase of life expectancy is not necessarily accompanied by a similar increase in healthy life span, and understanding determinants of successful aging and in particular the role played by endocrine factors has gained increased interest [1]. Women are likely to live a third of their lives in a state of relative oestrogen deficiency, which is held responsible for unfavourable long-term effects on bone metabolism, the cardiovascular system, and probably cognitive function culminating in dementia. There is growing evidence that oestrogens impact on memory, affect, and motor co-ordination in women, and they also appear to have a neuroprotective effect for Alzheimer's disease [2;3]. In contrast, results from studies with hormonal replacement therapy (HRT) have not supported a protective effect of exogenous oestrogens on cognitive functions. [4-6] A few studies have addressed the role of the remaining circulating postmenopausal oestrogens in cognition, but were hampered by small sample sizes or improper adjustment for potential confounders. [7-10] Two studies reported protective effect of higher oestradiol levels on cognitive decline [3,11] while a recent study found that higher serum oestradiol levels were associated with a higher risk of Alzheimer's disease. [12]

The aim of this study was to determine the relation between circulating oestrogen measured with new more sensitive methods and sex hormone binding globulin levels and cognition in healthy postmenopausal women.

Subject and methods

Study population

Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC). [13] In PROSPECT a total of 17,395 healthy participants who came for breast cancer screening, aged 49-70 years, living in Utrecht and surroundings, were enrolled between 1993 and 1997. Using baseline data from PROSPECT, we selected women who had experienced a natural menopause between 8 to 30 years ago. In addition, inclusion criteria were an intact uterus and at least one intact ovary, and no use of sex steroids after the reported date of last menstruation. Out of 1803 eligible women, 902 women were invited and 553 (61%) answered positively.

402 participants were finally included in our study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study centre independently. Each participant underwent all tests and assessments during two visits to the study centre. The study was approved by the Institutional Review Board of the University Medical Centre, Utrecht, and written informed consent was obtained from all participants. Data collection took place between September 1999 and March 2000.

MMSE

Cognitive function was assessed by specially trained research assistants using the Dutch version of the 30-point Mini- Mental State Examination questionnaire (MMSE). [14] This short general cognitive test has been used extensively in epidemiological studies and allows assessment of orientation to time and space, concentration, language, calculation, and immediate and delayed memory. Among older people a score below 24 indicates cognitive impairment. Because of our selection of a relatively young population, with exclusion of disabled and those dependent on others for activities of daily living, we did not expect any woman participating in this study to qualify for the definition of clinical dementia. We used two different cut-off points, the widely used threshold of 26 below which moderate cognitive impairment is defined, [15] and a threshold of 27 below which mild cognitive impairment was considered to occur.

Biochemical assessments

Venous blood samples were collected in the morning between 8.00 and 11.00 a.m. after an overnight fast. Levels of oestradiol were estimated using an ultra-sensitive double-antibody radioimmunoassay (RIA) purchased from Diagnostic Systems Laboratories (Webster, TX, USA). Oestrone levels were measured using a double antibody RIA from the same supplier. SHBG was measured using a chemoluminescence based immunometric assay on the Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, CA, USA).

Other measurements

Information on health status was obtained by medical history, registration of current medication and physical examination. A standardised questionnaire on oestrogen use, alcohol

consumption and smoking was obtained from all women as part of the medical history. Subjects were categorised in current smokers, former smokers and those who had never smoked. Height, weight, waist and hip circumference were measured with the subject in standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters, and body fat distribution was assessed by the ratio of waist and hip circumferences. Blood pressure and heart rate were measured during the first visit by the oscillometric automated device DINAMAP 8100 (Critikon, Johnson-Johnson, Tampa, FL, USA) according to the orthostatic hypotension protocol. [16] Measurements were conducted before 11.00 a.m. after an overnight fast. [17] After five minutes of rest, blood pressure was taken at the right brachial artery simultaneously with heart rate measure, twice with the participant lying down and three times with the participant in standing position, with a time laps of one minute between each measurement. Systolic as well as diastolic blood pressure was defined as the average of the two supine measurements. Mean arterial blood pressure (MAP) was calculated by the following formula: diastolic blood pressure + 1/3 x (systolic blood pressure – diastolic blood pressure).

Data analysis

Distributions of population characteristics are expressed according to the quintile distributions of oestradiol level. Associations between sex steroid levels and cognition were quantified using logistic regression models and odds ratios and their 95% confidence intervals are presented. Adjustments were made for age and other potential confounders. Statistical analyses were done using SPSS for Windows (version 11.0).

Results

General characteristics of the entire study population and according to the quintile distribution of oestradiol serum levels are given in Table 1.

Table 1. Characteristics of the study population ($n = 402$) per quintiles oestradiol

Characteristic	Study population ($N = 402$)	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
		Oestradiol:				
		0–10 pmol/l ($N = 83$)	10–15 pmol/l ($N = 87$)	15–20 pmol/l ($N = 87$)	20–29 pmol/l ($N = 74$)	29–102 pmol/l ($N = 80$)
Age (years)	66·3 (3·8)	66·2 (3·8)	66·4 (3·9)	66·4 (3·7)	66·5 (4)	66 (3·9)
Time since menopause (years)	15·2 (5·4)	15·8 (5·8)	17·6 (6·5)	17·5 (6·5)	18 (6·5)	16·6 (6·8)
Age at menopause (years)	49·6 (4·5)	50·8 (3·4)	49·2 (4·4)	49·4 (4·7)	48·9 (4·6)	49·9 (5)
MMSE (points)	26·8 (1·9)	26·7 (2·1)	26·7 (2)	26·6 (1·9)	26·9 (2)	27·2 (1·7)
Moderate cognitive impairment (< 26) % (n)	22 (89)	24·1 (20)	24·4 (19)	21·8 (19)	23 (17)	17·5 (14)
Mild cognitive impairment (< 27) % (n)	37·1 (149)	38·6 (32)	44·9 (35)	41·4 (36)	33·8 (25)	26·3 (21)
Cardiovascular risk factors						
Body Mass Index (kg/m^2)	26·2 (4·4)	24·3 (3·1)	25·9 (4·3)	26 (4·1)	26·4 (4·6)	28·4 (4·9)
Waist (cm)	82·9 (10·8)	79·7 (9·4)	81·7 (10·2)	81·6 (9·8)	83·5 (11·6)	88·2 (11·3)
Mean arterial pressure (mmHg)	100 (14·8)	96·3 (14·5)	99·1 (15·9)	101·6 (15·6)	99·4 (12·7)	104 (14·6)
Educational level of participants % (n)						
Primary school completed	22·6 (91)	26·5 (22)	23·1 (18)	17·2 (15)	28·4 (21)	18·8 (15)
Technical/professional education	45·3 (182)	39·8 (33)	43·6 (34)	49·4 (43)	41·9 (31)	51·3 (41)
Secondary education	13·7 (55)	15·7 (13)	15·4 (12)	16·1 (14)	8·1 (6)	12·5 (10)
Academic education	18·2 (73)	18·1 (15)	16·7 (13)	17·2 (15)	21·6 (16)	17·5 (14)
Hormones						
Oestradiol (pmol/l)	20·2 (13·5)	5·3 (3·1)	12·6 (1·3)	18 (1·5)	24·9 (2·4)	41 (12·2)
Oestrone (pmol/l)	47 (35·9)	21·5 (16·25)	33·4 (18·7)	40·1 (21·3)	50 (25)	81·6 (53·8)
SHBG (nmol/l)	59·06 (26·7)	67·7 (24·7)	63·2 (26·7)	60·4 (23·5)	55·5 (28·1)	47·8 (26·9)

Values are expressed as mean (standard deviation) unless otherwise stated.

Increasing oestradiol levels were associated with higher BMI and waist circumference consistent with adipose tissue being an important source of postmenopausal oestrogen production. Oestrogens were also associated with increasing blood pressure independently of BMI. Circulating oestradiol and oestrone levels were not associated to age, age at menopause, time since menopause or educational level of participants.

The distribution of MMSE scores is given in Fig. 1, and the educational level of participant is shown in Fig. 2.

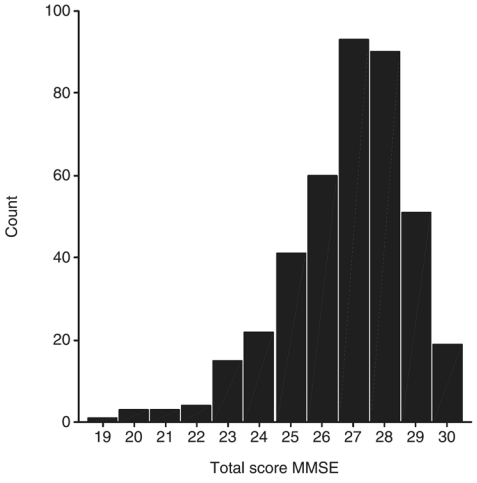

Fig. 1 Distribution of MMSE scores.

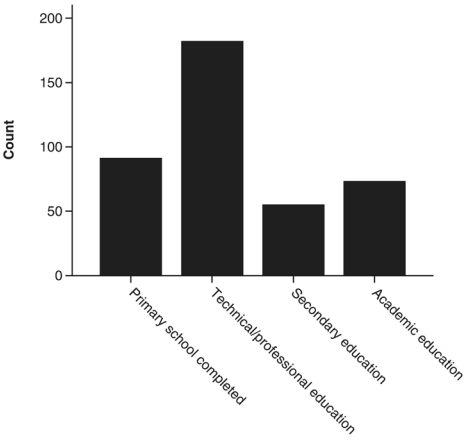

Fig. 2 Educational level of participants.

The frequency of cognitive impairment increased with age, with time since menopause, and with higher blood pressure, and both levels of cognitive impairment were decreasing with higher age at menopause and with higher educational level of participants (Table 2). The frequency of cognitive impairment was not related to BMI or fat mass, even after adjustment for blood pressure and/or age.

A slight decrease in the frequency of cognitive impairment was seen with increasing quintiles of oestradiol concentration (Table 1) and higher oestradiol and oestrone levels were significantly associated with lower frequency of mild cognitive impairment (MMSE<27) (Table 3). These associations were stronger after adjustment for mean arterial blood pressure and/or SHBG, and remained unchanged after adjustment for BMI, and educational level.

Analyses with moderate cognitive impairment (MMSE<26) showed essentially the same results, with variations explained by the smaller number of women in this category (Table 4).

Further adjustment for other possible confounders did not significantly change the associations. Sex hormone-binding globulin was not related to the level of cognitive decline. The same analyses performed after exclusion of early menopausal women (age at menopause ≤ 45 year) showed virtually the same results.

Table 2: Determinants of impaired cognition

Characteristics	MMSE score < 26 Odds ratio, 95% CI	MMSE score < 27 Odds ratio, 95% CI
Age (years)	1·064 (1·001–1·132)*	1·044 (0·991–1·1005)
Age at menopause (years)	0·911 (0·865–0·958)***	0·917 (0·875–0·96)***
Time since menopause (years)	1·073 (1·034–1·114)***	1·061 (1·028–1·096)***
Mean arterial pressure (mmHg)	1·017 (1·002–1·033)*	1·02 (1·006–1·034)**
Body mass index (kg/m^2)	1·011 (0·96–1·07)	1·018 (0·97–1·06)
Level of education	0·52 (0·391–0·69)***	0·452 (0·353–0·58)***

*$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$; ***$P \leq 0{\cdot}001$.

Table 3. Odds ratios for the relations between quintiles of serum hormone levels and mild cognitive impairment (MMSE < 27)

Hormone OR (95%CI)	Quintile 1 Reference	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P*-value for trend
Oestradiol	1	1·30 (0·69–2·43)	1·13 (0·61–2·08)	0·81 (0·42–1·56)	0·57 (0·29–1·10)	0·039
Oestradiol*	1	1·28 (0·68–2·41)	1·11 (0·60–2·06)	0·80 (0·42–1·54)	0·57 (0·29–1·11)	0·040
Oestradiol* Edu	1	1·31 (0·66–2·57)	1·22 (0·63–2·36)	0·75 (0·37–1·52)	0·56 (0·28–1·13)	0·038
Oestradiol*†	1	1·22 (0·65–2·31)	0·99 (0·53–1·87)	0·75 (0·39–1·46)	0·48 (0·24–0·95)	0·013
Oestradiol*‡	1	1·24 (0·66–2·34)	1·06 (0·57–1·96)	0·73 (0·37–1·42)	0·48 (0·24–0·97)	0·015
Oestradiol*†‡	1	1·19 (0·63–2·26)	0·95 (0·51–1·79)	0·69 (0·35–1·35)	0·41 (0·20–0·84)	0·005
Oestradiol*†‡§	1	1·17 (0·61–2·23)	0·94 (0·50–1·77)	0·68 (0·34–1·34)	0·40 (0·19–0·83)	0·005
Oestradiol*†‡§ Edu	1	1·30 (0·65–2·60)	0·93 (0·58–2·23)	0·72 (0·35–1·49)	0·50 (0·23–1·08)	0·028
Oestrone*	1	0·85 (0·46–1·60)	0·52 (0·27–1·00)*	0·71 (0·37–1·34)	0·51 (0·27–0·99)	0·038

*Adjusted for age; †Adjusted for MAP; ‡Adjusted for SHBG. §Adjusted for BMI. Edu: Adjusted for educational level.

Table 4. Odds ratios for the relations between quintiles of serum hormone levels and moderate cognitive impairment (MMSE < 26)

Hormone OR (95%CI)	Quintile 1 Reference	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P*-value for trend
Oestradiol	1	1·01 (0·49–2·09)	0·88 (0·43–1·8)	0·94 (0·45–1·97)	0·67 (0·31–1·44)	0·32
Oestradiol*	1	1 (0·48–2·06)	0·86 (0·42–1·78)	0·92 (0·44–1·94)	0·67 (0·31–1·45)	0·32
Oestradiol Edu	1	1·05 (0·5–2·21)	0·94 (0·45–1·96)	0·91 (0·42–1·96)	0·68 (0·31–1·49)	0·32
Oestradiol†	1	0·95 (0·46–1·98)	0·78 (0·38–1·62)	0·88 (0·41–1·85)	0·58 (0·27–1·28)	0·198
Oestradiol‡	1	0·97 (0·47–2·01)	0·83 (0·4–1·71)	0·85 (0·4–1·81)	0·59 (0·26–1·3)	0·194
Oestradiol†‡	1	0·93 (0·45–1·94)	0·75 (0·36–1·57)	0·82 (0·38–1·74)	0·52 (0·23–1·17)	0·121
Oestradiol†‡§	1	0·94 (0·45–1·95)	0·76 (0·37–1·59)	0·83 (0·39–1·77)	0·51 (0·22–1·17)	0·124
Oestradiol†‡§ Edu	1	1·03 (0·48–2·21)	0·88 (0·41–1·87)	0·89 (0·4–1·94)	0·62 (0·27–1·45)	0·265
Estrone	1	0·95 (0·46–1·96)	0·85 (0·4–1·74)	0·7 (0·33–1·5)	0·75 (0·35–1·58)	0·299

*Adjusted for age; †adjusted for MAP; ‡adjusted for SHBG; §adjusted for BMI. Edu: adjusted for educational level.

Discussion

The results of this study among healthy women 8 to 30 years after menopause suggest that higher circulating oestrogen concentrations (predominantly oestradiol) are associated with a lower risk of cognitive decline. This effect was more pronounced after adjustment for blood pressure and SHBG, and was independent of BMI.

Before interpreting these data, some issues need to be addressed. We used the MMSE test to evaluate cognitive function because of its wide use and simplicity to be performed on a large scale allowing subjective interpretation to be avoided. A cut-off value of 26 points was chosen to mark intact cognition because our objective was to determine cognitive function rather than to diagnose dementia. [18] Although detection of subtle cognitive change or specific cognitive function testing are not possible with the MMSE, its validity and usefulness as a test for overall cognitive screening has been well documented [19,20]. On an individual level the MMSE is probably not a very precise test to assess cognitive function, but on a population level it adequately reflects the distribution of cognition. [21] Because of a small number of women with MMSE<26 in this relatively healthy group of independently living women in which individuals with clearly impaired cognition were excluded at the start of the study, we used a threshold of 27 below which mild cognitive impairment was considered to occur. Both cognitive impairments analyses showed essentially the same results, with variations in significance explained by the smaller number of women in the category below 26.

There is a moderate increase of MMSE score with increasing quintiles of oestradiol concentration in this population. Although associations between higher oestrogen levels and lower frequency of mild cognitive impairment were significant, a risk of over interpretation cannot be totally excluded. It is once again stressed that this cross-sectional study was carried out in a group of menopausal women in relative health and of relatively good cognition.

Our findings are consistent with previously reported postmenopausal evolution of sex steroids serum concentrations, with intra-individual oestrone and oestradiol levels remaining relatively stable from 3 years after the postmenopausal decline. [22-24] The principal source of oestrogens in postmenopausal women is peripheral aromatisation of androstenedione to oestrone in adipose tissue and skin, which is subsequently reduced to oestradiol in peripheral tissues. Extragonadal oestrogen biosynthesis occurs in a number of sites, including adipose tissue, bone, various sites of the brain, and vascular endothelial and smooth muscle cells. [25]

Postmenopausal oestrogen production is thus primarily influenced by body weight but not by age.

The protective effect of oestrogens on cognitive decline was more pronounced after adjustment for SHBG, suggesting that the bioavailable oestrogen level is the principal determinant for an effect on the brain. This is consistent with the findings of Yaffe *et al.*[3] in 425 postmenopausal women 65 years and older, but not with the findings of Drake *et al.*[11] in a smaller group of 39 postmenopausal women aged 65 to 90 years, mean 78.8 ± 7.1 (SD). Apart from a lower power in the study of Drake *et al.*,[11] adjustment for SHBG probably better estimates oestrogen activity, apart from a possible additional intrinsic effect of SHBG. Recent evidence has shown that SHBG bound to specific SHBG receptors might promote steroid activity by activation of adenylate cyclase, thus without steroids entering the cell. [26] In both studies mentioned above[3,11] blood pressure was not measured. However, we found this to be a probable confounding factor in the relation between circulating oestrogen levels and cognitive decline, which could have weakened the association between oestradiol and cognition.

Interestingly, in our population serum oestradiol levels increased with BMI and frequency of impairment in cognitive functions decreased with higher serum oestradiol levels, but higher BMI did not protect against cognitive impairment. Fat mass or higher BMI was not related to cognition, which suggest the importance of other sources of oestradiol production than adipose tissue in postmenopausal women. Also local oestrogen biosynthesis by aromatase activity in the brain may be important in the regulation of various cognitive and hypothalamic functions.[27] Simpson[28] and Labrie[29] have hypothesised that in postmenopausal women, and also in men, extragonadal oestradiol plays an important paracrine, autocrine and indeed, intracrine role.

Our findings do not agree with a recent study where higher oestradiol levels were (although not significantly) associated with higher risk of dementia. [12] Two other studies on Alzeimer disease (AD) patients found the same trend, one with higher oestrone and androstenedione levels in the AD group, [10] and the other with not significantly increased levels of oestradiol in the AD group compared to healthy elderly.[9] AD is associated with a disorder of the hypothalamic-pituitary-adrenal (HPA) axis and with increased levels of adrenocortical and gonadal hormones. The hypothesis that AD itself could be responsible for an abnormality of sex steroid production has been previously mentioned and deserves further investigation. [9,10]

In conclusion, postmenopausal women with higher “remaining” oestradiol levels appear less likely to suffer from cognitive decline. This effect seems independent of age at

menopause, time since menopause or body mass index, and stronger after adjustment for blood pressure. The findings of this study support the hypothesis that higher postmenopausal endogenous oestrogen levels may protect against cognitive impairment.

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Chapter 4

Bone mineral density

Local contribution of bone mineral density determinants at various locations in healthy postmenopausal women

Abstract

Objective: The aim of this study in healthy postmenopausal women was to determine the variations of effect on bone mineral density at various sites of the following determinants: fat mass, lean mass, appendicular lean mass and muscle strength, and endogenous hormones.

Method: We performed a cross-sectional study among 402 randomly selected women aged 56-73 years, not using exogenous hormones. Serum concentrations of estradiol (E2), estrone (E1), testosterone (T) and sex hormone-binding globulin (SHBG) were determined by immunoassay. Bone mineral density and body composition including lean mass and fat mass were assessed using dual-energy x-ray absorptiometry at the following sites: the whole body, skull, arms, legs and the total hip. We measured grip and knee muscle strength using dynamometry. To compare the strength of effects of various determinants, we used standardised β of linear regression.

Results: Well-known determinants of BMD were confirmed. For whole body BMD, lean and fat mass were equal independent determinants, but at local sites, BMD was mainly determined by total fat mass (standardised β of linear regression 0.21 to 0.32) followed by total lean mass (0.19 to 0.24). Skull BMD was further determined by circulating estradiol level (0.14), while weak and not significant effects of muscle mass and strength were observed. Grip force was strongly associated with arm BMD (0.21), while the relation with leg extensor strength and arm BMD was dependent on appendicular lean mass (0.19). Serum estradiol was also independently related to arm BMD (0.13). Other independent determinants of hip BMD were leg muscle strength (0.24) and appendicular lean mass (0.14), while no significant relation was found with estradiol.

Conclusions: The postmenopausal changes in body composition have considerable effects on BMD determinants. Fat mass plays the major role, followed by lean mass. We present evidence for specific local effects of lean mass en muscle strength on arm and hip BMD. The effect of circulating estradiol was predominantly localized at the skull and the arm.

Introduction

Postmenopausal women all share a drop in circulating estrogen level, known to be associated with decreased bone mineral density (BMD) and increased risk of osteoporotic fractures, a significant cause of disability among postmenopausal elderly women in Western societies. The increasing life expectancy augments the time span lived in a postmenopausal state of relative estrogen deficiency and the consecutive unfavorable effects on bone metabolism may reduce the chance of living an independent life until death [1]. There is however much variation between women in tendency of suffering from osteoporotic fractures. Differences in remaining serum levels of estradiol might in part explain this variation, with evidence that estradiol levels under a given threshold are strongly associated with rapid bone loss and sudden increased risk of fractures [2,3]. In addition, sex hormone binding globulin (SHBG), which determines free levels of sex steroid hormones, may also play a substantial role in postmenopausal estrogen physiology. The age related decrease of BMD is known to be attenuated by higher body mass index, serum estrogen levels [2,4,5], and muscle strength [6-9]. A protective effect of muscle strength and physical exercise on BMD has also been demonstrated [10-12]. However, the mechanisms by which these determinants are leading to changes on BMD among postmenopausal women as well as their respective roles are not fully understood. As far as we know, a site-specific analysis of BMD determinants has only be done for muscle strength and lean mass [13,14]. Additional insight in the specific local effects of these factors is of etiological interest and might have implications in the prevention of osteoporotic fractures.

