Exercise, weight loss and biomarkers for breast cancer risk

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CONTENTS

1

GENERAL INTRODUCTION

Exercise, weight loss and postmenopausal breast cancer risk

Lifestyle plays a substantial role in the risk of breast cancer, in addition established reproductive-linked risk factors and family history or genetics[1]. As breast cancer is the most prevalent disease among Western women and the incidence is rising[2], options to reduce risk and, thereby, lower incidence rates are an important subject under study. Live a more healthy lifestyle is a form of primary prevention of breast cancer as it decreases exposure to certain risk factors.

In this thesis, we mainly focus on overweight/obesity and physical inactivity as modifiable breast cancer risk factors. Observational studies have provided strong evidence for an association between these factors and an increased risk of several types of cancer, including postmenopausal breast cancer[3].

The World Cancer Research Fund estimated that postmenopausal breast cancer risk increases by 13% for every five unit increase in body mass index (BMI)[3]. Regarding physical inactivity: a meta-analysis concluded that breast cancer risk is 20% to 40% lower in active women compared to inactive women[4]. One of the pathways whereby physical activity influences postmenopausal breast cancer risk is via exercise-induced weight maintenance and weight loss[5]. It is also suggested that there is an independent beneficial effect of exercise on cancer risk, since in most observational studies the effect remains after adjustment for BMI[4].

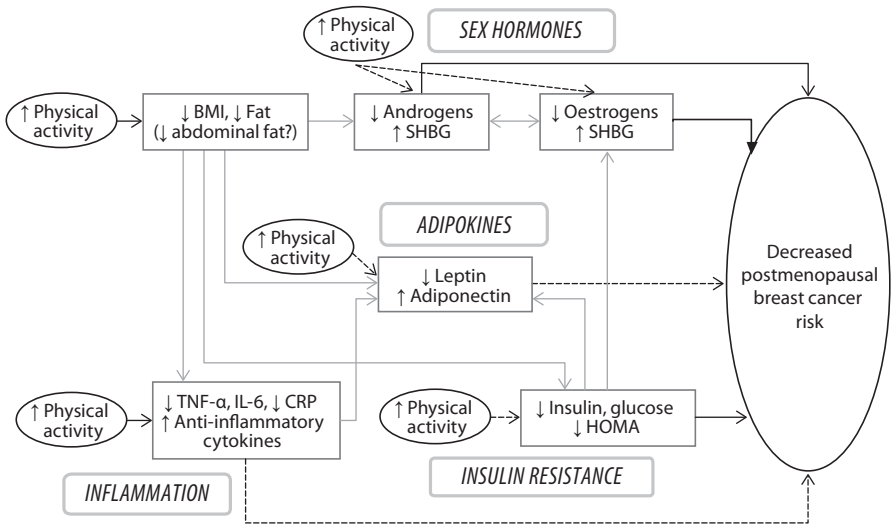
A limitation to observational research is that it does not establish a direct causal link between physical activity and breast cancer risk[6]. Especially exercise exposure is difficult to assess in observational studies due to recall bias or misclassification. Furthermore, residual confounding may bias the results. Randomised clinical trials (RCTs) are less prone to these types of bias[7] and are a valuable addition to observational research in unravelling the effect of exercise on breast cancer risk.

Theoretically, the most optimal study design to investigate the effects of weight loss and exercise on breast cancer risk would be to randomise large groups of women lifelong to a diet or exercise intervention or control group and follow them until breast cancer, the primary outcome, occurs. Unfortunately, this approach would simply not be feasible due to practical constraints and high costs. Moreover, it is unethical to withhold a large group of women from exercise or weight loss attempts, as this has been proven to have beneficial health effects considering cardiovascular diseases, diabetes, different types of cancer and various other chronic diseases[8]. An alternative is to use breast cancer risk associated biomarkers as an intermediate outcome in a randomised trial design. Surrogate endpoints generally allow clinical trials to be shorter in duration and smaller in sample size.