The aim of this study was to investigate the local effects of fat and lean mass, muscle mass and strength, and endogenous estrogens and SHBG on the whole body, skull, arms and hip BMD in healthy women having experienced menopause 8 to 30 years ago.

Subject and methods

Study population

Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) [15]. In PROSPECT a total of 17,395 healthy participants who came for breast cancer screening,

aged 49-70 years, living in Utrecht and surroundings, were enrolled between 1993 and 1997. Using baseline data from PROSPECT, we selected women who had experienced a natural menopause between 8 and 30 years ago. In addition, inclusion criteria were an intact uterus and at least one intact ovary, and no use of sex steroids after the reported date of last menstruation. Out of 1803 eligible women, 902 women were invited and 553 (61%) answered positively. Four hundred and two participants were finally included in our study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study center independently. Each participant underwent all tests and assessments during two visits to the study center. The study was approved by the Institutional Review Board of the University Medical Center, Utrecht, and written informed consent was obtained from all participants. Data collection took place between September 1999 and March 2000.

Measurements

Information on health was obtained by medical history, registration of current medication and physical examination. Height, weight, waist, and hip circumference were measured with the subject in standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Bone mineral density and body composition

Bone mineral density and body composition were measured using dual energy x-ray absorptiometry (DXA) (Hologic QDR1000, Hologic Europe, Zaventem, Belgium). Total body DXA scan allows the simultaneous determination of regional quantities of three distinct compartments: bone mass, fat mass and lean body mass, the sum of these giving the total body weight. Using the default Hologic definitions we could get beside total body BMD, fat en lean masses, separate measures of these compartments for the skull, arms and legs [16]. Hip BMD was measured at the proximal femur, the femoral neck, the greater trochanter, and Ward's triangle. Standard positioning was used with the left proximal femur. If there was a history of left sided prosthesis implantation, the right femur was scanned. Quality control including calibration, was performed every morning, using the standard provided by the manufacturer.

Fat mass and lean mass percentages were calculated dividing total fat or lean mass (kg) by body weight (kg) multiplied by 100. Appendicular fat mass and appendicular lean mass (ALM) were calculated as the sum of legs and arms fat and lean mass as described by Heymsfield et al [17]. DXA-estimated appendicular lean mass comprises muscle and other components such as skin, tendons, connective tissues, and the lean portion of adipose tissue.

Hormone measurements

Venous blood samples were collected in the morning between 8.00 and 11.00 a.m. after an overnight fast. Serum was separated by centrifugation and deeply frozen. Serum concentrations of estradiol (E2, nmol/l), estrone (E1, nmol/l), and total testosterone (T, nmol/l) were estimated using commercial ultra-sensitive double-antibody radioimmunoassay kits purchased from Diagnostic Systems Laboratories (Webster, TX, USA). Sex hormone binding globulin (SHBG, nmol/l) was measured using a chemoluminescence immunometric assay on the Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, CA, USA).

Muscle strength

Isometric grip strength was measured using an adjustable hand-held dynamometer (JAMAR dynamometer) at the non-dominant hand. Each test was done in triplicate, and the average was used in the analysis [18]. Leg (or knee) extensor strength was measured as described previously [1,19], using the Hoggan MicroFET hand-held dynamometer. To obtain one main outcome measurement for leg extensor strength, maximum LES (MLES) was defined as the maximum strength for the right or the left leg, whichever was largest, in a position of 120 degrees extension. Statistical analyses were based on the physical unit momentum [Newton meters (Nm)], obtained by multiplying the maximum strength (in N) and the distance of the dynamometer to the knee joint (in meters).

Data analysis

Results are expressed as the mean and SD, unless otherwise stated. Independent determinants of BMD were determined using multiple linear regression analysis. We further

analysed the relation between those determinants and BMD at the following sites: total body, skull, arms, legs and total hip. Because of the similarity of the results obtained by analyses performed with all individual hip BMD measures (femoral neck, Ward's triangle, and femoral trochanter), and with legs BMD, we restricted the presented results to total hip. Associations between variables and bone mineral density at different sites were first assessed using a linear regression model and multiple linear regression analysis was performed to control for possible confounders. In order to compare the strength of the associations between determinants and bone mineral density at various locations, we used the standardized beta coefficient. All variables - determinants and bone mineral densities - are standardized by subtracting the mean and dividing by the standard deviation. When using these variables in the regression functions, the resulting standardized regression coefficients represent the change in bone mineral density (expressed as a fraction of the standard deviation) for a change of one standard deviation in a determinant. Statistical analyses were done using the SPSS 12.0 statistical package for Windows.

Results

General characteristics of the study population are given in Table 1.

Bone mineral density determinants

Well-known independent determinants of BMD were confirmed. Total body BMD decreased with 0.041 ± 0.001 gr/cm² per 10 years of advancing age, and was higher with higher age at menopause and shorter time since menopause. Total body BMD increased with a mean of 0.004 ± 0.001 gr/cm² per kg fat mass increase, with 0.0076 ± 0.001 gr/cm² per kg lean mass increase, with 0.003 ± 0.001 gr/cm² per N grip strength, and with 0.0012 ± 0.001 gr/cm² per Nm leg extensor muscle strength (Table 2).

After adjustment for fat mass, total body BMD increased with 0.008 ± 0.003 gr/cm² per 10 pmol/l estradiol, and with 0.14 ± 0.05 gr/cm² per 10 nmol/l Testosterone (Table 3). When estradiol and testosterone were included in the same model, associations with BMD where lost, suggesting a common pathway. Higher SHBG levels were associated with lower total body BMD, with a decrease of 0.005 ± 0.003 gr/cm² per 10 pmol/l SHBG. In order to investigate the relation between SHBG, BMD and estradiol, we first did a trend analysis

between quintiles of SHBG levels and BMD where a threshold of effect around 45 nmol/l was seen (Table 3a). Thereafter we compared the effect of E2 on BMD between women with SHBG levels < 45 nmol/l with those with SHBG level ≥ 45 nmol/l. The trends of associations are shown in table 3b. In the group with low SHBG, only women with estradiol levels < 10 pmol/l had a lower BMD. In the group with high SHBG, estradiol was linearly and significantly related to BMD (Figure 1).

Table 1. Characteristics of the study population (n = 402).

Characteristic Mean (SD)	Study population N = 402
Age (year)	66.3 (3.9)
Time since menopause (year)	17.1 (6.4)
Age at menopause (year)	49.6 (4.5)
Mean arterial pressure (mmHg)	100 (14.8)
Body Mass Index (kg/m²)	26.2 (4.4)
Waist (cm)	82.9 (10.8)
Fractures after 50 yrs of age % (n)	22.9 (92)
Total fat mass (kg)	23.5 (8.4)
Total lean mass (kg)	44.9 (5)
Appendicular lean mass (kg)	17.2 (2.3)
Lean mass arms (kg)	3.9 (0.6)
Lean mass legs (kg)	13.3 (1.8)
Fat mass percentage (%)	32.8 (6.5)
Lean mass percentage (%)	65.1 (6.4)
Grip force (N)	24.8 (4.8)
Maximum Leg Extensor Strength (Nm)	119.8 (22)
Bone mineral density (BMD)	
Total Body Bone Mineral Density (g/cm²)	1.02 (0.09)
BMD skull (g/cm²)	1.84 (0.28)
BMD dominant arm (g/cm²)	0.74 (0.06)
BMD not dominant arm (g/cm²)	0.73 (0.06)
BMD arms (g/cm²)	0.73 (0.07)
BMD legs (g/cm²)	1.08 (0.12)
Total hip BMD (g/cm²)	0.83 (0.13)
BMD neck of the hip (g/cm²)	0.69 (0.11)
BMD trochanter (hip) (g/cm²)	0.64 (0.11)
BMD wards triangle (hip) (g/cm²)	0.51 (0.13)
Hormones	
Estradiol (pmol/l)	20.2 (13.5)
Non-SHBG bound E2 (pmol/l)	12.7 (9.9)
Estrone (pmol/l)	45 (36.4)
SHBG (nmol/l)	59.07 (26.7)
Testosterone (nmol/l)	1.97 (0.8)
Non-SHBG bound T (nmol/l)	0.90 (0.5)

Values are expressed as mean (standard deviation)

Table 2: Determinants of BMD at different location

	Total body BMD ß ± SE [95% CI]; standardised coefficient ß		**BMD Skull** ß ± SE[95% CI]; standardised coefficient ß		**BMD arms** ß ± SE [95% CI]; standardised coefficient ß		**BMD legs** ß ± SE [95% CI]; standardised coefficient ß		**BMD total hip** ß ± SE [95% CI]; standardised coefficient ß	
Age (year)	-0.004 ± 0.001	-0.16	-0.0065 ± 0.004	-0.09	-0.006 ± 0.002	-0.19	-0.012 ± 0.003	-0.19	-0.0045 ± 0.002	-0.13
	[-0.006;-0.002]**		[-0.014;0.001]		[-0.010;-0.003]***		[-0.018;-0.006]***		[-0.008;-0.001]**	
Age at menopause (year)	0.0024 ± 0.001	0.11	0.0024 ± 0.003	0.04	0.0024 ± 0.001	0.08	0.0052 ± 0.003	0.09	0.0032 ± 0.001	0.11
	[0.000;0.004] *		[-0.004;0.009]		[0.000;0.005]		[0.000;0.011]		[0.000;0.006] *	
Time since menopause (years)	-0.0027 ± 0.001	-0.18	-0.0033 ± 0.002	-0.08	-0.0036 ± 0.001	-0.18	-0.007 ± 0.002	-0.18	-0.003 ± 0.001	-0.16
	[-0.004;-0.001]***		[-0.008;0.001]		[-0.006;-0.002]***		[-0.011;-0.003]***		[-0.005;-0.001] **	
Body mass index (kg/m2) #	0.0086 ± 0.001	0.40	0.029± 0.003	0.46	0.0095 ± 0.001	0.32	0.025 ± 0.002	0.44	0.013 ± 0.001	0.43
	[0.007;0.011]***		[0.024;0.035]***		[0.007;0.012]***		[0.020;0.030]***		[0.010;0.015]***	
Total fat (kg) #	0.0045 ± 0.001	0.40	0.016 ± 0.001	0.49	0.0047 ± 0.001	0.31	0.0125 ± 0.001	0.43	0.0064 ± 0.001	0.42
	[0.003;0.005] ***		[0.013;0.019]***		[0.003;0.006]***		[0.010;0.015]***		[0.005;0.008]***	
Total lean (kg) #	0.0076 ± 0.001	0.40	0.020 ± 0.003	0.36	0.008 ± 0.0012	0.30	0.0197 ± 0.002	0.40	0.009 ± 0.001	0.36
	[0.006;0.009] ***		[0.015;0.025]***		[0.005;0.010]***		[0.015;0.024]***		[0.007;0.012]***	
Total lean (kg) # §	0.005 ± 0.001	0.26	0.008 ± 0.003	0.14	0.0050 ± 0.001	0.19	0.012 ± 0.003	0.24	0.0048 ± 0.001	0.19
	[0.003;0.007] ***		[0.002;0.013]***		[0.002;0.008]***		[0.007;0.017]***		[0.002;0.007]***	
Appendicular lean (kg) #	0.014 ± 0.002	0.34	0.034 ± 0.006	0.28	0.017 ± 0.003	0.29	0.038 ± 0.005	0.36	0.018 ± 0.003	0.31
	[0.010;0.018]***		[0.022;0.046]***		[0.012;0.022]***		[0.029;0.048]***		[0.012;0.023]***	
Appendicular lean (kg) # §	0.008 ± 0.002	0.19	0.007 ± 0.006	0.06	0.011 ± 0.003	0.19	0.021 ± 0.005	0.20	0.008 ± 0.003	0.14
	[0.004;0.013]***		[-0.004;0.019]		[0.005;0.017]***		[0.011;0.032]***		[0.002;0.014]***	
Grip strength (N) #	0.003 ± 0.001	0.14	0.0049 ± 0.003	0.08	0.005 ± 0.001	0.20	0.006 ± 0.003	0.12	0.0025 ± 0.001	0.09
	[0.001;0.005]**		[-0.001;0.011]		[0.003;0.008]***		[0.001;0.011]*		[0.000;0.005]	
Grip strength (N) # §	0.003 ± 0.001	0.15	0.0053 ± 0.003	0.09	0.006 ± 0.001	0.21	0.0065 ± 0.002	0.13	0.0028 ± 0.001	0.11
	[0.001;0.005]**		[0.000;0.010]*		[0.003;0.008]***		[0.002;0.011]*		[0.000;0.005]*	
Maximum leg extensor strength (N/m)	0.0012 ± 0.001	0.27	0.0027 ± 0.001	0.21	0.0012 ± 0.001	0.20	0.0037 ± 0.001	0.33	0.0018 ± 0.001	0.31
#	[0.001;0.002]***		[0.001;0.004]***		[0.001;0.002]***		[0.003;0.005]***		[0.001;0.002]***	
Maximum leg extensor strength (N/m)	0.0008 ± 0.001	0.18	0.0012 ± 0.001	0.10	0.0007 ± 0.001	0.13	0.0026 ± 0.001	0.24	0.0013 ± 0.001	0.22
# §	[0.000; 0.001]***		[0.000; 0.002]*		[0.000; 0.001]**		[0.002; 0.004]***		[0.001; 0.002]***	

ß coefficient reflects changes in BMD (gr/cm²) per unit variable. # Adjusted for age. § Adjusted for fat mass.

*p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001

Table 3: Relations between serum levels of hormones and BMD at different sites

	Total body BMD ß ± SE [95% CI]; standardised coefficient ß		**BMD Dominant arm** ß ± SE [95% CI]; standardised coefficient ß		**BMD not dominant arm** ß ± SE [95% CI]; standardised coefficient ß		**BMD total hip** ß ± SE [95% CI]; standardised coefficient ß	
Estradiol per 10 pmol/l	0.015 ± 0.003 [0.008;0.022] ***	0.22	0.009 ± 0.002 [0.004;0.014] ***	0.19	0.011 ± 0.002 [0.007;0.016] ***	0.24	0.018 ± 0.005 [0.008;0.027] ***	0.18
Estradiol per 10 pmol/l §	0.008 ± 0.003 [0.001;0.015]**	0.11	0.005 ± 0.002 [0.000;0.010]**	0.11	0.007 ± 0.002 [0.002;0.011] **	0.14	0.004 ± 0.005 [-0.005;0.013]	0.04
Non SHBG bound Estradiol per 10 pmol/l	0.023 ± 0.005 [0.014;0.033] ***	0.24	0.014 ± 0.003 [0.008;0.021] ***	0.22	0.017 ± 0.003 [0.011;0.023] ***	0.26	0.030 ± 0.006 [0.017;0.042] ***	0.23
Non SHBG bound Estradiol per 10 pmol/l §	0.013 ± 0.005 [0.003;0.023] **	0.13	0.008 ± 0.003 [0.002;0.015] **	0.13	0.010 ± 0.003 [0.004;0.017] **	0.15	0.010 ± 0.007[-0.003;0.023] **	0.07
Estrone per 10 pmol/l	0.003 ± 0.001[0.000;0.005]*	0.10	0.002 ± 0.001[0.000;0.003]*	0.10	0.002 ± 0.001[0.001;0.004] **	0.13	0.004 ± 0.002 [0.000;0.007]*	0.11
Estrone per 10 pmol/l §	0.000 ± 0.001[-0.002;0.003]	0.004	0.001 ± 0.001 [-0.001;0.002]	0.02	0.001 ± 0.001 [-0.001;0.002]	0.04	0.000 ± 0.002 [-0.004;0.003]	-0.007
Testosterone per 10 nmol/l	0.171 ± 0.059 [0.054;0.288] **	0.14	0.140 ± 0.039 [0.067;0.221] ***	0.18	0.172 ± 0.039[0.095;0.248] ***	0.22	0.089 ± 0.082 [-0.072;0.249]	0.05
Testosterone per 10 nmol/l§	0.137 ± 0.055 [0.029;0.245] **	0.12	0.125 ± 0.037 0.052;0.198]***	0.16	0.150 ± 0.037 [0.077;0.222] ***	0.19	0.027 ± 0.075 [-0.120;0.174]	0.02
Non SHBG bound Testosterone per 10 nmol/l	0.497 ± 0.099 [0.303;0.692] ***	0.12	0.346 ± 0.066 [0.217;0.475] ***	0.26	0.386 ± 0.065 [0.258;0.514] ***	0.29	0.477 ± 0.137 [0.207;0.746] ***	0.17
Non SHBG bound Testosterone per 10 nmol/l§	0.339 ± 0.096 [0.150;0.527] ***	0.10	0.253 ± 0.065[0.132;0.387]***	0.19	0.289 ± 0.064[0.162;0.415] ***	0.22	0.190 ± 0.131 [-0.067;0.447]	0.07
SHBG per 10 nmol/l	-0.009 ± 0.002[-0.013;-0.006]***	-0.14	-0.005 ± 0.001[-0.007;-0.003] ***	-0.22	-0.006 ± 0.001[-0.008;-0.003] ***	-0.24	-0.011 ± 0.002 [-0.016;0.007] ***	-0.23
SHBG per 10 nmol/l §	-0.005 ± 0.002[-0.005;0.002] **	-0.14	-0.003 ± 0.001[-0.005;-0.001] **	-0.13	-0.003 ± 0.001[-0.005;-0.001] **	-0.14	-0.005 ± 0.002 [-0.009;0.000] *	-0.09

Adjusted for age. § Adjusted for fat mass. *p ≤ 0.05. **p ≤ 0.01. ***p ≤ 0.001.

Table 3a: Relation of quintiles of SHBG with total body bone mineral density

Full model*	**ß coefficient**	**SE**	**P**
Quintile 2 shbg (36.5 thru 48.7 nmol/l)	-0.0169	0.015	0.254
Quintile 3 shbg (48.7 thru 61.9 nmol/l)	-0.0439	0.015	0.003
Quintile 4 shbg (61.9 thru 76.7 nmol/l)	-0.0460	0.015	0.002
Quintile 5 shbg (>76.7 nmol/l)	-0.0795	0.015	< 0.001

Table 3b : Trend analysis between quintiles E2 and BMD in both SHBG groups

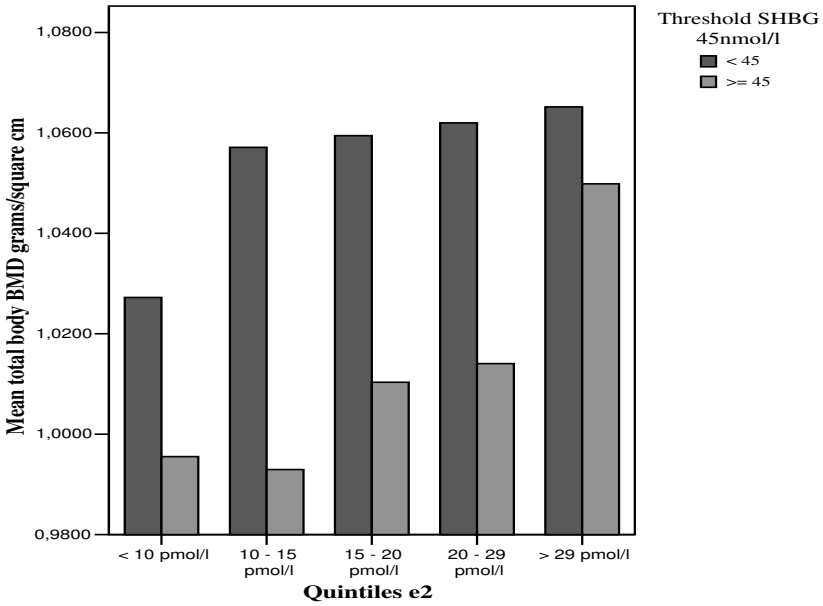
	SHBG level < 45 nmol/l ß ± SE	p	**SHBG level >= 45** ß ± SE	p
Total body BMD	0.007 ± 0.006	0.245	**0.012 ± 0.004**	**0.003**
Skull BMD	0.026 ± 0.018	0.146	**0.043± 0.011**	**<0.001**
Arms BMD	**0.007 ± 0.004**	**0.043**	**0.009± 0.003**	**0.004**
Legs BMD	0.011 ± 0.008	0.169	0.010± 0.005	0.059
Total hip BMD	**0.017 ± 0.008**	**0.040**	0.003± 0.006	0.547

ß coefficient reflects BMD changes in gr/cm² per quintile E2*p ≤ 0.05. **p ≤ 0.01. ***p ≤ 0.001.

BMD determinants at different sites

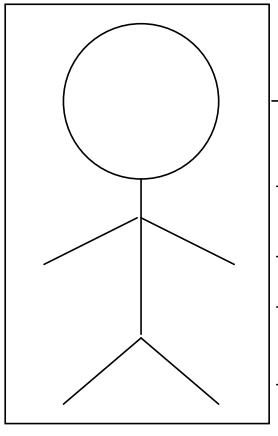
We used the standardised beta coefficient with standard error to compare the strength of the associations between the determinants mentioned above and bone mineral density at various locations. The results are shown in figure 2. At all locations, BMD was mainly determined by total fat mass (standardised β of linear regression between 0.21 and 0.42) and total lean mass (0.14 to 0.24). Local differences were found for estradiol, appendicular lean mass and muscle strength. Skull BMD was further determined by circulating estradiol level (0.14), while weak and non significant effects of muscle mass and strength were observed. At the arm, grip force (0.21) was strongly associated with higher BMD, the relation with leg extensor strength being weak and dependent on appendicular lean mass (0.19). Serum estradiol was also independently related to arm BMD (0.13). Independent other determinants of higher leg and hip BMD were leg muscle strength (0.22 and 0.24) and appendicular lean mass (0.20 and 0.14), while no significant relation was found with estradiol. The relation with grip strength at those sites was lost after adjustment for appendicular lean mass.

Figure 1. Relation between quintiles estradiol and BMD at different sites within both SHBG groups.



Relative contribution of determinants at different sites

In order to analyse the relative contribution of the different determinants at various sites standardised beta coefficient were estimated in a multiple regression model containing age, fat mass, total or appendicular lean mass, grip or leg muscle strength, and estradiol (Table 4). The negative effect of age was more pronounced at arm-BMD site. Total fat mass and lean mass were both independently associated with BMD, with a stronger effect of fat mass on BMD at most sites. The Pearson correlation between appendicular lean and total lean mass was 93%, and putting both determinants in the same model demonstrates a dominant effect of lean mass on total body and skull BMD, suggesting the presence in lean mass of other important factors influencing BMD (Table 4).



Location	Total fat mass # Θ	Total lean mass # §	Muscle mass # §	Grip force # §	Leg strength # §	Estradiol §
Whole body	0.26±0.006***	0.26±0.010***	0.20±0.02***	0.15±0.001**	0.18±0.001***	0.11±0.003 **
Skull	0.42±0.016***	0.14±0.028***	0.06±0.054	0.09±0.003*	0.10±0.001*	0.14±0.010**
Arms	0.21±0.008***	0.19±0.014***	0.19±0.027***	0.21±0.001***	0.13±0.001**	0.13±0.005**
Legs	0.31±0.015***	0.24±0.025***	0.20±0.048***	0.13±0.002*	0.24±0.001***	0.07±0.009
Hip	0.32±0.008***	0.19±0.014***	0.14±0.026***	0.11±0.001*	0.22±0.001***	0.04±0.005

Figure 2. Comparisons of strength (standardized beta coefficient ± SE) of associations between determinants and BMD at various locations. # Adjusted for age. § Adjusted for fat mass. Θ Adjusted for lean mass. *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001

Table 4a: Relative effects of BMD determinants at various BMD locations (all determinants together in the same linear regression model)

	Total body BMD	**BMD arms**	**BMD total hip**
	ß ± SE [95% CI]	ß ± SE [95% CI]	ß ± SE [95% CI]
Age (10 years)	-0.030 ± 0.011 [-0.052; -0.008] **	-0.051 ± 0.016 [-0.082; -0.020] ***	-0.029 ± 0.015 [-0.059; 0.000]*
Total fat mass (10 kg)	0.025 ± 0.006 [0.013; 0.037] ***	0.026 ± 0.009 [0.009; 0.043] **	0.048 ± 0.008 [0.032; 0.064] ***
Total lean mass (10 kg)	0.079 ± 0.024 [0.032; 0.125] ***	0.005 ± 0.034 [-0.062; 0.072]	0.065 ± 0.032 [0.001; 0.128] *
Appendicular lean (10 kg)	-0.089 ± 0.050 [-0.188; 0.010]	0.091 ± 0.072 [-0.052; 0.233]	-0.096 ± 0.069 [-0.232; 0.040]
Leg extensor muscle strength (Nm)	0.0004 ± 0.0002 [0.000;0.001] *	0.0003 ± 0.0003 [0.000;0.001]	0.001 ± 0.0003 [0.000;0.002]***
Estradiol (10 pmol/l)	0.008 ± 0.003 [0.002; 0.015] **	0.014 ± 0.005 [0.005; 0.023]**	0.004 ± 0.005 [-0.005; 0.013]

ß coefficient reflects BMD changes in gr/cm² per unit variable *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001

Table 4b: Relative effects of BMD determinants at various BMD locations (all determinants together in the same linear regression model), grip force instead of leg extensor strength

	Total body BMD	**BMD arms**	**BMD total hip**
	ß ± SE [95% CI]	ß ± SE [95% CI]	ß ± SE [95% CI]
Age (10 years)	-0.029 ± 0.011 [-0.050; -0.007] **	-0.044 ± 0.016 [-0.074; -0.013] **	-0.032 ± 0.015 [-0.062; -0.002]*
Total fat mass (10 kg)	0.027 ± 0.006 [0.015; 0.039] ***	0.031 ± 0.009 [0.014; 0.048] ***	0.049 ± 0.008 [0.033; 0.066] ***
Total lean mass (10 kg)	0.088 ± 0.023 [0.042; 0.134] ***	0.016 ± 0.033 [-0.049; 0.082]	0.084 ± 0.033 [0.019; 0.148] **
Appendicular lean (10 kg)	-0.103 ± 0.051 [-0.203; -0.002]*	0.047 ± 0.073 [-0.097; 0.190]	-0.096 ± 0.071 [-0.237; 0.044]
Grip force (N)	0.002 ± 0.001 [0.000;0.004] *	0.004 ± 0.001 [0.002;0.007]***	0.002 ± 0.001 [-0.001;0.004]
Estradiol (10 pmol/l)	0.008 ± 0.003 [0.002; 0.015] **	0.014 ± 0.005 [0.004; 0.023]**	0.004 ± 0.005 [-0.005; 0.013]

ß coefficient reflects BMD changes in gr/cm² per unit variable *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001

Discussion

In this cross sectional study among healthy women aged 56 to 73 years, 8 to 30 years after menopause, whole body BMD decreased with age, and increased with higher fat mass, lean mass, muscle strength, higher circulating levels of estradiol and lower levels of SHBG. Fat mass was found to be at equal strength with lean mass in terms of its effect on whole body BMD, but fat mass seems to be the major determinant of BMD at the local sites studied. Differences were present for the other determinants according to the site considered. Skull BMD was mainly determined by circulating estradiol level and total lean mass, but not significantly by appendicular lean mass, total skeletal muscle mass or SHBG. Interestingly, the relation with lean mass was independent of muscle mass. At the arm and hip locations, the contribution of appendicular lean mass to BMD was stronger than that of total lean mass. Grip strength was independently related to arm BMD and leg extensor muscle strength to hip BMD. The respective associations of leg extensor strength with arm BMD and of grip strength with hip BMD were dependent on lean mass, supporting a direct and local role of strength on stimulation of bone formation at those sites.

Our results are consistent with previously reported data concerning the determinants of bone mineral density among postmenopausal women: age, BMI, estrogens, and muscle strength [6-9] and they add a detailed insight in the strength of their effect at various locations. Also consistent with our results, earlier studies have provided support for the presence of site-specific effect of muscular strength on bone [11,13,14,20]. Bone bending strength adaptation to mechanical loading represented by lean mass and physical activity is the probable way of action[21,22]. Furthermore, skull BMD has been shown to be an interesting bone location because it is less sensitive to mechanical influences [23-25].

Lean mass has been shown to be the most important predictor of bone mass compared to fat mass in men [26], in premenarcheal girls [27] and in pre- and perimenopausal women [28,29]. In postmenopausal women, lean mass and fat mass become equally important contributors to BMD [21,30-32].

Estrogen deficiency at menopause is a major factor in the development of osteoporosis. The associated loss of trabecular bone mass and structure leads to an increased incidence of postmenopausal osteoporotic fractures [33]. The mechanisms by which skeletal effects of sex steroids are mediated are not completely understood. Even after menopause, circulating levels of the biologically active estrogen, estradiol, seem to play an important role in bone mineral

density [2,4]. Postmenopausal estradiol is produced by aromatization of androstenedione to estrone in adipose tissue and skin, which is subsequently reduced to estradiol in peripheral tissues [34]. Moreover, all enzymes necessary for estrogen metabolism have been shown to be expressed and to be biologically active in differentiating human osteoblasts [35]. Increasing circulating levels of estradiol and increasing local estrogen concentrations in bone can slow down the rate of postmenopausal bone loss. Furthermore, administration of DHEA to postmenopausal women showed beneficial effects on bone mineral density through transformation of the precursor steroid DHEA into androgens and/or estrogens in specific peripheral intracrine tissues [36,37].

The stronger effect of estrogens at the arm location shown in our study can be explained by the preferential trabecular bone-sparing effect of estrogen, considering the higher proportion of trabecular bone at this site [38]. The threshold for effect of estrogen deficiency in cortical bone in women appears to be lower than that in trabecular bone [39,40]. The loss of trabecular bone soon after ovariectomy occurs by allowing bone formation to continue in previously activated bone remodelling units while suppressing the production of new remodelling units. Hormone therapy reduces bone turnover significantly after ovariectomy, resorption being suppressed more than formation and restores bone density, but not trabecular connectivity. This may be the mechanism by which prompt intervention with estrogen and other antiresorptive agents can restore bone mass that has been lost as result of the increase in remodelling space [41]. Also in an animal study, trabecular bone connectivity and volume have been shown to deteriorate rapidly in acute estrogen deficiency, supporting the importance of treatment institution very early in the estrogen-deficient state to preserve fully trabecular bone structure[42].

Small variations in endogenous serum estradiol and high serum SHBG have been shown to result in differences in BMD and rate of bone loss in elderly women and to affect the response to treatment with estrogen. Accordingly, women with a low serum estradiol level are optimal candidates for estrogen therapy for osteoporosis prevention [43]. A clear relation exists between fat mass and serum estrogen levels. A 12-month moderate-intensity exercise intervention study in postmenopausal women resulted in significant decreases in serum estrogens, an effect which was limited to women who lost body fat [44]. Plasma SHBG levels decrease with higher fat mass while insulin also suppresses SHBG levels [45-47]. The great influence of fat mass on BMD is most likely due to the combination of a number of factors: the known direct modulation of bone cell function by the mechanical load resulting from a greater weight,

together with a greater conversion of androgens to estrogens in adipocytes, and a decrease in SHBG level resulting in a higher concentration of free levels of sex steroids.

In conclusion, the present study among healthy postmenopausal women confirms the major role of fat mass on BMD at all sites and demonstrates the presence of a local stimulation of appendicular lean mass and muscle strength on appendicular BMD. Furthermore, skull BMD seems to be mainly influenced by circulating estradiol and by lean mass independently of muscle mass. Finally the effects of circulating estradiol levels were weak at leg and hip BMD sites. The preponderant role of fat mass is probably due to the combination in this group of healthy postmenopausal women of an increased peripheral aromatization, a decrease in SHBG level and a mechanical load stimulating the maintenance of bone mass. This suggests that the postmenopausal changes in body composition have great consequences for BMD determinants.

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Chapter 5

Body composition and development of disability

Fat mass rather than muscle strength is the major determinant of physical function and disability in postmenopausal women younger than 75 years of age

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Abstract

Objective: Few studies have investigated the relations between body composition, functional ability and age-related disability in postmenopausal women. We investigated the relative role of fat mass, lean mass, and muscle strength in the development of disability in a group of healthy postmenopausal women younger than 75 years.

Design: We performed a cross-sectional study among 396 independently living women aged 56-73 years, randomly selected between 8 and 30 years after menopause. Lean mass and fat mass were assessed by dual-energy x-ray absorptiometry. Muscle strength (grip and leg extensors) was assessed using dynamometry. Functional ability was estimated by Physical Performance Score, physical activity during the preceding year, and impairment in activities of daily living.

Results: 43.7 % of participants were overweight ($25 \geq BMI < 30$ kg/m²), and 17.7 % were obese (BM I ≥ 30 kg/m²). Higher muscle strength was observed with increasing lean body mass, and participants with higher muscle strength scored better in the physical performance score and ADL.

Higher fat mass was significantly associated with a lower physical performance score, a lower physical activity and a higher frequency of disability. Increasing fat mass went together with increasing lean mass, and decreasing lean/fat ratio. The increase of lean mass and muscle strength associated with higher fat mass was mainly localized in the legs.

Conclusions: Our results support the role of fat mass as the primary risk marker for disability, which might later accelerate by the age-related decrease in lean mass and the development of sarcopenia after the age of 75 years.

Introduction

The aging process is accompanied by a gradual loss of physical capacity, mobility, balance and endurance, which eventually may result in the loss of living an independent life until death[1]. Muscle mass is the main determinant of sex-related differences in strength, and a decrease in muscle mass - in terms of muscle size as well as of number of muscle fibers - is the main reason for the decline in muscle strength during aging [2,3]. Due to the loss of skeletal muscle, the aging body is less able to resist protein catabolism induced by acute illness or inadequate protein intake, and loss of skeletal muscle mass is considered to be the most important determinant of functional impairment and disability in the elderly [4-6]. A second mechanism which might contribute to the decrease in physical function is the age-related change in muscle composition due to fat infiltration as well as an increase in fat mass that is redistributed from a peripheral to a central location[7]. In several studies in elderly individuals a higher level of disability was demonstrated with increasing BMI or fat mass[8-14].

Studies investigating the association between low muscle mass and physical function have shown inconsistent results[11,12,14,15]. In many studies the percentage of muscle mass has been used, making it difficult to recognize the role played by fat mass in the development of physical impairment[16]. The use of calculated values of muscle mass might also explain some of the inconsistent results in previous studies[6,14,16].

The aim of our study was to investigate in more detail the relative contribution of muscle and fat mass and their regional distribution, as directly measured by dual-energy x-ray absorptiometry (DEXA), to the physical functional status and the development of disability in a large group of healthy independently living postmenopausal women between the age of 56 and 74 years.

Materials and methods

Subjects

Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC)[17]. In PROSPECT a total of 17,395 healthy participants who came for breast cancer screening, aged

49-70 years, living in Utrecht and surroundings, were enrolled between 1993 and 1997. Using baseline data from PROSPECT, we selected women who had experienced a natural menopause. In addition, women should have an intact uterus and at least one intact ovary and should not have used sex steroids after the reported date of last menstruation. Out of 1803 eligible women, 902 women were invited and 553 (61%) answered positively. Four hundred and two participants were finally included in our study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study centre independently. Each participant underwent all tests and assessments during two visits to the study centre. The study was approved by the Institutional Review Board of the University Medical Center Utrecht and written informed consent was obtained from all participants. Data collection took place between September 1999 and March 2000.

Measurements

Information on health was obtained by medical history, registration of current medication and physical examination. Height, weight, waist and hip circumference were measured with the subject in standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters, and body fat distribution was also assessed by calculating the ratio of waist to hip circumferences. Overweight was defined as BMI $\geq$ 25 and < 30 kg/m², and obesity as a BMI $\geq$ 30 kg/m² [18]. Comorbidity was categorized as no chronic conditions, one chronic condition or two or more chronic conditions, based on medication use and self-reported physicians' diagnosis of cardiovascular disease, stroke, diabetes mellitus, COPD, and severe osteoarthritis.

Body composition

Body composition was assessed using dual energy x-ray absorptiometry (DXA) (Hologic QDR1000, Hologic Europe, Zaventem, Belgium). This allows the simultaneous measure of three distinct compartments: total bone mass, fat mass and lean body mass, the sum of the three giving the total body weight, and the determination of regional quantities of these components. DXA-estimated appendicular lean mass (ALM) - which comprises muscle and other components such as skin, tendons, connective tissues, and the lean portion of adipose tissue - was used to calculate total body skeletal muscle mass (SM) according to the

prediction model developed by Kim et al [19] using the formula: Total-body SM = (1.13 x ALM) – (0.02 x age) +0.97. This prediction model has been derived from a large healthy adult population of men and women, aged 18 to 88 y, mean 49 ± 19 for women, with a BMI<35, mean 24.8 ± 4.3 for women. Quality control for dual energy x-ray absorptiometry, including calibration, was performed every day, using the standard provided by the manufacturer.

Fat mass and lean mass percentages were calculated dividing total fat or lean mass (kg) by body weight (kg) multiplied by 100.

Muscle strength

Isometric grip strength was measured using an adjustable hand-held dynamometer (JAMAR dynamometer) at the non-dominant hand. Each test was done in triplicate, and the average was used in the analysis [20]. Leg (or knee) extensor strength was measured using the Hoggan MicroFET hand-held dynamometer. To obtain one main outcome measurement for lee extensor strength, maximum leg extensor strength was defined as the maximum strength for the right or the left leg, whichever was largest, in a position of 120-degree extension. Statistical analyses were based on the physical unit momentum [Newton meters (Nm)], obtained by multiplying the maximum strength (in N) and the distance of the dynamometer to the knee joint (in meters).

Parameters of functional ability

1- Physical performance score

Lower extremity function, or physical performance, was measured as described by Guralnik et al.[21], and included measurement of standing balance, walking speed, and ability to rise from a chair. Three tests of standing balance were considered as hierarchical in difficulty in assigning a single score of 0 – 4 for standing balance. For the 8-ft walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1– 4, corresponding to the quartiles of time needed to complete the task in this population, with the fasted times scoring as 4. A summary performance scale was created by summing the category scores for the walking, chair stand,

and balance tests, yielding possible sum scores between 0 and 12. Impaired physical performance was defined as a score under 7; impaired standing balance was defined as a score < 4 and impaired chair standing and walking speed were defined as a score ≤ 1.

2- Physical activity

The questionnaire on mobility in the elderly developed and validated by L.E. Voorrips et al [22] was used to assess physical activity. It includes three items of physical activities during the preceding year: scores in household activities, sporting activities, and other physically active leisure time activities. Household activities concern 10 questions and are ranging from very active to inactive. Sport and other activities were asked for in terms of type of activity and hours per week, and month per year spent on it. A classification was done using an intensity code based on net energetic costs of activities according to work posture and movements. The three parts together yield a total physical activity score [22], for which there is no maximum by design.

3- Activities of daily living

Information on the participant's satisfaction with performing activities of daily living was obtained by a trained research assistant using the Stanford Health Assessment Questionnaire. The disability index was composed of the mean score (0 indicating no impairment, 3 unable to perform) for six component questions on arising, walking, bending, and getting in and out of a car [23]. This score potentially ranges from 0-3 (maximum sum of 18 divided by 6 components), the higher the worse. Impairment in activities of daily living was defined as a score > 0 on the Health Assessment Questionnaire. This does not imply total impairment, such as wheel chair dependency, as women had to be able to come to our department.

Data analysis

Body composition data were not available for 6 women, leaving 396 women for analyses. Distributions of population characteristics, with anthropometrical measures, body composition and physical function parameters were expressed as means and standard deviations for continuous variables, and frequency and percentage for categorical variables.

Because the distribution of the health assessment questionnaire disability score was skewed, it was converted into a dichotomous variable, 0 for no disability, 1 when disabilities were present (score > 0). Multivariate regression analysis was used to determine the association between body composition and physical function, to adjust for age and BMI, and further possible confounders. Linear regression analysis was used for continuous variables, e.g., grip and leg extensor strength, physical performance score, and physical activity. Logistic regression analysis was used to analyze dichotomous outcomes. Statistical analyses were done using SPSS for Windows (version 9.0).

Results

General characteristics of the study population are given in Table 1.

One hundred and seventy three participants were overweight (BMI ≥ 25 kg/m², 43.7%) and 70 were obese (BMI ≥ 30 kg/m², 17.7%). The following parameters decreased with age: lean body mass (-1.5 kg/ 10 yrs, p=0.02), as well as maximal leg extensor muscle strength (-10.6 Nm/ 10 yrs, p<0.01) grip strength (-2.7 kg/ 10 yrs, p<0.01), and physical performance score (-1.16 points/ 10 yrs, p<0.01). Impairment in activities of daily living increased with 10%/ yr (p<0.01). BMI, fat mass and hip circumference were not related to age, whereas waist to hip ratio significantly increased with age, due to an increase in waist circumference (+3 cm/10 yrs, p<0.01). Higher fat mass was closely associated with higher lean mass (Pearson correlation coefficient r=0.52, p<0.01). Also higher fat mass was associated with higher maximum leg extensor strength (r=0.24, p<0.01), while grip strength did not differ (r=-0.02, p=0.75).

Relations between fat mass, lean mass, and muscle strength

An increase in lean mass was significantly associated with higher grip and leg muscle strength. Higher fat mass was associated with a non-significant decrease in grip strength, and with a higher leg muscle strength (Table 2).

TABLE 1. *Characteristics of the study population (N = 396) per quintiles fat mass*

Characteristic	Study population, mean (SD) (N = 396)
Age, y	66.3 (3.8)
Time since menopause, y	17 (6.4)
Age at menopause, y	49.6 (4.5)
No. (%) of chronic conditions	
1	19.7 (78)
2	5.1 (20)
Body composition	
Body mass index, kg/m^2	26.0 (4.2)
Height, m	1.64 (6.1)
Waist, cm	82.7 (0.54)
Total fat mass, kg	23.5 (8.45)
Fat mass percentage, %	32.8 (6.47)
Overweight, % (no.)	43.7 (173)
Obesity, % (no.)	17.7 (70)
Total lean mass, kg	44.9 (5.0)
Lean mass percentage, %	65.06 (6.4)
Total body skeletal muscle, kg	19.7 (2.57)
Physical function tests	
Physical activity, points	13.3 (7)
Physical performance score	9 (1.7)
Standing balance score	3.7 (0.65)
Chair standing score	2.5 (1.12)
Walking speed score	2.7 (0.83)
Grip strength, N	24.8 (4.8)
Maximal leg extensor strength, Nm	119.9 (22)
Impaired activities of daily living (Health Assessment Questionnaire), % (no.)	24.2 (96)

After adjustment for lean mass, the decrease in grip strength with higher fat mass became significant, and the relation with muscle strength of the knee was lost suggesting an increase in leg strength due to the higher total mass that had to be carried. Lean/fat ratio and lean mass percentage were inversely associated with maximum leg extensor strength, which may again be explained by an effect of weight itself on leg muscle strength. Indeed, this negative relation was not observed for grip strength.

TABLE 2. *Relations of body composition with muscle strength as dependent variable adjusted for age*

	Grip strength (N) ß coefficient ± SE (95% CI)	Maximum leg extensor strength (Nm) ß coefficient ± SE (95% CI)
Total fat mass (10 kg)	−0.09 ± 0.28 (−0.65 to 0.47)	6.24 ± 1.26 (3.77 to 8.72)[c]
Total fat mass (10 kg)[a]	−1.14 ± 0.32 (−1.76 to −0.52)[c]	−0.83 ± 1.31 (−3.40 to 1.74)
Total lean mass (10 kg)	2.42 ± 0.46 (1.50 to 3.33)[c]	22.23 ± 1.88 (18.52 to 25.93)[c]
Total lean mass (10 kg)[b]	3.42 ± 0.53 (2.37 to 4.47)[c]	22.96 ± 2.21 (18.62 to 27.31)[c]
Total body skeletal muscle mass (10 kg)	5.83 ± 0.90 (4.07 to 7.59)[c]	43.33 ± 3.72 (36.01 to 50.64)[c]
Total body skeletal muscle mass (10 kg)[b]	7.56 ± 0.99 (5.61 to 9.51)[c]	42.83 ± 4.20 (34.58 to 51.08)[c]
Fat mass percentage (10%)	−0.91 ± 0.37 (−1.64 to −0.19)[d]	3.51 ± 1.69 (0.19 to 6.83)[e]
Lean mass percentage (10%)	0.89 ± 0.37 (0.15 to 1.62)[d]	−4.35 ± 1.70 (−7.70 to −1.00)[d]
Lean/fat ratio	1.06 ± 0.38 (0.31 to 1.80)[d]	−4.38 ± 1.72 (−7.76 to −0.99)[d]

All regression models were adjusted for age. In multivariate models, additional adjustment was done for [a]lean mass or [b]fat mass.
[c]$P \leq 0.001$; [d]$P \leq 0.01$; [e]$P \leq 0.05$.