Mechanisms that link physical inactivity and overweight/obesity with breast cancer risk

It is not fully clear how physical inactivity and overweight or obesity influence breast cancer risk. Several underlying pathological mechanisms have been suggested. These include sex hormones, markers of inflammation, adipokines and insulin resistance (Figure 1).

Figure 1 Hypothesised mechanisms whereby physical activity influences breast cancer risk



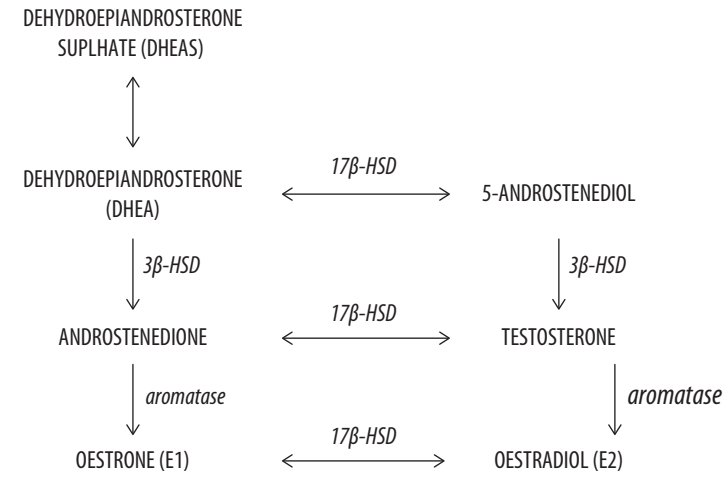
The hypothesised mechanisms whereby physical activity may influence breast cancer risk that are investigated in this thesis. Figure based on Neilson et al.[9]
SHBG=Sex hormone binding globulin. TNF-α=Tumor necrosis factor alpha. IL-6=Interleukine 6. CRP=C-reactive protein. HOMA=Homeostatic model assessment of insulin resistance.

Sex hormones

One of the pathways described, with the largest body of evidence and the most consistent associations, is through serum sex hormones levels[9]. Sex hormones include oestrogens, such as oestradiol and oestrone, and androgens, such as testosterone and androstenedione. Sex hormone binding globulin (SHBG) is a protein synthesised in the liver which is also associated with breast cancer risk. It binds to oestradiol and testosterone and, thereby, reduces their (harmful) free fractions or bioavailability. High levels of sex hormones and low levels of SHBG have been consistently shown to associate with higher postmenopausal breast cancer risk up to two-fold[10,11].

Before menopause sex hormones are mainly produced by a woman's ovaries and, as a secondary site, the adrenal glands[12]. After menopause the ovaries become non-active and the main source of oestrogens and androgens is via conversion in peripheral fat tissue of precursors as DHEAS by enzymes such as aromatase and hydroxyl-dehydrogenase[13,14] (Figure 2).

Figure 2 Sex hormone synthesis in adipose tissue



3β-HSD, 17β-HSD and aromatase are enzymes catalysing the conversion of sex hormones.
3β-HSD=3β-hydroxysteroid dehydrogenase-$\Delta^{5,4}$-isomerase.
17β-HSD=17β-hydroxysteroid dehydrogenase.

Women who are overweight or obese and inactive have been shown to have higher levels of circulating sex hormones than their lean and active counterparts[15]. As exercise may concordantly reduce body weight, this could explain at least part of the beneficial effect on sex hormones. However, even in the absence of weight loss, exercise may reduce sex hormone bioavailability via decreased insulin. As insulin decreases, SHBG rises in reaction, leading to lower free fractions of oestrogens and androgens[16].

Insulin resistance

Insulin resistance and consequent hyperinsulinaemia are associated with an increased risk of several cancer types, including postmenopausal breast cancer[17-19]. Diabetic women

have been shown to have a substantial (estimated 16% to 32%) increase in breast cancer risk compared to healthy women[18,19]. Insulin levels are higher among overweight and obese women and can be reduced with weight loss. Also, exercise may lower serum insulin and enhance insulin sensitivity[20].