The relation between fat mass and lean mass in individuals with increasing BMI is shown in Figure 1.

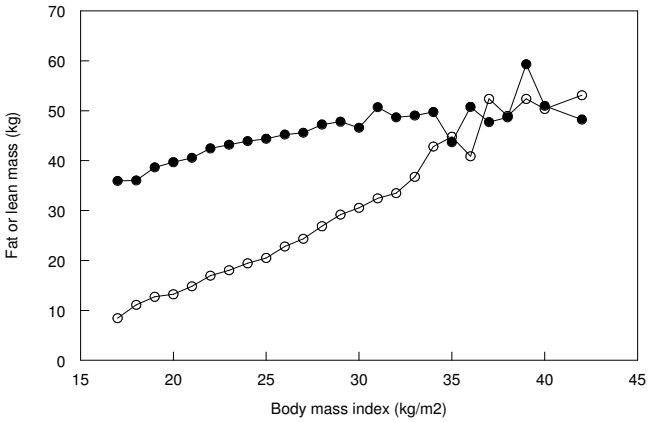

Figure 1. Proportions of fat mass (○ open circles) and lean mass (● closed circles) with increasing BMI.

Increasing BMI was predominantly due to increase of fat mass, which went together with a much lower increase of lean mass. Although there was no absolute decline of lean mass observed with increasing fat mass, lean mass relative to fat mass (lean/fat ratio) sharply decreased with increasing BMI (ß = -0.116 ± 0.005; $p<0.001$) (Figure 2). For BMI values under 27, lean mass represented more then twice the fat mass in weight. For BMI values above 27, the proportion decreased and tended to equality (Figure 2).

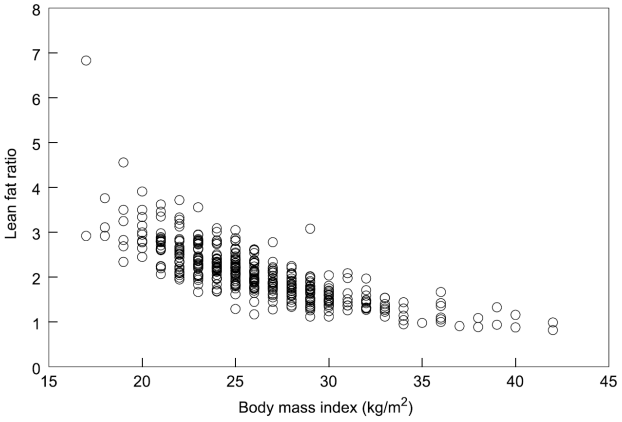

Figure 2. Lean/fat ratio values with increasing BMI

Relations between fat mass, lean mass, muscle strength and functional ability (physical performance score, physical activity and impaired activities of daily living)

Twenty four percent of the participants had a decreased activities of daily living function (n=96), but as expected due to the inclusion criteria used, no participant showed severe disability (Health Assessment Score = 3, Figure 3).

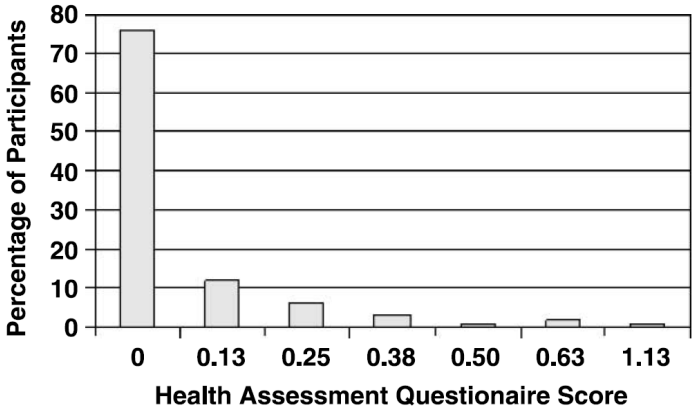

Figure 3. Distribution of disability in activities of daily living.

10 Kg increase in total fat mass was associated with 0.5 points lower physical performance and 1.1 points lower physical activity, and with 64 % higher frequency of impaired activities of daily living, demonstrating an strong adverse effect of fat mass which does not seem to be compensated by the observed parallel increase in leg muscle mass (Table 3).

Higher lean mass and total skeletal muscle mass were not significantly associated with higher physical performance, physical activity and lower frequency of impaired activities of daily living. After adjustment for fat mass, 10 kg increase in total lean mass was associated with 2.2 points higher physical activity, and 10 kg increase in total body skeletal muscle mass was associated with 1.14 points higher physical performance score, 4.7 points higher physical activity and 71 % lower frequency of impaired activities of daily living.

Grip and leg extensor muscle strength were significantly associated with higher physical performance, physical activity and lower frequency of impaired activities of daily living, independently of fat mass (Table 3).

TABLE 3. *Relations of body composition with parameters of physical function as a dependent variable adjusted for age*

	Physical performance (points): ß coefficient ± SE (95% CI)	Physical activity (points): ß coefficient ± SE (95% CI)	Impaired ADL (Health Assessment score > 0) OR (95% CI)
Total fat mass (10 kg)	−0.53 ± 0.11 (−0.75 to −0.31)[c]	−1.06 ± 0.41 (−1.87 to −0.26)[d]	1.64 (1.26 to 2.14)[c]
Total fat mass (10 kg)[a]	−0.66 ± 0.13 (−0.91 to −0.40)[c]	−1.74 ± 0.47 (−2.67 to −0.81)[c]	1.91 (1.39 to 2.61)[c]
Total lean mass (10 kg)	−0.17 ± 0.19 (−0.56 to 0.21)	0.65 ± 0.70 (−0.72 to 2.02)	1.08 (0.67 to 1.72)
Total lean mass (10 kg)[b]	0.41 ± 0.22 (−0.03 to 0.84)	2.19 ± 0.80 (0.61 to 3.77)[d]	0.60 (0.34 to 1.05)
Total body skeletal muscle mass (10 kg)	0.07 ± 0.38 (−0.68 to 0.82)	2.07 ± 1.37 (−0.61 to 4.76)	0.87 (0.34 to 2.19)
Total body skeletal muscle mass (10 kg)[b]	1.14 ± 0.42 (0.32 to 1.96)[d]	4.72 ± 1.52 (1.74 to 7.70)[d]	0.29 (0.10 to 0.87)[e]
Low skeletal muscle mass	−0.01 ± 0.24 (−0.48 to 0.46)	−0.62 ± 0.87 (−2.32 to 1.08)	1.33 (0.73 to 2.22)
Fat mass percentage (10%)	−0.78 ± 0.15 (−1.07 to −0.50)[c]	−1.80 ± 0.53 (−2.84 to −0.75)[d]	2.09 (1.45 to 3.01)[c]
Lean mass percentage (10%)	0.76 ± 0.15 (0.47 to 1.05)[c]	1.77 ± 0.54 (0.71 to 2.83)[c]	0.47 (0.32 to 0.68)[c]
Lean/fat ratio	0.77 ± 0.15 (0.47 to 1.06)[c]	1.91 ± 0.54 (0.84 to 2.98)[c]	0.43 (0.28 to 0.67)[c]
GripF (N)	0.06 ± 0.02 (0.02 to 0.10)[d]	0.18 ± 0.07 (0.03 to 0.32)[d]	0.94 (0.90 to 0.99)[e]
GripF (N)[b]	0.06 ± 0.02 (0.02 to 0.10)[d]	0.17 ± 0.07 (0.03 to 0.32)[d]	0.94 (0.90 to 0.99)[e]
Maximum leg extensor strength (Nm)	0.018 ± 0.004 (0.01 to 0.03)[c]	0.09 ± 0.01 (0.06 to 0.12)[c]	0.98 (0.97 to 0.99)[c]
Maximum leg extensor strength (Nm)[b]	0.025 ± 0.004 (0.02 to 0.03)[c]	0.11 ± 0.01 (0.08 to 0.14)[c]	0.97 (0.96 to 0.98)[c]

ß Coefficients reflect changes in points per unit of the physical parameters. For example, physical performance score increases 0.018 points per Nm maximal leg extensor strength.

All regression models were adjusted for age. In multivariate models, additional adjustment was done for [a]fat mass or [b]lean mass.

[c]$P \leq 0.001$; [d]$P \leq 0.01$; [e]$P \leq 0.05$.

Discussion

In this group of independently living postmenopausal women younger than 75 years, higher lean mass was related to higher muscle strength, and muscle strength was associated with better functional ability. However, higher fat mass was significantly related with lower functional ability (lower physical performance score, lower physical activity and higher frequency of disability). Increased lean mass and muscle strength associated with higher fat mass were mainly localized in the legs, most likely due to the higher mass to be carried. This increase in lean mass and leg extensor muscle strength was not sufficient to overcome the negative effects of fat mass on physical function.

Before further interpreting these data some issues have to be addressed. In this study, we obtained direct body composition measures using DEXA. This allows better insight in the respective associations of fat mass and muscle mass with disability. Only about one quarter of the participants of this study reported the presence of some chronic diseases (hypertension, heart disease, COPD, and diabetes mellitus). The inclusion criteria used excluded participants with important limitations in physical performance and physical activities or likely to impede daily living activities. No women included in our study had yet developed a state of clear frailty or disability, such as wheelchair dependency. The selection of this age group, however, was expected to allow the detection of early determinants that are associated with disability during the aging process.

We did not include any test of "fitness" in this study. Some obese women are very fit and have increased muscle mass and fat. If included, such individuals may blunt the relationship. However, in our study population, there are no obese (BMI >/ 30) women with >70% lean mass (as percentage of body weight), and only 14 women with BMI >/ 25 have >70% lean mass, which reduces the likelihood that we enrolled very fit obese women.

The increase in waist to hip ratio with age seen in the individuals studied is consistent with redistribution of body fat, and the accumulation of fat mass before age 60 [14, 25], which is eventually followed by a decrease with older age [26].

The decrease of lean and skeletal muscle mass with age is consistent with results from both cross-sectional and longitudinal studies, which have shown a lean mass peak in the third to fourth decade of life, followed by a steady decline with advancing age among healthy elderly people [6,26,27].

An unanswered question in many studies is how the increase in fat load with age and the age-related decrease in lean mass interact in their effects on the functional impairment developing during the aging process. Being overweight impairs physical activity in all age groups: the increased prevalence of functional limitations and disability with increasing BMI has been widely reported, while the relative increase in muscle mass with increasing BMI might explain some of the discrepant results in previous studies between men and women [9,10,15,28]. Epidemiological studies have shown that the prevalence of being overweight climbs in women from age 20 to 60 and particularly increases among non-hispanic white women in their 50's [29]. The factors implied in this weight gain among mid-aged women are probably modifiable and include behavior and life-style. Longitudinal studies in elderly individuals indicate that lean mass remains stable when fat mass increases, decreases slightly in weight stable subjects, and decreases sharply in elderly people that lose weight, despite normal to high levels of recreational and sport activities [30].

It is probable that at this age group of healthy independent living women, fat mass is a more important determinant of disability, while sarcopenia becomes important as a determinant much later in the aging process.

Unequivocal conclusions are limited by the fact that the different compartments of body composition have different relationship to morbidity, disability, and health status. Loss of muscle is in the aging process initially replaced by fat. DXA- scan does not allow to make this discrimination. Therefore it remains often difficult to conclude which factor in the interrelation between the changes in fat mass and muscle mass plays the overriding role in disability among older people.

In this study in healthy independent living women younger than 75 years, fat mass appears to be a major risk marker for physical functional status and disability. An increase in fat mass and body weight leads to a compensatory increase in the muscle mass and muscle strength of the legs, which, however, only partially prevents the decrease in physical function and activity and the development of disability. However, due to the cross-sectional nature of the study, causal inference is limited, and future studies with prospective designs should further explore our findings.

These observations might indicate a different approach to the prevention of disability in this age group of elderly women, than is currently considered for the oldest old (>75 years) where the role of sarcopenia in the disability seems much more outspoken.

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Chapter 6

Body composition and hormones

The relationship between estrogens, other hormones and physical functional status in healthy postmenopausal women

Abstract

Objective: The aim of this study was to investigate whether serum levels of circulating hormones, in particular estrogen concentrations, are associated with functional ability in healthy postmenopausal women younger than 75 years of age.

Method: We performed a cross-sectional study among 402 healthy postmenopausal women, aged 56-73 years, randomly selected between 8 and 30 years after menopause. Serum concentrations of estradiol (E2), estrone (E1), and sex hormone-binding globulin (SHBG), testosterone (T), IGF-1, DHEA and DHEAS, and insulin were measured by immunoassay. Body composition measures included bone mineral density (BMD), lean mass and fat mass assessed by dual-energy x-ray absorptiometry. Functional ability was estimated by measuring muscle strength (grip and leg extension) using dynamometry, and physical performance (PPS), and by assessing the number of problems in activities of daily living (impaired ADL or disability).

Results: Higher fat mass was associated with a lower PPS and higher disability. Higher lean mass was associated with lower disability on basis of muscle strength. A higher lean mass, muscle strength, and fat mass were also independently associated with a higher BMD. Estrogen levels were positively related to BMD, and high estrogen levels were negatively correlated with ability. T had small effects on BMD, which were lost after adjustment for E2, and was negatively correlated with PPS independent of E2 levels, and lean and fat mass. After adjustment for BMI, SHBG was negatively associated with BMD and leg muscle strength, independent of insulin. Insulin showed positive associations with lean, fat and bone mass, and IGF-1 was positively related to leg muscle strength, physical performance and a negatively to disability.

Conclusion: In this population of relatively healthy postmenopausal women, fat mass was the major factor negatively affecting ability. Estrogens, T and SHBG levels were related to BMD and fat mass. Known positive effects of IGF-1 on muscle strength and activity of daily living were confirmed. The remaining estrogen levels in the postmenopausal state do not exert positive effects on lean mass, muscle strength or physical performance. Activities of daily living were even negatively related to E2 in those women with the highest BMI. Unexpectedly and unexplained, T was a negatively associated with physical performance.

Introduction

Aging is known to induce profound changes in body composition, particularly an accumulation and redistribution of body fat and a decrease of skeletal muscle mass. However, since declining muscle mass explains loss of strength only partially, other yet undetermined factors have to play a role in the loss of strength seen with aging [1]. Sex hormones are known to be important determinants of body composition, but less is known about their role within the aging process. There is evidence that the age-related changes in body composition, together with the age-associated somatopause, are related to declining levels of serum testosterone (T) in men [2]. In women, menopausal transition and early postmenopausal status are associated with a selective increase in intra-abdominal fat independently of age and total adiposity [3,4]. The remaining circulating levels of sex hormones are also known to play an important physiological role in bone metabolism after the menopause [5-7], with evidence that estradiol levels under a given threshold are strongly associated with rapid bone loss [7]. In addition, sex hormone binding globulin (SHBG), which determines free levels of sex steroids, may affect postmenopausal estrogen physiology substantially. Investigation of hormonal factors associated with the decline in muscle mass and strength, and the development of age-related disability is important in the light of the probably increasing proportion of older persons expected over the next decennia.

The aim of this study in healthy postmenopausal women younger than 75 years was to investigate the effects of endocrine determinants, particularly estrogens, testosterone and SHBG concentrations, on functional abilities. Lean body mass, fat mass and muscle strength were measured in order to get better insight in direct or indirect actions of these endocrine effects.

Subject and methods

Study population

Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) [8]. In PROSPECT a total of 17,395 healthy participants who came for breast cancer screening, aged 49-70 years, living in Utrecht and surroundings, were enrolled between 1993 and 1997. Using baseline data from PROSPECT, we selected women who had experienced a natural menopause between 8 and 30 years ago. In addition, inclusion criteria were an intact uterus and at least one intact ovary, and no use of sex steroids after the reported date of last menstruation. Out of 1803 eligible women, 902 women were invited and 553 (61%) answered positively. Four hundred and two participants were finally included in our study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study center independently. Each participant underwent all tests and assessments during two visits to the study center. The study was approved by the Institutional Review Board of the University Medical Centre, Utrecht, and written informed consent was obtained from all participants.

Measurements

Information on health was obtained on basis of medical history, registration of current medication and physical examination. Height, weight, waist and hip circumference were measured with the subject in standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Hormone measurements

Venous blood samples were collected in the morning between 8.00 and 11.00 a.m. after an overnight fast. Serum was separated by centrifugation and frozen at –80^{o} C. Serum concentrations of estradiol (E2, pmol/l), estrone (E1, pmol/l), dehydroepiandrosterone (DHEA nmol/l), DHEAS (μmol/l) and testosterone (T, nmol/l) were estimated using radioimmunoassay (RIA) kits purchased from Diagnostic Systems Laboratories (Webster, TX, USA). Sex hormone binding globulin (SHBG, nmol/l) was measured using a chemoluminescence based immunometric assay on the Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, CA, USA).

Total IGF-1 was determined by a IGFBP-blocked RIA in the presence of a large excess of IGF-II as described previously[9]. Insulin was measured by a commercially available RIA (Medgenix Diagnostics, Nivelles, Belgium). Intra- and interassay coefficients of variation, determined on basis of duplicate results of samples and of results of internal quality control serum pools with three different levels of analyse, respectively, were less than 20% for oestrone, less than 15% for oestradiol and androstenedione, less than 12% for testosterone and DHEA and less than 8% for cortisol, DHEAS and SHBG.

Non-SHBG-bound T and E2 were calculated using the method described by Sodergard et al.[10], using a fixed plasma albumin concentration of 40 g/l [11].

Bone mineral density and body composition

Total body bone mineral density and body composition were measured using DXA (Hologic QDR1000, Hologic Europe, Zaventem, Belgium). Quality control for dual energy x-ray absorptiometry, including calibration, was performed every morning, using the standard provided by the manufacturer. Total body DXA scan allows the simultaneous determination of regional quantities of three distinct compartments: bone mass, fat mass and lean body mass, the sum of the them giving the total body weight.

Muscle strength

Isometric grip strength was measured using an adjustable hand-held dynamometer (JAMAR dynamometer) at the non-dominant hand. Each test was done in triplicate, and the average was used in the analysis [12]. Leg (or knee) extensor strength (LES) was measured using the Hoggan MicroFET hand-held dynamometer. To obtain one main outcome measurement for LES, maximum LES (MLES) was defined as the maximum strength for the right or the left leg, whichever was largest, in a position of 120-degree extension. Statistical analyses were based on the physical unit momentum [Newton meters (Nm)], obtained by multiplying the maximum strength (in N) and the distance of the dynamometer to the knee joint (in meters).

Physical performance score

Lower extremity function, or physical performance, was measured as described by Guralnik et al. [13], and included measurement of standing balance, walking speed, and ability to rise from a chair. Three tests of standing balance were considered as hierarchical in difficulty in assigning a single score of 0 – 4 for standing balance. For the 8-ft walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1– 4, corresponding to the quartiles of time needed to complete the task in this population, with the fastest times scoring as 4. A summary performance scale was created by summing the category scores for the walking, chair stand, and balance tests, yielding possible sum scores between 0 and 12. Impaired physical performance was defined as a score under 7; impaired standing balance was defined as a score < 4 and impaired chair standing and walking speed were defined as a score ≤ 1.

Activities of daily living

Information about satisfaction on performing activities of daily living (ADL) was obtained by a trained research assistant using the Stanford Health Assessment Questionnaire (HAQ). The disability

index (HAQ) was composed of the mean score (0 indicating no impairment, 3 unable to perform) for six component questions on arising, walking, bending, and getting in and out of a car [14]. This score potentially ranges from 0-3 (maximum sum of 18 divided by 6 components), the higher the worse. Impairment in activities of daily living was defined as a score > 0 on the Health Assessment Questionnaire. This does not imply total impairment, such as wheel chair dependency, as women had to be able to come to our department.

Data analysis

Body composition data were not available for 6 women, leaving 396 women for analyses. Distributions of population characteristics, with anthropometrical measures, body composition and physical function parameters were expressed as means and standard deviations for continuous variables, and frequency and percentage for categorical variables. Multivariate regression analysis was used to determine the association between determinants of functional status and hormones and physical parameters to adjust for age and BMI, and further possible confounders. Additional analyses were performed within tertiles of fat mass in order to discriminate between cause and effect. Because the distribution of the health assessment questionnaire disability score was skewed, it was converted into a dichotomous variable, 0 for no disability, 1 when disabilities were present (score > 0). Correlations between hormones were assessed by using Pearson's correlation coefficient *r*. Statistical analyses were done using SPSS for Windows (version 12.0).

Results

General characteristics of the study population including results of hormone measurements are given in Table 1.

TABLE 1. Characteristics of the study population (n = 396).

Characteristic	Mean (SD)
Age (year)	66.3 (3.8)
Time since menopause (year)	17 (6.4)
Age at menopause (year)	49.6 (4.5)
Physical characteristics	
Body Mass Index (kg/m²)	26.0 (4.2)
Physical Performance score (points)	8.97 (1.7)
Impaired activity of daily living HAQ (points)	0.064 (0.15)
Total Bone Mineral Density (g/cm²)	1.025 (0.095)
Total fat mass (kg)	23.5 (8.45)
Total lean mass (kg)	44.9 (5.0)
Grip strength (N)	24.8 (4.8)
Maximum Leg Extensor Strength (Nm)	119.9 (22)
Hormones	
Estradiol (pmol/l)	19.8 (13)
Non SHBG bound E2 (pmol/l)	12.44 (9.41)
Estrone (pmol/l)	44.6 (36.3)
Testosterone (nmol/l)	1.97 (0.8)
Non SHBG bound T (nmol/l)	0.89 (0.46)
Dehydroepiandrostendione (DHEA) (nmol/l)	13.24 (8.1)
DHEAS (umol/L)	2.25 (1.4)
SHBG (nmol/l)	59.4 (26.7)
Insulin (mU/l)	11.8 (7.15)
Insulin-like growth factor-1 (IGF-1) (nmol/l)	17.9 (7.18)

Values are expressed as mean (standard deviation).

Relations between body composition and characteristics of physical functional status

BMD, lean body mass, grip strength, leg muscle strength and physical performance decreased with age and impaired ADL increased with age, while fat mass and BMI were not related to age (Table 2).

Table 2: Relations between physical characteristics and age

	ß coefficient± SE	**P**
Physical Performance Score (points)	**-1.16± 0.22**	**< 0.001**
Disability HAQ (log)	**0.40 ± 0.13**	**0.002**
Total Body BMD (g/cm²)	**-0.04 ± 0.01**	**0.001**
Body Mass Index (kg/m²)	0.60 ± 0.55	0.277
Total fat mass (kg)	0.18 ± 1.11	0.913
Total lean mass (kg)	**-1.51 ± 0.65**	**0.021**
Grip force (N)	**-2.77 ± 0.62**	**< 0.001**
Max Leg Extension Strength (Nm)	**-10.64 ± 2.84**	**< 0.001**

ß coefficient reflects changes in unit per 10 year. For example, PPS decreases 1.16 points per 10 year.

An algorithm representing the observed independent and significant relationships between the compartments of body composition and the parameters of functional status is shown in Figure 1.

Higher muscle strength was observed with increasing lean body mass, and participants with higher muscle strength scored better in the PPS and had lower impaired ADL. Higher lean mass association with lower impaired ADL was lost after adjustment for muscle strength. Higher fat mass was significantly and independently associated with a lower PPS, and with a higher impaired ADL. Lean mass and fat mass were positively related to each other. Finally, a higher lean mass, muscle strength, and fat mass were independently associated with a higher BMD. There was no significant relation found between PPS and BMD.

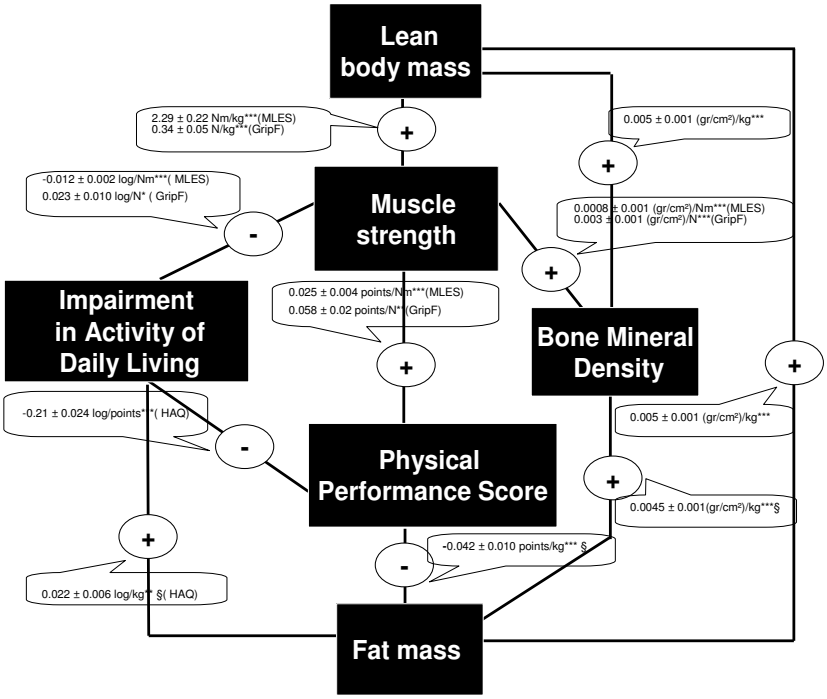

Figure 1. Algorithm of mutual correlations between parameters which determine validity

All adjusted for age and BMI, except for §: Not adjusted for BMI. Lean mass was adjusted for fat mass.

***P-value ≤ 0.001. **P-value ≤ 0.01. *P-value ≤ 0.05

Mutual relations between hormone levels

High Pearson correlation coefficients were found for the relationships between the circulating levels of E2 and E1, between non-SHBG-bound T and non-SHBG-bound E2, and between SHBG and non-SHBG-bound E2, non-SHBG-bound T, and insulin. Among the adrenal hormones, we further restricted the presentation of the results to T because of the high correlations and similarity of results between T, DHEA and DHEAS (Table 3).

Table 3: Pearson correlation coefficients between hormones levels and bindings proteins

	E2	Non SHBG E2	E1	T	Non SHBG T	DHEA	DHEAS	SHBG	Ins
IGF-1	0.03	0.06	0.05	**0.13** **	**0.19** **	0.05	**0.15** **	**-0.17** **	0.04
Insulin	**0.12** **	**0.18** **	**0.13** **	0.02	**0.17** **	**-0.12** *	**-0.15** **	**-0.30** **	-
SHBG	**-0.27** **	**-0.45** **	**-0.24** **	**-0.11** *	**-0.54** **	**0.11** *	- 0.03	-	
DHEAS	**0.32** **	**0.28** **	**0.28** **	**0.35** **	**0.29** **	**0.63** **	-		
DHEA	**0.23** **	**0.16** **	**0.17** **	**0.43** **	**0.26** **	-			
Non SHBG T	**0.48** **	**0.57** **	**0.41** **	**0.85** **	-				
T	**0.40** **	**0.38** **	**0.35** **	-					
E1	**0.62** **	**0.63** **	-						
Non SHBG E2	**0.96** **	-							

Pearson correlation coefficient. ** P-value <0.01, * P-value <0.05

Relations between hormone levels and physical characteristics

The associations between hormone levels and age and BMI are shown in Table 4.

Table 4: Relation between hormones and age or BMI

	Age ß coefficient ± SE	P	**BMI** ß coefficient ± SE	P
Estradiol (pmol/l)	-0.31 ± 1.70	0.856	**0.94 ± 0.148**	**< 0.001**
Non-SHBG bound E2 (pmol/l)	-0.01 ± 1.23	0.991	**0.83 ± 0.104**	**< 0.001**
Estrone (pmol/l)	6.50 ± 4.75	0.173	**2.32 ± 0.417**	**< 0.001**
Testosterone (nmol/l)	0.16 ± 0.10	0.126	**0.020 ± 0.009**	**0.036**
Non-SHBG bound T (nmol/l)	0.086 ± 0.06	0.156	**0.03 ± 0.005**	**< 0.001**
DHEA (nmol/L)	**-3.91 ± 1.05**	**< 0.001**	**-0.25 ± 0.096**	**0.011**
DHEAS (umol/L)	**-0.76 ± 0.18**	**< 0.001**	-0.026 ± 0.016	0.106
SHBG (nmol/l)	-0.83 ± 3.49	0.813	**-2.59 ± 0.290**	**< 0.001**
Insulin (mU/L)	0.105 ± 0.94	0.262	**0.610 ± 0.080**	**< 0.001**
IGF 1 (nmol/L)	-1.42 ± 0.94	0.130	-0.092 ± 0.085	0.285

ß coefficient reflects changes in unit per 10 year and per kg/m² BMI. For example, DHEA decreases 3.91 nmol/l per 10 years and 0.25 nmol/l per kg/m².

Only DHEA and DHEAS were associated with age, and most hormone levels measured were associated to BMI, excepted for DHEAS, and IGF-1.

1- Estrogen

Associations of non-SHBG bound E2 levels with measured physical parameters were stronger than for estradiol levels (Table 5). Non-SHBG bound E2 was positively related to fat mass, lean mass, BMD, leg strength and disability, and was negatively related to PPS. After adjustment for BMI only the relations with BMD and disability remained significant (Table 5). Similar results were obtained with E2 and E1 consistent with the high correlation between levels of both hormones. After adjustment for BMI, all E1 relations were lost (results not shown).

Table 5: Relation between hormones and physical parameters

	Fat Mass(kg) β±SE	**Lean mass(kg)** β±SE	**BMD (gr/cm²)** β±SE	**MLES (Nm)** β±SE	**GripF (N)** β±SE	**PPS (points)** β±SE	**Impaired ADL (logHAQ)** β±SE
E2 pmol/l	**0.18± 0.03****	**0.045± 0.02***	**0.0016± 0.0004****	0.11± 0.085	0.002± 0.02	**-0.024± 0.008****	**0.012± 0.004****
Non SHBG bound E2 pmol/l	**0.30± 0.04****	**0.09± 0.03****	**0.003± 0.0005****	**0.26± 0.12***	-0.01± 0.03	**-0.04± 0.01****	**0.02± 0.005****
Non SHBG bound E2 pmol/l (BMI)	**-**	-0.026± 0.024	**0.001± 0.0005***	0.08± 0.12	-0.002± 0.03	-0.020± 0.011	**0.012± 0.006***
T nmol/l	0.786± 0.535	0.349± 0.318	**0.017± 0.006****	-1.97 ± 1.40	0.096± 0.31	**-0.41± 0.11****	0.089± 0.064
Non SHBG bound T nmol/l	**4.14± 0.9****	**1.77± 0.54****	**0.05± 0.01****	1.8 ± 2.4	-0.31± 0.53	**-0.84± 0.21****	**0.23± 0.11***
Non SHBG bound T nmol/l (BMI)	**-**	0.04± 0.48	**0.03± 0.01****	-1.34 ± 2.45	-0.23± 0.55	**-0.60± 0.22****	0.13± 0.11
SHBG nmol/l	**-0.11± 0.015****	**-0.045± 0.009****	**-0.0009± 0.0002****	**-0.151± 0.040****	0.004± 0.009	**0.008± 0.003***	-0.003± 0.002
SHBG nmol/l (ins)	**-0.08± 0.016****	**-0.04 ± 0.010****	**-0.001 ± 0.0002****	**-0.15 ± 0.044****	0.001± 0.01	0.007 ± 0.004	-0.002± 0.002
SHBG nmol/l (BMI)	-	-0.003 ± 0.009	**-0.0004 ± 0.0002***	**-0.09 ± 0.044***	0.002± 0.01	0.004 ± 0.004	-0.0006± 0.002
Insulin mU/l	**0.40± 0.057****	**0.183± 0.034****	**0.002± 0.001****	-0.22± 0.156	0.016± 0.034	**-0.04± 0.012***	**0.021± 0.007****
Insulin mU/l (SHBG)	**0.31± 0.057****	**0.14± 0.035****	**0.002± 0.001***	0.06± 0.16	0.017± 0.036	**-0.041± 0.016****	**0.019± 0.007***
Insulin mU/l (BMI)	**-**	0.05 ± 0.032	0.0007± 0.001	-0.026± 0.164	0.03± 0.04	-0.023± 0.013	0.014± 0.008
IGF-1 nmol/l	-0.11± 0.06	0.034± 0.035	0.001± 0.001	**0.36± 0.15***	-0.008± 0.034	**0.029± 0.014***	**-0.015± 0.007***

β coefficient reflects changes in unit physical parameters per unit hormone concentration.

(BMI) Adjusted for BMI. (ins) Adjusted for insulin. (age) Adjusted for age. (SHBG) Adjusted for SHBG **p≤0.005 * p≤0.05

2- Testosterone

While Testosterone was positively associated with BMD and negatively related to PPS, non SHBG bound T was positively related to fat mass, lean mass, BMD and disability. T was negatively related with PPS. After adjustment for BMI the relations with disability and lean mass were lost, and the relation with BMD was lost after adjustment for E2. The negative relation of T with PPS was independent of E2, or BMI.

3- SHBG

Serum SHBG concentrations were inversely related to fat mass, lean body mass, BMD, and MLES and positively related to PPS. After adjustment for BMI or fat mass, only the association with BMD and MLES remained significant. The relation between SHBG and muscle strength was lost after adjustment for lean mass. Adjustment for insulin did not alter these relations.

4- Insulin and IGF-1

Insulin was positively associated with fat mass, lean mass and impaired ADL and was negatively associated with PPS. All those relations were lost after adjustment for BMI, while adjustment for SHBG did not materially alter these relations.

IGF-1 was positively related to MLES and PPS, and negatively associated with disability.

Further analyses on the unexpected relationships between E2 and ADL and T and PPS were performed within tertiles of fat mass (data not shown). The relation of E2 with disability was found only in the highest tertile, suggesting a causal role of fat mass. The relation of T with PPS was found in the lowest tertile, which confirms that the T effect is independent of fat mass, and the relation was independent of fat mass, SHBG and insulin. E2, DHEA and DHEAS did not demonstrated the same relationships.

A summary of these hormonal effects in the algorithm in figure 1 is shown in Figure 2.

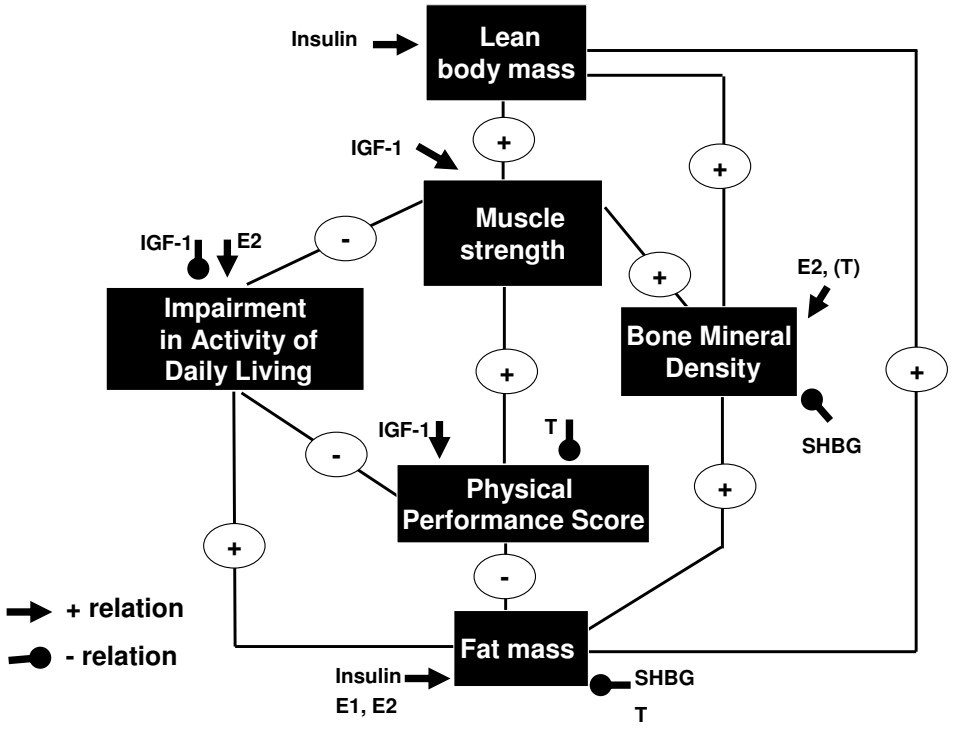

Figure. 2 Independent significant relations between hormone levels and physical parameters

Discussion

In this cross sectional study among healthy, independently living women younger than 75 years, the significant and independent relations between body composition, muscle strength, PPS and impaired ADL could be presented in an algorithm which demonstrated a direct negative effect of muscle strength and PPS on disability, an indirect negative effect of lean mass through muscle strength, and a direct positive effect of fat mass on disability. Lean mass and fat mass were positively related to each other. Lean mass, fat mass and muscle strength had all three direct independent effects on BMD.

The role of hormones measured varied considerably. Besides the strong effect of estrogens on BMD, this study did not demonstrate any effects of estrogens on muscle mass, muscle strength and physical performance, while high estrogen levels even negatively affect ability. Hormone levels of adrenal androgens (DHEA, DHEAS) and T, which is presumably mainly produced from adrenal precursors, demonstrated small effects on bone mineral density, lost after adjustment for E2, indicating that the effect is caused through aromatization of T to E2. T had independent negative effects on PPS which were in particular independent of E2 levels, SHBG, lean mass or fat mass. After adjustment for BMI, SHBG was negatively associated with BMD and leg muscle strength, independently of insulin. Insulin showed positive effects on lean, fat and bone mass, and IGF-1 had positive effects on leg muscle strength, physical performance and negative effect on disability.

Before interpreting these data, some issues need to be addressed. In this study a relatively healthy group of independently living women was studied and individuals with clear disability were excluded at the start of the study. The presence of disability was predominantly related to overweight. The lack of direct relation between lean mass and disability is most likely due to this selection. We have shown in an earlier study in this population how lean and fat mass were related to each other [15]. Lean/fat ratio sharply decreased with increasing BMI due to increasing fat mass accompanied by a much lower increase of lean mass principally located at the legs.

The strong relationship of estrogen levels with BMD can be explained by the direct effect of E2 on bone metabolism [16-18], with the additional effect of increased fat mass resulting in higher mechanical load and higher E2 levels [19,20]. The most probable explanation for the positive relation between E2 and disability is the role of overweight, confirmed by the presence of this relation in the highest fat mass tertile only.

A study among older men has shown an algorithm where significant relations were found between PPS and BMD [21]. In our population this relation was absent. A probable explanation is the strong effect of fat mass on BMD through mechanical force with increase of aromatization in addition to the negative effect of fat mass on PPS. The relationship between PPS and BMD seemed masked because when we put BMI, estradiol and PPS in the same model, the positive association of PPS with total body BMD became significant.

In this population of postmenopausal women with low androgen levels, we observed an unexplained negative relation between T and physical performance. This relationship seems due to a unknown confounder, since it was independent of fat mass, lean mass, SHBG, and insulin.

Although high levels of E2 are known to increase hepatic SHBG synthesis [22], SHBG was negatively related with E2 in the present study. This negative relation is probably due to the predominant negative relation of SHBG with fat mass in this population: a higher fat mass is associated with a higher E2 and a lower SHBG. When adjustment for fat mass was performed, both hormones were positively but not significantly associated.

It is also known that insulin suppresses the liver SHBG synthesis [23-25]. Interestingly, the effects of SHBG were independent of insulin in our population. In particular, when put together in the same linear regression model, both hormones were independently and significantly related to fat mass.

In this population of healthy postmenopausal women without marked disability, fat mass negatively affected physical performance and exerted a major role in the presence of disability. Estrogens, T and SHBG levels were related to BMD and fat mass. Known effects IGF-1 on muscle strength and activity of daily living were confirmed. The remaining estrogen levels in the postmenopausal state do not exert positive effects on lean mass, muscle strength or physical performance. Activities of daily living were even negatively related to E2 in those women with the highest BMI. Unexpectedly and unexplained, T exerted a negative effect on physical performance.

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Chapter 7

Determinants of Quality of life in healthy postmenopausal women

Relations between body composition, functional and hormonal parameters and quality of life in healthy postmenopausal women

Maturitas 55(1):82-92. aug 20 2006

Abstract

Objective: To investigate whether body composition, functional status and serum hormone levels are associated with quality of life in healthy postmenopausal women.

Design: A cross-sectional study among 402 women aged 56-73 years, 8 to 30 years postmenopausal. Quality of life (QoL) was assessed using the questionnaire on life satisfaction (QLS), with two modules directed at general factors (QLS-General) and health factors (QLS-Health). Muscle strength was measured using dynamometry. Functional ability was estimated by physical performance (PPS), physical activity during the preceding year, and impairment in activities of daily living (ADL). Bone mineral density, lean mass and fat mass were assessed by dual-energy x-ray absorptiometry. Fasting levels of serum oestradiol, oestrone, and sex hormone-binding globulin (SHBG), testosterone, cortisol, androstenedione, DHEA and DHEAS, Insulin-like Growth Factor (IGF-1), its binding proteins (IGFBP –1 and –3) and insulin, were determined.

Results: Both QLS modules did not decrease with age. The major positive predictor of QLS-general module was the presence of a partner. Higher physical performance and higher educational level of participants' partners were significantly related to higher QLS-general, while smoking and presence of co-morbidities were significantly associated with a lower QLS-general. The determinants studied were mostly related to the QLS-health module, the major negative predictor of QLS-health being the presence of co-morbidities, followed by physical activity, physical performance and grip strength. Higher educational level of participants was related with higher QLS-health module, while higher BMI, fat mass and presence of disability were associated with significantly lower QLS-health. No consistent relation was found between serum levels of hormones measured and both QLS modules.

Conclusions: The most important and specific determinant for psychological well being was having a partner. Physical and psychological well being are further strongly associated in this population of healthy postmenopausal women below 75 years of age, while increasing fat mass was related to decreased well being. Our results suggest that in elderly and late postmenopausal women hormonal factors do not predict quality of life.

Introduction

As the number of postmenopausal women in our society is increasing, the importance of the prevention of frailty in these women is increasing together with the growing demand for an active old age and for interventions improving the quality of life. In addition to menopausal oestrogen deficiency, older age is associated with a decreased hormonal production of growth hormone and IGF-1, changes in body composition with osteopaenia, sarcopaenia, and fat redistribution,[1] and to lower physical activity and muscle strength.[2-4] Different definitions of successful aging, a concept dependent on individual perspectives and value systems, have been proposed.[5,6] The model of successful aging proposed by Rowe and Kahn in which the combination of absence of disease, maintenance of functional capacities, and active engagement with life are fundamental,[7] can be assessed by means of different instruments and questionnaires making it more practical. Based on this model, we investigated the interrelationships between serum levels of hormones, body composition, physical function parameters and quality of life in a population of postmenopausal women between 55 and 75 years, i.e. before old age and overt physical frailty.

Material and methods

Subjects

Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC).[8] In PROSPECT a total of 17,395 healthy participants who came for breast cancer screening, aged 49-70 years, living in Utrecht and surroundings, were enrolled between 1993 and 1997. Using baseline data from PROSPECT, we selected women who had experienced a natural menopause between 8 and 30 years ago. In addition, women should have an intact uterus and at least one intact ovary and should not have used sex steroids after the reported date of last menstruation. Out of 1803 eligible women, a random sample of 902 women were invited and 553 (61%) answered positively. 402 participants were finally included in our study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study centre independently. None of the women had obvious cognitive

impairment. Each participant underwent all tests and assessments during two visits to the study centre. The study was approved by the Institutional Review Board of the University Medical Center Utrecht and written informed consent was obtained from all participants.

Measurements

Information on health status was obtained by medical history, registration of current medication and physical examination. Height, weight, waist and hip circumference were measured with the subject in standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Information on educational level of participants and their partners was available out of the baseline data of PROSPECT. Co-morbidity was defined as the presence of one or more chronic conditions based on medication use and self-reported physician diagnosis of cardiovascular disease, stroke, diabetes mellitus, COPD, and severe osteoarthritis.

Quality of life

Psychological well being or quality of life was assessed using the standardized multidimensional questionnaire of life satisfaction from Henrich and Herschbach,[9] where subjective QoL is reflected by weighting the response to each item for its importance to the respondent. The questionnaire of life satisfaction (QLS) used consists of two modules covering general quality of life and health, and is easy to complete in less than 20 minutes. The first module addresses 8 general dimensions of life satisfaction (QLS-general): friends/acquaintances, leisure time/hobbies, general health, income/financial security, occupation/work, housing/living conditions, family life/children and partner relationship/sexuality. The module QLS-general correlates best with scales assessing psychological well being.[9] The second module consists of 8 questions regarding general health (QLS-health): physical condition/fitness, ability to relax, energy/zest for life, mobility, vision and hearing, freedom from anxiety, freedom from aches and pain, and independence from help/care. Individual weighing of the items was realized as follows: for each module's item, subjects were first asked to rate the subjective importance of the given area of life; then they were asked about the degree of satisfaction in that area. All items are evaluated on a 5-

point scale, ranging from 0 to 4, with the highest score indicating respectively extremely important and very satisfied. We calculated the combination of importance (I) and satisfaction (S), for each item ((I-1) x [(Sx2)-5]) according to Herschbach,[10] and the sum was calculated for each module. Scores on the two modules can vary between –192 and 160. The questionnaire produces total scores for each module separately.

Biochemical assessments

Venous blood samples were collected in the morning between 8.00 and 11.00 a.m. after an overnight fast. Serum was separated by centrifugation and frozen at –80^{o} C. Serum concentrations of oestradiol (E2, nmol/l), oestrone (E1, nmol/l), DHEA (nmol/l) and DHEAS (μmol/l) and total testosterone (T, nmol/l) were estimated using radioimmunoassay kits purchased from Diagnostic Systems Laboratories (Webster, TX, USA). Sex hormone binding globulin (SHBG, nmol/l) was measured using a chemoluminescence immunometric assay on the Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, CA, USA).

Total IGF-1 was determined by a IGFBP-blocked RIA in the presence of large excess of IGF-II as described previously.[11] IGFBP-1 and IGFBP-3 were measured with in-house RIAs as described previously. Insulin was measured by a commercially available RIA (Medgenix Diagnostics, Nivelles, Belgium). Intra- and interassay coefficients of variation, determined on basis of duplicate results of samples and of results of internal quality control serum pools with three different levels of analyse, respectively, were less than 20% for oestrone, less than 15% for oestradiol and androstenedione, less than 12% for testosterone and DHEA and less than 8% for cortisol, DHEA sulphate and SHBG.

Body composition

Body composition was assessed using dual energy x-ray absorptiometry (DXA) (Hologic QDR1000, Hologic Europe, Zaventem, Belgium). This allows the simultaneous measure of three distinct compartments: total bone mass, fat mass and lean body mass, the sum of the three giving the total body weight, and the determination of regional quantities of these components. Quality control for dual energy x-ray absorptiometry, including calibration, was performed every morning, using the standard provided by the manufacturer.

Parameters of functional ability

1- Muscle strength

Isometric grip strength was measured using an adjustable hand-held dynamometer (JAMAR dynamometer, Memphis, TN) at the non-dominant hand. Each test was done in triplicate, and the average was used in the analysis.[12] Leg (or knee) extensor strength (LES) was measured using the MicroFET hand-held dynamometer (Hoggan Health industries, Draper, Utah, USA). To obtain one main outcome measurement for LES, maximum LES (MLES) was defined as the maximum strength for the right or the left leg, whichever was largest, in a position of 120-degree extension. Statistical analyses were based on the physical unit momentum [Newton meters (Nm)], obtained by multiplying the maximum strength (in N) and the distance of the dynamometer to the knee joint (in meters).

2- Physical performance score

Lower extremity function, or physical performance, was measured as described by Guralnik et al.,[13] including measurement of standing balance, walking speed, and ability to rise from a chair. Three tests of standing balance were considered as hierarchical in difficulty in assigning a single score of 0 – 4 for standing balance. For the 2.4 meter walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1– 4, corresponding to the quartiles of time needed to complete the task in this population, with the fasted times scoring as 4. A summary performance scale was created by summing the category scores for the walking, chair stand, and balance tests. Impaired physical performance was defined as a score under 7; impaired standing balance was defined as a score < 4 and impaired chair standing and walking speed were defined as a score ≤ 1.

3- Physical activity

The questionnaire on mobility in elderly developed and validated by Voorrips et al.[14] was used to assess physical activity. It includes three items of physical activities during the preceding year: scores in household activities, sporting activities, and other physically active

leisure time activities. Household activities concern 10 questions and are ranging from very active to inactive. Sport and other activities were asked for as type of activity, hours per week and month per year spent on it. A classification was done using an intensity code based on net energetic costs of activities according to work posture and movements. The three parts together yield a total physical activity score.

4- Activities of daily living

Information on the participant's satisfaction with performing activities of daily living was obtained by a trained research assistant using the Stanford Health Assessment Questionnaire (HAQ). The disability index (HAQ) was composed of the mean score (0 indicating no impairment, 3 unable to perform) for six component questions on arising, walking, bending, and getting in and out of a car.[15] Impairment in ADL was defined as a HAQ score above 0.

Data analysis

Population characteristics including anthropometrical measures, body composition and physical function parameters, quality of life modules and serum hormones levels were expressed as the mean and SD, unless otherwise stated. Because distributions of the HAQ disability score was skewed, it was converted into dichotomous variables, 0 for no disability, 1 when disabilities were present (HAQ score > 0). Multiple linear regression analysis was used to determine the association between body composition, physical function, serum hormone levels on the one hand, and quality of life modules on the other and was also used to adjust and control for possible confounders. The change in the R squared statistic produced by adding an independent variable was used to estimate the importance of predictor of both QLS modules. Statistical analyses were done using the SPSS 12.0 statistical package for Windows.

Results

General characteristics of the study population are given in Table 1.

Table 1
Characteristics of the study population ($n = 402$)

Characteristic	Mean (S.D.)
Age (year)	66.3 (3.8)
Time since menopause (year)	17.1 (6.4)
Age at menopause (year)	49.6 (4.5)
Body mass index (kg/m^2)	26.2 (4.4)
Waist (cm)	82.9 (10.8)
Partner	73.1 (294)*
Co-morbidity	25.1 (101)*
Fractures after 50 yrs of age	22.9 (92)*
Civil status of participants	
Married/living together	68.2 (274)*
Widow	15.2 (61)*
Separated	5 (20)*
Living with friend	2.7 (11)*
Single	9 (36)*
Cardiovascular risk factors	
Mean systolic arterial pressure (mmHg)	148 (21)
Mean diastolic arterial pressure (mmHg)	76 (14)
Smoker	12.4 (50)*
Past smoker	37.3 (150)*
Body composition	
Total fat mass (kg)	23.5 (8.4)
Total lean mass (kg)	44.9 (5)
Fat mass percentage (%)	32.8 (6.5)
Total bone mineral density (g/cm^2)	1.02 (0.09)
Parameters of physical function	
Grip force (N)	24.8 (4.8)
Maximum leg extensor strength (N m)	119.8 (22)
Physical performance score (points)	8.7 (2)
Standing balance	3.7 (0.65)
Chair standing	2.5 (1.1)
Walking speed	2.5 (1.1)
Physical activity (Voorrips) (points)	13.3 (7)
Disability HAQ	24.9 (100)*
Quality of life questionnaire (QLS)	
QLS-general ($n = 377$)	81.1 (23.5)
QLS-health ($n = 386$)	78.5 (31)
Hormones	
Estradiol (pmol/l)	20.2 (13.5)
Oestrone (pmol/l)	47 (35.9)
SHBG (nmol/l)	59 (26.7)
Testosterone (nmol/l)	1.97 (0.8)
Androstenedione (nmol/l)	3.4 (1.5)
Dehydroepiandrostendione (DHEA) (nmol/l)	13.2 (8.1)
DHEAS (μmol/l)	2.26 (1.4)
Cortisol (nmol/l)	464 (142)
Insulin (mU/l)	11.8 (7.2)
IGFBP-1 (mg/l)	34.3 (28)
Insulin-like growth factor-1 (IGF-1) (nmol/l)	17.8 (7.2)
IGFBP-3 (mg/l)	3.34 (0.56)

Values are expressed as mean (S.D.) expect the values marked with '*' for which the values are expressed as in % (n).

Educational level of participants and their partners (Table 2) shows a similar distribution as the total cohort of the prospect-EPIC study where they have been selected.[8]

Table 2
Educational level of participants and their present partners ($n = 402$)

	Participants % (n)	Prospect EPIC (%)	Partners of participants % (n)	Prospect EPIC (%)
Primary school completed	22.6 (91)	22.1	8.5 (34)	9.3
Technical/professional education	45.3 (182)	46.9	28.4 (114)	29.2
Secondary education	13.7 (55)	14	15.2 (61)	17.8
Academic education	18.2 (73)	16.1	21.1 (85)	21.9
Missing	0.2 (1)	0.8	1.2 (5)	2.3
No partners			25.6 (103)	19.4
Total number	402	17357	402	17357

Relations between physical characteristics and QLS-modules are described in Table 3.

None of the QLS scores was decreasing with age and they were not related with age at menopause or time since menopause.

QLS-general

Partners education level but not participants' one was significantly associated with higher QLS-general score (P<0.001) (Table 3), and was the most important predictor of QLS-general (Table 4).

In decreasing order of R squared values, higher physical performance score was associated with 1.5 points higher QLS-general (P=0.01), smoking was associated with 4 points lower QLS-general score (P=0.02), and the presence of co-morbidities was associated with 6 points lower QLS-general score (P=0.03). Presence of disability was related to 5 points lower QLS-general (P=0.04), but was dependent on fat mass and co-morbidities.

None of the twelve circulating hormone levels measured was related to QLS-general score (data not shown).

QLS-health

Presence of co-morbidities was significantly associated with lower QLS-health (p<0.01) (Table 3), with a R squared value explaining an important part of the variation (Table 4). Higher education level of participants but not of their partners was significantly associated with higher QLS-health, but with a weak R squared change value. The following associations are given in decreasing value of R squared change.

Table 3
Relations between body composition, muscle power and physical functioning and quality of life modules

	QLS-general		QLS-health	
	$\beta \pm$ S.E.	P	$\beta \pm$ S.E.	P
Age (year)	0.06 ± 0.32	0.85	0.19 ± 0.41	0.64
Time since menopause (year)	−0.16 ± 0.19	0.39	−0.12 ± 0.24	0.62
Age at menopause (year)	0.41 ± 0.27	0.13	0.40 ± 0.35	0.26
Partner (0–1)	17.58 ± 2.69	<0.01	−0.73 ± 3.61	0.84
Civil status (1–5)	−5.59 ± 0.97	<0.01	−0.21 ± 1.25	0.87
Educational level (1–4)	0.32 ± 1.22	0.79	3.05 ± 1.56	0.05
Educational level of partner (1–4)	2.50 ± 1.28	0.05	0.70 ± 1.76	0.69
MAP (mmHg)	−0.09 ± 0.08	0.28	−0.14 ± 0.10	0.17
Alcohol quartiles (g l/wk)	1.03 ± 1.01	0.31	0.23 ± 1.31	0.86
Smoking class	−4.00 ± 1.74	0.02	−3.58 ± 2.27	0.12
Comorbidity (0–1)	−6.08 ± 2.80	0.03	−11.6 ± 3.63	<0.01
Body mass index (kg/m^2)	−0.45 ± 0.27	0.09	−0.86 ± 0.37	0.02
Fat percentage (%)	−0.34 ± 0.19	0.07	−0.56 ± 0.24	0.02
Total fat mass (kg)	−0.27 ± 0.14	0.06	−0.38 ± 0.19	0.04
Total fat mass[a] (kg)	−0.24 ± 0.14	0.09	−0.32 ± 0.18	0.08
Total lean mass (kg)	−0.36 ± 0.25	0.15	−0.13 ± 0.31	0.68
Total lean mass[b] (kg)	−0.17 ± 0.29	0.56	0.28 ± 0.36	0.44
Total bone mineral density (g/cm^2)	9.34 ± 12.6	0.46	16.3 ± 16.7	0.33
Grip force (N)	0.03 ± 0.26	0.91	0.74 ± 0.32	0.02
Grip force[b] (N)	0.06 ± 0.26	0.95	0.72 ± 0.32	0.03
MLES (N m)	−0.01 ± 0.06	0.85	0.11 ± 0.07	0.12
MLES[b] (N m)	0.01 ± 0.06	0.85	0.14 ± 0.07	0.05
Physical performance score (points)	1.53 ± 0.60	0.01	2.70 ± 0.77	<0.01
Physical performance score[b] (points)	1.35 ± 0.62	0.03	2.26 ± 0.79	<0.01
Standing balance	2.72 ± 1.88	0.14	4.82 ± 2.44	0.05
Chair standing	3.05 ± 1.08	<0.01	3.98 ± 1.41	<0.01
Walking speed	0.97 ± 1.07	0.36	3.12 ± 1.40	0.03
Physical activity (points)	0.33 ± 0.17	0.06	0.90 ± 0.22	<0.01
Physical activity[b] (points)	0.29 ± 0.18	0.10	0.79 ± 0.22	<0.01
Disability HAQ (0–1)	−5.72 ± 2.81	0.04	−20.7 ± 3.51	<0.01
Disability HAQ[b] (0–1)	−5.10 ± 2.92	0.08	−18.7 ± 3.61	<0.01
Disability HAQ[a,b] (0–1)	−4.09 ± 2.96	0.17	−17.1 ± 3.68	<0.01

[a] Adjusted for comorbidity.
[b] Adjusted for fat mass.

Table 4
Predictors of quality of life modules: R^2 changes

	QLS-general	QLS-health
Presence of a partner	0.103	–
Civil status	0.082	–
Educational level	–	0.010
Educational level of partner	0.014	–
Smoking class	0.014	–
Comorbidity (0–1)	0.012	0.026
Body mass index (kg/m^2)	–	0.014
Fat percentage (%)	–	0.014
Total fat mass (kg)	–	0.011
Grip force (N)	–	0.013
Physical performance score (points)	0.017	0.031
Chair standing	0.021	0.020
Physical activity (points)	–	0.042
Disability HAQ (0–1)	0.011	0.083

1- Parameters of physical function-QLS-health:

Presence of disabilities was related to 20 points lower QLS-Health independent of fat mass and chronic disease (P<0.01). One point higher physical activity was related to 0.9 point higher QLS-Health score independently of fat mass and chronic disease (P<0.01). Higher physical performance was related with 2.7 points higher QLS-health (P<0.01), with chair standing as the major factor determining these relations. Grip strength was associated with 0.7 point higher QLS-Health score per N (p=0.02) independently of fat mass. Leg muscle strength was related to 0.1 point higher QLS-Health. This relation became significant after adjustment for fat mass (P=0.05).

2- *Body composition-QLS-health*:

Ten kg higher fat mass was related to 3.8 points lower QLS-health (p=0.04), and adjustment for chronic disease attenuated this relation. Lean mass was not related to QLS.

3- Circulating hormones levels- QLS-health:

There was a negative relation between oestradiol and QLS-health (p=0.04) (Table 5a) which was only seen in the highest quintile of fat mass and oestradiol (table 5b).
A negative relation between the insulin and both modules of QLS were only present in the highest quintile of insulin and were attenuated after adjustment for fat mass (Table 5b and 5c). We did not find any consistent relation between serum levels of the other hormones measured and QLS-health.

Table 5a
Relations between circulating serum hormone levels and quality of life modules (linear regression analysis with continuous variables)

	QLS-general		QLS-health		QLS-hormone	
	$\beta \pm$ S.E.	*P*-value	$\beta \pm$ S.E.	*P*-value	$\beta \pm$ S.E.	*P*-value
Oestradiol (pmol/l)	−0.093 ± 0.09	0.295	−0.24 ± 0.12	0.041	−0.38 ± 0.21	0.072
Oestradiol[a] (pmol/l)	−0.053 ± 0.10	0.589	−0.14 ± 0.12	0.252	−0.25 ± 0.23	0.268
Oestrone (pmol/l)	−0.032 ± 0.04	0.392	−0.066 ± 0.05	0.184	−0.18 ± 0.08	0.024
Oestrone[a] (pmol/l)	−0.013 ± 0.04	0.748	−0.024 ± 0.05	0.644	−0.15 ± 0.08	0.073
SHBG (nmol/l)	0.009 ± 0.045	0.838	0.11 ± 0.06	0.072	0.24 ± 0.11	0.025
SHBG[a] (nmol/l)	0.024 ± 0.048	0.623	0.07 ± 0.06	0.298	0.19 ± 0.11	0.095
Testosterone (nmol/l)	−0.60 ± 1.53	0.696	−1.59 ± 2.01	0.430	−1.77 ± 3.75	0.637
Androstenedione (nmol/l)	0.23 ± 0.82	0.782	0.10 ± 1.06	0.924	2.01 ± 1.89	0.288
DHEA (nmol/l)	−0.063 ± 0.15	0.675	0.037 ± 0.20	0.856	0.004 ± 0.35	0.990
DHEAS (μmol/l)	0.095 ± 0.88	0.914	−1.57 ± 1.16	0.174	−3.11 ± 2.05	0.131
Cortisol (nmol/l)	0.02 ± 0.009	0.022	0.019 ± 0.011	0.086	0.018 ± 0.02	0.361
Insulin (mU/l)	−0.29 ± 0.17	0.080	−0.38 ± 0.22	0.083	−0.45 ± 0.40	0.260
IGFBP-1 (mg/l)	0.05 ± 0.043	0.250	0.03 ± 0.088	0.715	0.033 ± 0.10	0.744
IGF-1 (nmol/l)	0.14 ± 0.17	0.414	−0.07 ± 0.22	0.750	−0.10 ± 0.39	0.796
IGFBP-3 (mg/l)	1.06 ± 2.15	0.622	−6.02 ± 2.81	0.033	−10.32 ± 5.03	0.041
Albumine (mg/l)	0.37 ± 0.54	0.496	−0.09 ± 0.70	0.891	−0.221 ± 1.26	0.861

[a] Adjusted for fat mass.

Table 5b
Relations between quintiles of serum hormone levels and QLS-general module

Hormone	$\beta \pm$ S.E.					*P*-value for trend
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Oestradiol	1	−6.57 ± 3.82	−1.56 ± 3.71	−4.40 ± 3.84	−4.79 ± 3.77	0.354
Oestradiol[a]	1	−6.58 ± 3.83	−1.58 ± 3.73	−4.44 ± 3.89	−4.86 ± 3.90	0.366
Oestradiol[b]	1	−5.68 ± 3.87	−0.75 ± 3.75	−3.59 ± 3.88	−3.07 ± 3.98	0.616
Oestrone	1	−2.01 ± 3.77	5.11 ± 3.82	1.16 ± 3.79	−2.47 ± 3.72	0.763
Oestrone[a]	1	−2.01 ± 3.78	5.10 ± 3.82	1.16 ± 3.82	−2.47 ± 3.84	0.814
Oestrone[b]	1	−1.75 ± 3.80	5.44 ± 3.84	2.15 ± 3.86	−0.60 ± 3.93	0.791
Testosterone	1	0.26 ± 3.61	−0.63 ± 4.03	−5.99 ± 3.84	−0.44 ± 3.85	0.384
Testosterone [a]	1	0.25 ± 3.62	−0.60 ± 4.05	−5.97 ± 3.86	−0.41 ± 3.88	0.395
Testosterone[b]	1	0.46 ± 3.64	−0.35 ± 4.05	−5.40 ± 3.90	−0.05 ± 3.92	0.485
SHBG	1	−7.85 ± 3.82*	−5.90 ± 3.86	−6.77 ± 3.78	−2.26 ± 3.77	0.768
SHBG[b]	1	−9.53 ± 3.93*	−8.36 ± 4.04*	−10.36 ± 4.05**	−6.01 ± 4.06	0.254
Androstenedione	1	−1.38 ± 3.76	1.37 ± 3.73	1.75 ± 3.79	1.01 ± 3.85	0.451
Androstenedione[b]	1	−1.73 ± 3.78	1.13 ± 3.78	0.73 ± 3.85	0.05 ± 3.97	0.664
DHEA	1	2.26 ± 3.79	3.06 ± 3.76	4.58 ± 3.71	−1.61 ± 3.78	0.964
DHEA[b]	1	1.93 ± 3.82	2.85 ± 3.80	4.40 ± 3.77	−2.47 ± 3.83	0.826
DHEAS	1	−0.48 ± 3.65	2.70 ± 3.92	−1.39 ± 3.67	0.82 ± 3.80	0.837
DHEAS[b]	1	−0.33 ± 3.71	3.22 ± 3.96	−1.25 ± 3.71	1.14 ± 3.86	0.863
Cortisol	1	5.65 ± 3.83	2.48 ± 3.83	6.41 ± 3.77	5.77 ± 3.83	0.144
Cortisol[b]	1	5.19 ± 3.88	1.87 ± 3.89	6.20 ± 3.89	4.61 ± 3.95	0.245
Insulin	1	1.87 ± 3.77	−5.11 ± 3.72	−3.00 ± 3.73	−8.72 ± 3.73*	0.010
Insulin[b]	1	2.61 ± 3.83	−4.49 ± 3.80	−1.54 ± 4.04	−7.55 ± 4.14	0.063
IGFBP-1	1	−0.50 ± 3.78	−1.26 ± 3.81	8.41 ± 3.79*	2.60 ± 3.81	0.097
IGFBP-1[b]	1	−0.98 ± 3.92	−2.68 ± 4.01	6.52 ± 4.13	0.27 ± 4.30	0.411
IGF-1	1	0.44 ± 3.79	11.83 ± 3.77**	6.81 ± 3.77	3.75 ± 3.74	0.107
IGF-1[b]	1	0.40 ± 3.88	11.35 ± 3.84**	6.06 ± 3.87	3.31 ± 3.81	0.177
IGFBP-3	1	3.91 ± 3.46	1.22 ± 3.58	9.15 ± 4.22*	1.57 ± 3.73	0.471
IGFBP-3[b]	1	3.78 ± 3.51	1.02 ± 3.61	9.55 ± 4.28*	1.72 ± 3.79	0.446

[a] Adjusted for SHBG.
[b] Adjusted for fat mass.
* $P \leq 0.05$.
** $P \leq 0.01$.

Table 5c
Relations between quintiles of serum hormone levels and QLS-health module

Hormone	$\beta \pm$ S.E.					P-value for trend
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Oestradiol	1	−5.07 ± 4.95	−5.28 ± 4.8	−4.72 ± 4.95	−11.22 ± 4.93*	0.044
Oestradiol[a]	1	−4.82 ± 4.95	−4.71 ± 4.82	−3.78 ± 5.00	−9.54 ± 5.10	0.113
Oestradiol[b]	1	−4.44 ± 4.93	−4.31 ± 4.79	−3.83 ± 4.93	−7.75 ± 5.12	0.189
Oestrone	1	−2.92 ± 4.88	5.37 ± 4.93	3.75 ± 4.91	−7.76 ± 4.91	0.397
Oestrone[a]	1	−2.99 ± 4.88	5.64 ± 4.93	4.50 ± 4.94	−5.90 ± 5.09	0.734
Oestrone[b]	1	−3.09 ± 4.84	5.71 ± 4.88	5.27 ± 4.93	−4.07 ± 5.08	0.981
Testosterone	1	−2.30 ± 4.73	−5.39 ± 5.25	−4.41 ± 5.07	−2.01 ± 5.13	0.574
Testosterone[a]	1	−2.31 ± 4.72	−4.50 ± 5.26	−3.83 ± 5.07	−0.95 ± 5.15	0.756
Testosterone[b]	1	−2.26 ± 4.67	−5.00 ± 5.18	−2.16 ± 5.05	−0.73 ± 5.13	0.889
SHBG	1	1.31 ± 4.92	9.95 ± 4.99*	0.68 ± 4.91	10.81 ± 4.94*	0.061
SHBG[b]	1	1.02 ± 5.00	7.73 ± 5.16	−2.30 ± 5.19	7.79 ± 5.23	0.287
Androstenedione	1	−2.29 ± 4.89	−2.19 ± 4.82	5.65 ± 4.89	−4.30 ± 4.96	0.987
Androstenedione[b]	1	−2.67 ± 4.83	−2.11 ± 4.80	4.33 ± 4.87	5.16 ± 5.02	0.886
DHEA	1	−2.50 ± 4.99	−3.56 ± 4.90	−2.61 ± 4.83	−0.68 ± 4.95	0.717
DHEA[b]	1	−2.77 ± 4.92	−4.32 ± 4.86	−2.44 ± 4.82	−1.05 ± 4.93	0.716
DHEAS	1	−1.71 ± 4.77	−4.02 ± 5.13	2.38 ± 4.78	−6.04 ± 5.00	0.613
DHEAS[b]	1	−1.35 ± 4.75	−3.72 ± 5.08	2.06 ± 4.75	−4.35 ± 4.99	0.752
Cortisol	1	9.11 ± 4.97	2.90 ± 4.90	6.57 ± 4.86	9.35 ± 5.01	0.140
Cortisol[b]	1	9.22 ± 4.95	1.67 ± 4.91	6.52 ± 4.92	7.54 ± 5.06	0.230
Insulin	1	0.40 ± 4.91	−1.75 ± 4.86	−2.65 ± 4.87	−12.53 ± 4.84**	0.021
Insulin[b]	1	1.14 ± 4.91	−0.70 ± 4.89	0.76 ± 5.17	−9.97 ± 5.28	0.181
IGFBP-1	1	3.00 ± 4.95	10.78 ± 4.95*	10.42 ± 4.99*	8.23 ± 4.97	0.032
IGFBP-1[b]	1	2.77 ± 5.06	9.15 ± 5.12	7.77 ± 5.36	5.03 ± 5.53	0.240
IGF-1	1	3.86 ± 4.95	12.23 ± 4.95**	8.84 ± 4.98	2.38 ± 4.96	0.385
IGF-1[b]	1	4.17 ± 4.95	11.33 ± 4.94*	8.34 ± 5.02	1.56 ± 4.95	0.549
IGFBP-3	1	2.19 ± 4.50	−0.28 ± 4.65	2.08 ± 5.48	−7.97 ± 4.96	0.060
IGFBP-3[b]	1	1.37 ± 4.48	−0.84 ± 4.60	3.59 ± 5.45	−7.79 ± 4.94	0.084

[a] Adjusted for SHBG.
[b] Adjusted for fat mass.
* $P \leq 0.05$.
** $P \leq 0.01$.

Discussion

In this population of healthy postmenopausal relatively women between 55 and 75 years of age, quality of life did not decrease with age or age at menopause. Having a partner was the most important predictor of QLS-general. A lower physical performance score, smoking, a lower educational level of participants' partners, the presence of chronic diseases, and the presence of disability were associated with lower scores on the QLS-General. The determinants studied were mostly related to the QLS-health module. QLS-health was mostly determined by functional ability represented by impairments in activities of daily living, physical activity and physical performance, independently of fat mass and/or the presence of chronic diseases. Of the body composition components, BMD and lean mass were not associated with QoL and only fat mass was related to lower QLS-health, probably due to the higher frequency of chronic disease associated with overweight. None of the hormonal parameters measured were found to be related to QLS-health.

When interpreting our results, some methodological issues should be taken into account. There was no validated Dutch version available of published questionnaires especially designed for groups of postmenopausal women, like the WHQ,[16] and the specific quality of life questionnaire for menopause.[17] Furthermore, we did not focus on the perimenopause or on the menopausal transition in this study. The numerous general and disease-specific quality of life questionnaires share the presence of marked inter- and intra-individual differences in the perception and evaluation of objective aspects of life or disease.[18] We opted for the questionnaire of life satisfaction (QLS) recently developed and validated for the Dutch population,[19,20] which attempts to deal adequately with the problem of the relative importance of individual aspects of quality of life. It is based on the view that dimensions with little or great importance to an individual cannot contribute with the same weight to the overall quality of life score.[9] Recent studies with this QLS questionnaire have demonstrated that weighing of the individual items for their importance to the respondent is an effective way to incorporate the concept of subjectivity of quality of life in this quality of life instrument.[21] It is highly correlated with other instruments used to assess mainly psychological aspects of well-being,[22] such as the general well-Being-Schedule GWB,[23] the Beck Depression inventory-BDI,[24] and the general symptomatic index and the scale "depression" of the SCL-90-R Symptom Checklist.[25]

Age was not associated with quality of life, partly due to the moderate age-related increase of co-morbidities in this population of healthy postmenopausal women. However, the absence of association between age and quality of life gives a hopeful perspective and legitimates efforts spend on improving quality of life at older age. However, due to the cross-sectional nature of the study we cannot exclude the possibility that in this population physical condition in elderly women is already quite good, resulting in good QoL, so no investments need to be made for further improvement of QoL at older age.

The QLS-General module was strongly determined by the marital status, being without a partner affecting negatively the psychological well being of this population, but not the QLS-health. This can be explained by the fact that these women were not extremely old and the healthiness of this selected population, nevertheless having a psychological dissatisfaction resulting from the risk of living a more isolated life lacking an important source of love and support.

The QLS-Health module turned out to have the closest relation with the physical characteristics measured, consistent with previous observations that this module correlates better than the QLS-general module with scales addressing physical problems.[9]

Our results are consistent with the previously reported adverse impact of overweight on QoL through physical limitations due to overweight or secondary co-morbidities, impaired psychological functioning, and altered social functioning.[26,27] In our population the presence of chronic diseases associated with overweight explained a large part of this relation. This agrees with previous prospective data where BMI was shown to be a strong predictor of long-term risk for mobility disability in older women, which persists even to very old age.[28]

There was no significant positive relation between BMD and QoL, probably due to the selection of a healthy population. Nevertheless, a negative association between the presence of fractures after 50 years of age and QLS-health was present.

Physical performance and physical activity were related to higher QLS-Health which suggests an important role of physical well-being on the quality of life at this age. The strong physical functional disability relation with lower QLS-health could be expected because even modest physical impairment may limit participation in productive and recreational activities. In addition, several problems in activities of daily living as assessed by the Health Assessment Questionnaire are strongly associated with low values of all parts of QLS.

The absence of clear associations between circulating oestradiol levels and QLS modules is consistent with findings from previous studies. It has been well established that the positive effects of oestrogen substitution on quality of life at the time of menopause appear exclusively

due to menopausal symptoms relieve such as night sweating and hot flushes,[29] and no other effect of oestrogen on quality of life has been demonstrated.[30] However, we cannot exclude the possibility that adjustment for fat mass in fact was overadjustment, due to the fact that the fat mass is the tissue where conversion of androgens to extrogens primarily takes place in the postmenopause. Furthermore, the significant modification of quality of life perception during the peri/postmenopausal period seems to depend less upon biology than on socio-economic circumstances, individual experiences, resources and cultural morals.[31] Several studies have reported that oestrogens or combination of androgen and oestrogen replacement therapy of postmenopausal women improve wellbeing while providing beneficial effects on bone, lipids, sexual functioning, and quality of life,[32-35] but these effects are not perceptible in the ranges of circulating endogenous hormone levels.

Our findings are consistent with previous studies where a higher level of physical activity in the obese female population was positively associated with health related QoL.[36] Whether a good quality of life is a consequence or a cause of an overall good physical functional status cannot be established because of the cross-sectional nature of this study.

In conclusion, age itself is not associated with decrease in well being, and apart from the strong adverse psychological effect of being without a partner, this study suggests that physical and psychological well-being are strongly associated in healthy elderly and late postmenopausal women younger than 75 years of age. Our results do not support the presence of hormonal factors as predictors of quality of life in this age group. The data indicate an adverse effect of fat mass on overall well being.

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Chapter 8

General discussion

The main objective of the studies among postmenopausal women described in this thesis was to provide epidemiologic insight in factors associated with successful aging, such as avoidance of disability, maintenance of high physical and cognitive function, and sustained engagement in social activities. We investigated the associations between endogenous hormone levels and parameters of successful aging. For this purpose, a combination of measurements were used: arterial stiffness, cognition, body composition, bone mineral density, physical functioning and quality of life. The ultimate goal was to identify possible target groups for - preventive - intervention in order to increase the proportion of women that age successfully.

The main findings of our studies are discussed in this chapter and are placed in a broader context. Finally, views on further research regarding improvement of quality of life at older age are put forward.

METHODOLOGICAL CONSIDERATIONS

Study design

The studies in this thesis were conducted cross-sectionally, which implies that the determinants and outcome were measured at the same point in time. To appreciate the findings some possible limitations of this approach need to be addressed.

Selection bias

Selection bias occurs when the relation between the determinant and the outcome is different for those who participated to the study and those who would be theoretically eligible but did not participate. Selection bias can lead to either an underestimation or an overestimation of an association which affects the validity of the research an which generally cannot be corrected for in the analyses. For example, non responders may differ from responders with respect to their attitude towards health, risk factor status, socio-economic status, and health status. Therefore it is likely that non responders are older, have higher levels of risk factors, and more disease compared to subjects participating in the study. Selection of participants in studies is very common and does generally not create validity problems.

However, bias due to non-response only occurs when non-response is related to the determinant as well as to the outcome. In order to appreciate the presence of a selection bias in our study, we compared the characteristics of our population with the Prospect population and found no significant differences other than smoking behaviour (Table 1). Therefore, we do not think that selection bias plays a major role.

Table 1- Comparison of population characteristics between responders and non-responders

	Responders		**Non-responders**		
Variable	**mean**	**SD**	**mean**	**SD**	**p-value**
Age (years)	62.5	3.9	62.5	4.1	0.968
BMI (kg/m^2)	25.9	4.2	26.3	4.0	0.164
Systolic blood pressure (mmHg)	137	22	138	22	0.619
Diastolic blood pressure (mmHg)	79	11	79	11	0.569
Age at menarche (years)	13.6	1.6	13.7	1.8	0.373
Age at menopause (years)	49.4	4.7	49.4	4.7	0.911
	N	**%**	**N**	**%**	
Type of menopause					0.649
Natural	333	83	412	82	
Surgical	68	17	88	18	
Unknown	0	0	1	0	
Smoking behavior					0.011
Current	67	17	115	23	
Past	144	36	140	28	
Never	190	47	246	49	
Alcohol use					0.137
Never	47	12	68	14	
Yes	354	88	429	86	
Fractures before age 40 years					0.266
Yes	85	21	85	17	
No	315	79	416	83	
Myocardial infarction					0.332
Yes	7	2	12	2	
No	394	98	489	98	
Stroke					0.531
Yes	7	2	8	2	
No	394	98	493	98	

Another possible selection could be that participants are selected from a range of the determinant distribution at which the association addressed is less clear than at other parts of the distribution. We sampled from a base population, i.e. the Prospect cohort, comprising relatively young postmenopausal women, a healthy group who showed a very low rate of disability. This may explain that certain associations were less pronounced than expected for other populations. For example, we could not observe a relation between bone mineral density and disability. The reason to include this age group in our studies was because little data are available on determinants that are associated with disability early during the aging process, before sarcopenia has developed.

Confounding

Bias due to confounding occurs in etiologic analyses when a factor is related to both the determinant and the outcome and is not an intermediate in the causal pathway between determinant and outcome. There are several ways to deal with confounding. One of the most frequently applied methods is statistical adjustment by including the confounder as a covariate in the analyses. To be able to do so, knowledge is required on which variable might be confounder in the association of interest and data on the variable need to have been collected. When a confounder is present but not measured, adjustment is not possible and the effect estimate will be biased. Before each analysis, we decided based on our knowledge on disease mechanism which factors could be confounders, or intermediate factors in the causal relation, and we adjusted all analyses in the present thesis for the potential confounders, as for example age, blood pressure and body mass index.

MAIN FINDINGS

a- Post menopausal estrogen levels, SHBG and arterial distensibility

Despite the evidence of cardioprotective effects of late menopause, explained as a protective effect of higher premenopausal endogenous estrogen levels [1-4], we could not demonstrate an association between postmenopausal circulating estrogens and arterial distensibility. The analysis of this association is complicated by the fact that postmenopausal estrogen production primarily depends on the amount of peripheral fat mass, where conversion from precursors to estradiol takes place. The amount of peripheral fat is reflected in body weight, which is a strong determinant of blood pressure and atherosclerosis as well.

Furthermore, the population may already have been too old to display a wide enough range of estradiol levels, and among these women, with menopause 8 to 30 years ago, endogenous estradiol levels are probably far below a threshold at which any beneficial arterial effect can be demonstrated [5].

The relation we observed between SHBG and arterial stiffness is interesting although not fully understood. SHBG levels regulate plasma free and protein-bound androgens and

estrogens. SHBG decreases with increasing visceral fat, triglyceride and insulin concentrations, and increases with higher levels of HDL cholesterol. Therefore, SHBG seems to be an important and reliable marker of the interrelationships between sex steroid hormones, obesity, and cardiovascular disease risk [6-9]. Recently, SHBG has been shown to mediate extra-cellular steroid hormone actions after binding to specific high affinity SHBG receptors [10]. However, it is not known whether an intrinsic protective effect on the arterial wall works through SHBG-receptors.

b- Endogenous hormone levels and cognition

Our results support the hypothesis that higher postmenopausal endogenous estrogen levels protect against mild cognitive impairment. This effect seems independent of age at menopause, time since menopause or body mass index, and is stronger after adjustment for blood pressure. The more pronounced protective effect of estrogens on cognitive decline after adjustment for SHBG suggests that bio-available oestrogen level is the principal determinant for an effect on the brain. Although serum estradiol levels increase with BMI, higher fat mass or BMI were not related to cognition. This suggests a role for other sources of estradiol production than adipose tissue, and supports the hypothesis of local estrogen biosynthesis by aromatase activity in the brain[11]. However, studies with hormone therapy have not supported a protective effect of exogenous estrogens on cognitive functions [12-19]. As for the cardiovascular effects of estrogen, the populations studied in those trials may already have been too old at the time of substitution, where an irreversible stadium of estrogen deficiency has already been reached, explaining the absence of response to exogenous estradiol. To check this hypothesis a trial on premenopausal or early postmenopausal women should be performed.

c- Endogenous estrogens, body composition and bone mineral density

A major effect of fat mass on bone mineral density at all bone sites was demonstrated among our participants, and detailed data were provided on the strength of the effect of known BMD determinants at various locations. Our findings are compatible with an effect of

local stimulation of appendicular lean mass and muscle strength on appendicular BMD. Skull BMD seems to be mainly influenced by circulating estradiol and by lean mass independently of muscle mass. The effects of circulating estradiol levels were weaker at lower extremity and hip BMD sites.

While lean mass has been shown to be the most important predictor of bone mass compared to fat mass in men [20], in premenarcheal girls [21] and in pre- and perimenopausal women [22,23], lean mass and fat mass become equally important contributors to BMD in postmenopausal women [24-27]. Our results suggest that the importance of fat mass increases later in menopause. The postmenopausal changes in body composition thus have considerable consequences on BMD, with, apart from the mechanical load which stimulates the maintenance of bone mass, increased peripheral aromatization combined with a decrease in SHBG levels leading to higher bioactive estradiol-mediated effects.

d- Menopause, body composition and physical functional status

In our study population, increased fat mass was a major risk marker for decreased physical functional status and disability. An increase in fat mass and body weight leads to a compensatory increase in muscle mass and muscle strength of the legs, which, however, only partially prevents the decrease in physical function and the development of disability.

The cross sectional design of the study does not allow a definitive distinction between cause and consequences in the described associations. Because both directions are possible, we did not use arrows in the algorithm shown in figure 1.

While lean body mass is of major importance in men and premenopausal women, it appears that the importance of fat mass increases with age among postmenopausal women. Fat mass has an important effect on various outcomes: arterial stiffness with increased risk of cardiovascular complications, increased bone mineral density, lower physical function and disability, decreased health and well being. Fat mass also determines importantly levels of remaining endogenous oestrogens through several mechanisms of action: the load of fat mass, while the increased aromatisation produces higher levels of estrogens and lower levels of SHBG.

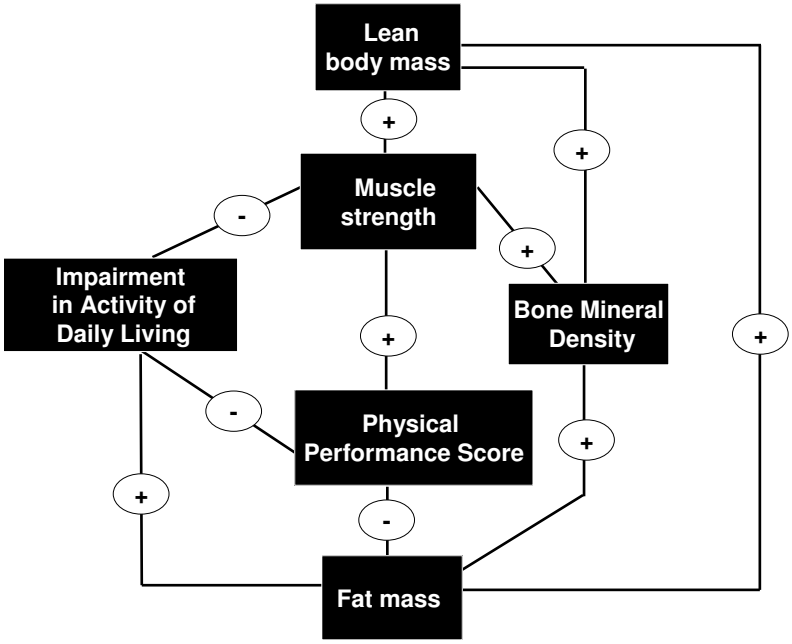

Fig. 1- Algorithm of mutual correlations between parameters which determine functional ability

Disability among more obese women is partly prevented thanks to a compensatory increase of muscle mass en strength localised at the legs. It is probable that in this age group of healthy independently living women, fat mass is a more important determinant of disability, while sarcopenia becomes important as a determinant much later in the aging process. It is also known that, during the aging process, lost muscle is initially replaced by fat, but how precisely the age-related increase in fat mass and decrease in muscle mass interact in their effects and lead to disability among older people is still unanswered. It remains difficult to understand because of the different relationships of the various compartments of body composition with morbidity, disability, and health status. The factors implied in weight gain among middle-aged women are probably at least partly modifiable and include behaviour and life-style. Our observations suggest a different approach for the prevention of disability in this age group of postmenopausal women, than is currently considered for the oldest old (>75 years) where the role of sarcopenia in the development of disability seems much more outspoken.

The question whether overweight accelerates the process of sarcopenia by the related disability cannot be answered.

e- Endogenous hormones, body composition and functional status

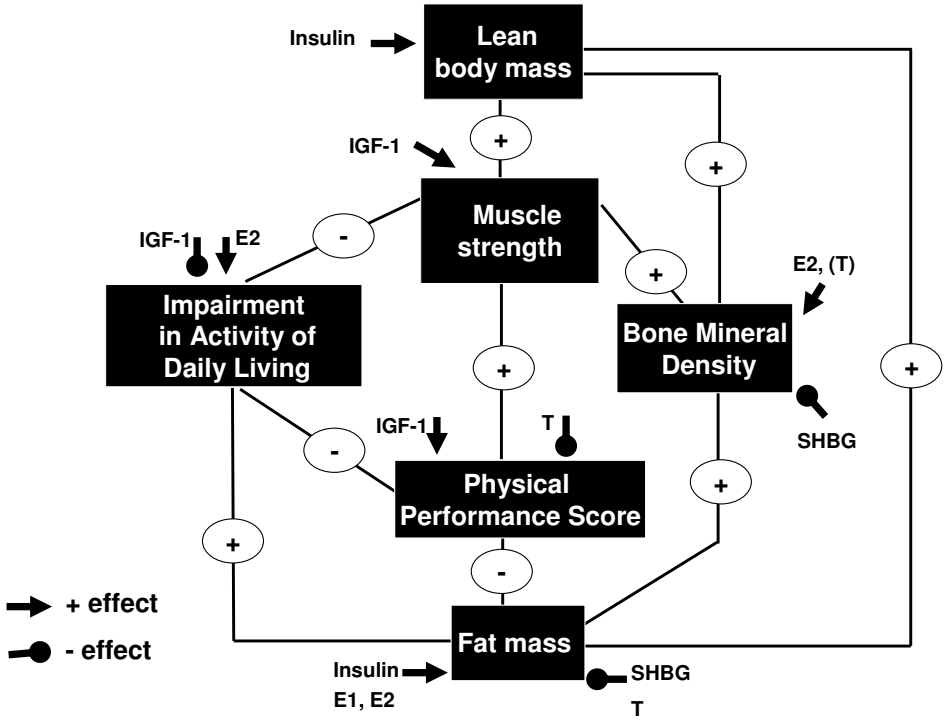

Fig. 2- Independent significant relations between hormone levels and physical parameters

On the basis of an algorithm of the significant and independent relations found in our study population between body composition, muscle strength, physical performance and disability, we studied the associations between endogenous hormones levels and various aspects of physical functioning. Apart from a strong effect on bone mineral density, no effects of circulating estrogens were found on muscle mass, muscle strength and physical performance. High estrogen levels were positively related to disability, most probably because the presence of disability was predominantly related to overweight in this healthy population. Adrenal androgens (DHEA, DHEAS) and testosterone, presumably mainly produced from adrenal precursors, showed modest associations with bone mineral density most likely through aromatization to estradiol. Higher testosterone levels were related with lower physical performance, independently of estradiol, SHBG or insulin levels, lean mass or fat mass. As this was unexpected and difficult to explain, the presence of an unknown confounder in this

relation cannot be excluded. Insulin was positively related to lean, fat and bone mass, as expected for this anabolic hormone. Finally known effects of IGF-1 were confirmed with positive effects on leg muscle strength, physical performance and a negative effect on disability.

f- Body composition, endogenous hormonal levels and quality of life

Findings for the QLS-Health module and the measured physical characteristics (muscle strength, functional ability, bone mineral density, lean mass and fat mass) suggest an important role of physical well-being on the quality of life at this age. The strong inverse relation between disability and QLS-Health may result from limitations in participation in productive and recreational activities even by modest physical impairment. Of the components of body composition, only fat mass was related to lower QLS-Health, probably reflecting physical limitations due to overweight or secondary co-morbidities, impaired psychological functioning, and altered social functioning [28,29].

Several hormonal factors were not found to be predictors of quality of life in this age group. Positive effects of estrogen therapy on quality of life at the time of menopause have been shown to be exclusively due to relieve of menopausal symptoms such as night sweating and hot flushes [30]. Reported positive effects of hormone therapy on wellbeing through beneficial effects on bone, lipids, sexual functioning, and quality of life [29,31-33], are probably not measurable in the ranges of circulating endogenous hormone levels in untreated postmenopausal women.

Whether a good quality of life is a consequence or a cause of an overall good physical functional status cannot be established because of the cross-sectional nature of this study.

CLINICAL IMPLICATIONS AND FURTHER RESEARCH

Because of our selection of participants living independently, maintaining a reasonable degree of physical capacity as well as a relatively good cognition, the population represents women that generally age successfully. To gain insight in the full spectrum of women of this

age group, it would be interesting to compare our results with those in a population also comprising institutionalised women, for example living in elderly homes.

The critical variable for protection against cardiovascular disease as well as poor cognition may be lifetime duration of premenopausal estrogen exposure. Factors affecting premenopausal estrogen levels could be as important as those influencing estrogen levels in postmenopausal women. It has been suggested that estrogen treatment has its optimal protective effect on vascular compliance and memory when administered immediately following ovariectomy of pre-menopausal women or to naturally menopausal women short after the cessation of their menstruations [34]. Consistent with the recent WHI-findings, estrogen given to older women would not have any beneficial effect on vascular and cognitive aging. Whether the critical period for the initiation of treatment is related to age, to a decrease of neuronal estrogen responsivity, or to the inability of the hormone to reverse neuronal loss and/or dysfunction which may have occurred during the estrogen deficiency state is not known and represents an important question for further research. Furthermore, research is needed to better understand the role of estrogens in cardiovascular disease prevention as well as in maintaining cognitive function in women and to identify ways in which we can translate this knowledge into clinical practice.

Another factor of importance explaining the conflicting results concerning the effects of observational studies and clinical trials with estradiol therapy on cardiovascular disease and cognition could be the mass of fat which has major effects on endogenous hormonal levels, health, disability and well-being. Consistent with the higher risk of generalized atherosclerosis associated with obesity and considering the fact that overweight women generally already have higher endogenous serum levels of estrogen en lower SHBG levels, negative effects of hormonal therapy have been obtained among overweight women. On the contrary, leaner women with low estrogen levels could eventually benefit from HT. Some evidence for this hypothesis is given in the subgroup analysis of the WHI-study [35] but this should be explored further in well-powered studies. Furthermore, most women from observational studies probably use HT as treatment for perimenopausal complaints. No study has, to our knowledge, assessed the association between postmenopausal complaints and disease risk, which could represent a potential confounder, a modifyer of HT effect, or an indicator of susceptibility to effects of exogenous estrogens [36]. When studying those relations, consideration of the time since menopause, the presence of postmenopausal complaints, and the BMI of individuals should be taken into account.

The question whether overweight accelerates the process of sarcopenia by the related disability cannot be answered in our population. Therefore, an intervention study with for example a training/exercise program and with quality of life as outcome measure should be performed.

In conclusion, hormone therapy could be of benefit for women with low endogenous serum estradiol levels, before major vascular and brain damage due to low estrogen exposition have occurred. Accordingly, thin women with a low serum estradiol level are better candidates for estrogen therapy for osteoporosis prevention [37]. Further research should take this into account.

Although estrogens have been proven useful in the prevention and treatment of osteoporosis, and with the increased risk for breast cancer [38], hormonal substitution in a state of obvious atherosclerosis has been proved ineffective [39], and even associated with increased risk of major coronary events [40,41]. Together with the shown increased risk of endometrial cancer with postmenopausal estrogen use [42], the preventive use of estrogen is not indicated to limit the aging process. Further research on selective estrogen receptor modulator (SERM) drugs could be a serious alternative in the future [43]. Tamoxifen has been proven to be protective against bone loss, but had similar side effects as estrogens on the uterus. The non-steroidal benzothiophene derivative raloxifene, is the best SERM available thus far. It does not increase the incidence of endometrial cancer, and like tamoxifen, it has the potential to prevent breast cancer, but has a better profile in its actions on bone (for example, it reduces the vertebral fracture rate more effectively than tamoxifen) [44,45]. Unlike estrogen, it decreases blood triglicerides as well as cholesterol. This should be confirmed in large population-based studies, before preventive measures can be taken.

An optimal individual management for every woman should be discussed with her caregiver. This should take into account the medical and family history as well as symptomatology, and the associated higher breast cancer risk. It can be uniformly recommended, however, that menopausal women should maintain appropriate nutrition, weight reduction, and exercise along with moderation in alcohol and caffeine intake and cessation of smoking. Data in early postmenopausal women show that exercise compensates at least partially for the negative effects of estrogen deficiency on bone loss, coronary heart disease, diabetes, and quality of life. A good physical activity, as walking more than 3 hours per week has been shown to be as effective as vigorous exercise to induce substantial reductions in the incidence of coronary events among women [46].

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Chapter 9

Summary/Samenvatting

The work presented in this thesis aims to gain further insight into the associations between endogenous estrogens and arterial stiffness (proxy of cardiovascular disease risk), cognition, bone mineral density, body composition, physical functions and quality of life. This to get answers about successful aging and to assess the need of hormonal substitution therapy or other intervention susceptible to ameliorate the quality of life of women at older age.

Chapter 1.

The purpose of the thesis is introduced in light of the growth of the elderly population and the need for a successful aging, in the light of actual knowledge about hormonal and body composition changes after menopause (bone mass, fat mass), and of relations with cardiovascular disease, cognition, and osteoporosis.

Chapter 2.

(**Chapter 2.1**). Among about 400 healthy postmenopausal women, aged 50-74 years, sampled from the general population, we cross-sectionally investigated the degree and potential cardiovascular determinants of arterial stiffness, assessed by aortic pulse wave velocity (PWV) measured using applanation tonometry. We found that PWV increased significantly with age, mean arterial pressure, pulse pressure, heart rate, and pack-years of cigarette smoking. Height and HDL cholesterol were found to be related with lower PWV. We also related arterial stiffness to absolute 10-12-year risks of stroke, coronary heart disease and death, as estimated by available risk functions. PWV is shown to be clear marker of an increased absolute risk of stroke, coronary heart disease, and death within 10-12 years

(Chapter 2.2).

We investigated whether postmenopausal levels of sex hormones, sex hormone-binding globulin, and a number of other hormones which could be implicated in the aging process - cortisol, androstenedione, DHEA and DHEAS, Insulin-like Growth Factor (IGF-1), its binding proteins (IGFBP –1 and –3) and insulin - are associated with arterial stiffness among our healthy population of 8 to 30 years postmenopausal women. None of the other circulating hormone levels measured was associated to pulse wave velocity.

Chapter 3.

In the same population, we cross-sectionally investigated whether levels of endogenous hormones, in particular circulating oestrogens and SHBG, are associated with cognition assessed using the mini-mental state examination questionnaire (MMSE). Our results support the hypothesis that higher postmenopausal endogenous estrogen levels may protect against cognitive impairment. This effect seems independent of age at menopause, time since menopause or body mass index, and stronger after adjustment for blood pressure. The protective effect of estrogens on cognitive decline was more pronounced after adjustment for SHBG, suggesting that the bio-available oestrogen level is the principal determinant for an effect on the brain. Serum estradiol levels increased with BMI and frequency of impairment in cognitive functions decreased with higher serum estradiol levels, but higher fat mass or BMI was not related to cognition. This suggests the importance of other sources of estradiol production than adipose tissue in postmenopausal women.

Chapter 4.

We investigated the variations of effects on bone mineral density at various sites of the following determinants: fat mass, lean mass, appendicular lean mass and muscle strength, and endogenous hormones. We compared the strength of effects of various determinants using standardised β of linear regression. Our results support an important effect of postmenopausal changes in body composition on BMD determinants. A major role of fat mass on bone mineral density at all bone sites was shown among our participants, and a detailed insight is given about the strength of the effect of known BMD determinants at various locations. This study supports a local stimulation of appendicular lean mass and muscle strength on appendicular BMD. Skull BMD seems to be mainly influenced by circulating estradiol and by lean mass independently of muscle mass. The effects of circulating estradiol levels were weaker at lower extremity and hip BMD sites.

Chapter 5.

We cross-sectionally investigated the relative role of fat mass, lean mass, and muscle strength in the development of disability in this group of healthy postmenopausal women younger than 75 years. Our results support the role of fat mass as the primary risk marker for disability, which might later accelerate by the age-related decrease in lean mass and the

development of sarcopenia after the age of 75 years. An increase in fat mass and body weight leads to a compensatory increase in the muscle mass and muscle strength of the legs, which, however, only partially prevents the decrease in physical function and the development of disability.

Chapter 6.

We investigated whether serum levels of circulating hormones, in particular estrogen concentrations, are associated with functional ability estimated by measuring muscle strength (grip and leg extension), physical performance, and by assessing the number of problems in activities of daily living (impaired ADL or disability) in the same healthy postmenopausal women population. We present an algorithm of the significant and independent relations found in our study population between body composition, muscle strength, physical performance and disability. On this basis, we studied the associations between measured endogenous hormones levels and the assessed various components of physical function. Aside from their strong effect on bone mineral density, no estrogen effects were found on muscle mass, muscle strength and physical performance. High estrogen levels were positively related to disability, most probably because the presence of disability was predominantly related to overweight in this healthy population. Hormone levels of adrenal androgens (DHEA, DHEAS) and testosterone, presumably mainly produced from adrenal precursors, demonstrated small effects on bone mineral density most likely through aromatization to estradiol. Still unexplained, higher testosterone levels were related with lower physical performance, independently of estradiol, SHBG or insulin levels, lean mass or fat mass. The presence of an unknown confounder in this relation can not be excluded. Insulin was positively related to lean, fat and bone mass, as expected for this anabolic hormone. Finally known effects of IGF-1 were confirmed with positive effects on leg muscle strength, physical performance and negative effect on disability.

Chapter 7.

We investigated whether body composition, functional status and serum hormone levels were associated with quality of life assessed using the questionnaire on life satisfaction (QLS), with two modules directed at general factors (QLS-General) and health factors (QLS-Health) in our healthy postmenopausal women population.

Both QLS modules did not decrease with age, which gives a hopeful perspective and motivates efforts to improve quality of life at older age. The most important and specific determinant for psychological well being was having a partner. Higher physical performance and higher educational level of participants' partners were significantly related to higher well being, while smoking and the presence of co-morbidities were significantly associated with a lower well being. Physical and psychological well being are strongly associated in this population, while increasing fat mass was related to decreased well being.

No consistent relation was found between serum levels of hormones measured and both QLS modules, suggesting that in elderly and late postmenopausal women hormonal factors do not predict quality of life.

Chapter 8.

Our results are briefly summarized and some methodological questions are discussed. The clinical relevance of the studies presented in this thesis is discussed and hypotheses on the physiopathology of estrogens effects on the different determinants studied are addressed in the light of our findings. Finally, some ideas for further research are given.

Samenvatting

Met het werk gepresenteerd in dit proefschrift is getracht meer inzicht te verkrijgen in de relatie tussen endogene circulerende oestrogenen en de volgende aspecten van de veroudering: vaatstijfheid (als maat van cardiovasculair risico), cognitie, bot massa, de lichaamssamenstelling, lichamelijke functies en de kwaliteit van het leven. Dit op zoek naar antwoorden omtrent succesvolle veroudering en om het nut van en behoefte aan hormonale substitutie therapie of eventuele andere interventies te kunnen inschatten ter verbetering van de kwaliteit van leven bij oudere vrouwen.

Hoofdstuk 1.

Het belang van succesvolle veroudering groeit met de toename van het aantal ouderen. Het doel van het proefschrift is geïntroduceerd in het licht van de actuele kennis over de verandering in hormonale- en lichaamssamenstelling na de menopauze (bot massa en vet massa) en over relaties met cardiovasculaire ziekte, cognitie en osteoporose.

Hoofdstuk 2.

(**Hoofdstuk 2.1**). Bij ongeveer 400 gezonde postmenopauzale vrouwen van 50 tot 74 jarige leeftijd, een steekproef van de algemene populatie, werd de graad en potentiële waarde van vaatstijfheid als determinant van het cardiovasculaire risico onderzocht, geschat middels aorta pulse wave velocity (PWV) meting. Vaatstijfheid nam significant toe met leeftijd, gemiddelde arteriële bloeddruk, pols druk, hart frequentie en het roken van sigaret uitgedrukt in “pack-year”. Lengte en HDL cholesterol waren geassocieerd met een lagere vaatstijfheid. Onze resultaten tonen aan dat PWV een duidelijke marker is van een toegenomen absoluut risico voor beroerte, coronaire hart ziekte, en dood binnen 10-12 jaar.

(Hoofdstuk 2.2).

Vervolgens werden de relaties onderzocht tussen vaatstijfheid en de postmenopauzale circulerende geslachtshormoon concentraties, sex hormoon bindend globuline en een aantal andere hormonen die mogelijk betrokken zijn in het verouderingsproces - cortisol, androsteendion, DHEA , DHEAS, Insulin-like Growth Factor1(IGF-1), zijn bindende

proteïnen (IGFBP –1 en –3) en insuline. Er werd geen relatie gevonden tussen circulerende hormoon concentraties en de vaatstijfheid in onze gezonde bevolking van 8 tot 30 jaar postmenopauzale vrouwen.

Hoofdstuk 3.

In dezelfde groep werd cross-sectioneel onderzocht of de endogene serum hormoon concentraties, in het bijzonder circulerende oestrogenen en SHBG, geassocieerd waren met cognitie gemeten met de Mini-Mental State Examination vragenlijst (MMSE). Onze resultaten steunen de hypothese, dat een hogere serum oestrogeen concentratie postmenopauzale vrouwen kan beschermen tegen cognitieve achteruitgang. Dit effect was onafhankelijk van menopauzeleeftijd, tijd sinds menopauze en body mass index, en was sterker na correctie voor de bloeddruk. Het beschermende effect van oestrogenen was meer uitgesproken na correctie voor SHBG, wat kan impliceren dat het biologische beschikbare oestrogeen gehalte de belangrijkste determinant is voor een effect op de hersenen. Het serum oestradiol gehalte steeg met toenemende BMI en de frequentie van cognitieve achteruitgang daalde bij hogere serum oestradiol concentraties, maar hogere vet massa en body mass index waren niet geassocieerd met cognitie. Dit suggereert de aanwezigheid en het belang van andere bronnen van oestradiol productie dan vetweefsel in postmenopauzale vrouwen.

Hoofdstuk 4.

Op verschillende bot locaties werden de effecten op bot minerale dichtheid (BMD) van de volgende determinanten onderzocht: vet massa, “spier”massa (lean mass), appendiculaire “spier”massa, spierkracht, en endogene hormoon concentraties. De effectsterkte van de diverse determinanten werd vergeleken door middel van de gestandaardiseerd β met lineaire regressie berekeningen. Onze resultaten ondersteunen een belangrijke invloed van de postmenopauzale veranderingen in lichaamssamenstelling op de determinanten van BMD. Een belangrijke rol van vet massa op BMD werd bij alle botlocaties aangetoond en een gedetailleerd inzicht over de effectsterkte van bekende determinanten van BMD per botlocatie werd verkregen. Deze studie toont een lokale stimulatie van appendiculaire spier massa en spierkracht op de appendiculaire BMD aan. Schedel BMD lijkt hoofdzakelijk beïnvloed te worden door het circulerend oestradiol en door de “spier”massa. Effecten van het circulerende oestradiol gehalte waren zwakker bij de benen en heup BMD.

Hoofdstuk 5.

De relatieve rol van vetmassa, “spier”massa, en spierkracht bij de ontwikkeling van functionele gebreken werd cross-sectioneel onderzocht in deze groep van gezonde postmenopauzale vrouwen. Onze resultaten suggereren de rol van vet massa als primaire risico factor voor functionele gebreken, die later door de leeftijdsafhankelijke daling van “spier”massa, de ontwikkeling van sarcopenie boven de leeftijd van 75 jaar zouden kunnen versnellen. Een toename van vet massa en lichaamsgewicht leidt tot een compensatoire toename van de “spier”massa en de spiersterkte van de benen, die de afname van fysieke functie en de ontwikkeling van functionele gebreken slechts gedeeltelijk tegen gaat.

Hoofdstuk 6.

De relatie tussen circulerende hormoon concentraties - met name oestrogeenconcentraties - en de functionele capaciteit werd onderzocht. Functionele capaciteit werd geschat door middel van spierkracht, meting van fysieke prestaties en het aantal problemen in activiteiten van het dagelijks leven (verminderde ADL of functionele gebreken) in dezelfde groep gezonde postmenopauzale vrouwen. Een algoritme werd samengesteld met de significante en onafhankelijke relaties tussen lichaamssamenstelling, spierkracht, fysieke prestaties en functionele gebreken. Op deze basis werden de relaties onderzocht tussen de gemeten endogene hormoon concentraties en de diverse componenten van de lichamelijke functies. Afgezien van hun welbekende effect op de bot minerale dichtheid, werden geen effecten van oestrogenen aangetoond op spiermassa, spierkracht en fysieke prestaties. Hogere serum oestrogeen concentraties waren geassocieerd met toegenomen functionele gebreken, meest waarschijnlijk omdat de aanwezigheid van functionele gebreken hoofdzakelijk geassocieerd was met overgewicht in deze gezonde groep. Serum concentraties van bijnierandrogenen (DHEA, DHEAS) en testosteron, toonden kleine effecten op de bot minerale dichtheid, waarschijnlijk via aromatisatie van oestradiol. Een onverklaarde omgekeerde relatie werd gevonden tussen het testosteron gehalte en fysieke prestaties, een effect dat onafhankelijk was van oestradiol, SHBG of insuline gehalte, “spier”massa of vetmassa. De aanwezigheid van een nog onbekende confounder in deze relatie is niet uitgesloten. Insuline werd positief geassocieerd met “spier”-, vet- en botmassa, zoals verwacht voor dit anabole hormoon. Tot slot werden de bekende effecten van IGF-1 bevestigd met positieve effecten op spierkracht, fysieke prestaties en een negatief effect op functionele gebreken.

Hoofdstuk 7.

Vervolgens werden in onze populatie van gezonde postmenopauzale vrouwen, de relaties onderzocht tussen lichaamssamenstelling, functionele status, circulerende hormoon concentraties en de kwaliteit van leven, beoordeeld door middel van een vragenlijst over levenstevredenheid (QLS), met twee modules: het QLS-Algemeen en het QLS-Gezondheid.

Beide QLS modules daalden niet met leeftijd, wat een hoopvol perspectief biedt en verdere inspanningen ter verbetering van de kwaliteit van leven op oudere leeftijd stimuleert. De belangrijkste determinant voor psychologisch welzijn was het hebben van een partner. Grotere fysieke prestaties en een hoger onderwijsniveau van de partners van de deelnemers waren significant gerelateerd met een beter algemeen welzijn, terwijl roken en de aanwezigheid van co-morbiditeit significant geassocieerd waren met een lager algemeen welzijn. Fysiek en psychologisch welzijn waren sterk geassocieerd en een grotere vetmassa was geassocieerd met een verminderd welzijn.

Er werden geen relaties gevonden tussen de gemeten serum hormoon concentraties en beide QLS modules. Dit suggereert dat bij oudere postmenopauzale vrouwen, hormonale factoren de kwaliteit van leven niet beïnvloeden.

Hoofdstuk 8.

Onze resultaten worden kort samengevat en enkele methodologische vragen worden besproken. De klinische relevantie van de studies gepresenteerd in dit proefschrift worden besproken en enkele pathofysiologische hypothesen omtrent de effecten van oestrogenen op de verschillende bestudeerde determinanten worden gepresenteerd in het licht van onze bevindingen. Tot slot worden ideeën voor toekomstige onderzoek gegeven.

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Curriculum Vitae

De schrijfster van dit proefschrift werd als Française op 13 maart 1964 geboren te Casablanca. In 1982 haalde zij het Baccalaureaat D eind examen te Marseille, Frankrijk. In 1986 aangevuld met de Brevet de Technicien Superieur en diététique en in 1987 met de Diplôme Universitaire en physiologie animale. Na het behalen in 1988 van het benodigde Nederlandse certificaat, werd in 1989 aangevangen met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam, alwaar in 1998 het artsexamen werd afgelegd. Per december 1998 begon zij als artsonderzoeker aan het promotie onderzoek onder leiding van prof. Dr. S.W.J. Lamberts, internist endocrinoloog en prof. Dr. D.E. Grobbee, epidemioloog. In mei 2001 werd een aanvang gemaakt met de opleiding tot internist in het voormalige Dijkzigt ziekenhuis te Rotterdam onder opleider prof. Dr. H.A.P. Pols. Zij is in april 2005 gestart met de opleiding tot reumatoloog in het Medisch Spectrum Twente te Enschede onder opleider prof. Dr. M.A.F.J. van de Laar, en sinds augustus 2007 in het Twenteborg ziekenhuis te Almelo onder opleider dr. C.J. Haagsma. Per april 2008 zal zij als reumatoloog werkzaam zijn in het Leveste Scheper ziekenhuis te Emmen.

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