Inflammation and adipokines

Obesity is associated with a chronic state of low-grade inflammation[21]. Adipose tissue can be seen as an endocrine organ secreting multiple hormones, cytokines and adipokines of which several have been associated with cancer risk[21]. High levels of C-reactive protein (CRP), interleukine-6 (IL-6), leptin and TNF-alpha might increase cancer risk[22-26]. Adiponectin is suggested to be protective for breast cancer[27,28]. However, the evidence for an association between these inflammatory markers and adipokines and postmenopausal breast cancer is limited. Exercise may lower levels of CRP, IL-6, and leptin. Although it is likely that these effects are largely mediated by weight or fat loss, independent effects of exercise have also been described, mainly for CRP[9,29].

Exercise, weight loss and sex hormones: current knowledge from intervention studies, and challenges

Three exercise trials in healthy postmenopausal women with sex hormones as the primary outcome have been conducted. One of these studies was the SHAPE(-1) trial[30]. In total, 189 normal- and overweight (BMI 22-40 kg/m^2), inactive postmenopausal women were randomised to either a one-year aerobic exercise intervention or control group. The exercise programme did not affect body weight or BMI[31], but exercisers showed a significantly larger loss of body fat (difference of -0.33 kg, -0.43%) and increase in lean mass (difference of +0.31 kg) compared to their peer controls[31]. No effects of the exercise intervention were observed on serum sex hormones and SHBG[32]. However, in those women who lost more than 2% body fat (measured by Dual-energy X-ray absorptiometry, DEXA) androgens had decreased significantly more in the exercise group compared to control. Also, a borderline significantly larger increase in SHBG (which is beneficial) was observed with exercise.

A comparable trial in the U.S. in overweight-to-obese and inactive women also found that sex hormones (both oestrogens and androgens) decreased in women who lost >2% body fat, but not in women who did not experience a loss in fat mass[33,34]. The third trial, among normal- and overweight inactive postmenopausal Canadian women, did observe an overall effect of exercise on (free) oestradiol and SHBG[35]. However, women in the exercise group concordantly lost more body weight (difference of -1.8 kg) and fat (difference of -2.0 kg, -1.8%) than controls[36], which could explain these results.

Dietary intervention studies investigating effects of low-fat and/or calorie restricted diets in postmenopausal women showed that SHBG[37,38] and testosterone[37], but not oestradiol, are reduced in the intervention groups that lost two to four kilogrammes of

body weight. It is however, not clear to which extent the effects are caused by the dietary change and to which extent by the weight loss.

After summarising the above study results the question arose whether weight loss alone is responsible for inducing favourable changes in sex hormones and other biomarkers of breast cancer risk, or whether there is an additional effect of exercise when weight loss is reached.

As we defined our research question, another weight loss and exercise trial was designed in the U.S. contemporaneously. This RCT among 439 healthy postmenopausal women aimed to get more insight in the independent and combined effects of exercise and weight loss on breast cancer risk[39]. Participating women were randomised to a calorie restricted diet intervention, an exercise intervention, a diet and exercise combined intervention or a control group. After one year of follow-up, the combined intervention induced the greatest weight loss (i.e., -8.9 kg, -10.8%), followed by diet (-7.2 kg, -8.5%) and exercise alone (-2 kg, -2.4%), compared with control (-0.07 kg)[40]. Beneficial effects were observed on oestrone and oestradiol with all three interventions. Furthermore, beneficial effects were observed on SHBG, free oestradiol and free testosterone in both diet intervention groups, but not in the exercise only group[39]. The combined diet and exercise intervention group induced the greatest effects. The authors drew the main conclusion that greater weight loss produced greater effects on sex hormones.

The Sex Hormones And Physical Exercise (SHAPE)-2 randomised controlled trial

We designed the Sex Hormones And Physical Exercise (SHAPE)-2 study to investigate whether exercise has an additional effect on serum sex hormones when weight loss is reached. The SHAPE-2 study is a three-armed randomised controlled trial among 243 healthy overweight-to-obese and inactive postmenopausal women (chapter 4). The study was conducted in the vicinities of Utrecht and Enschede, the Netherlands, from February 2012 to June 2013. All women who were eligible and willing to participate started with a four to six-week run-in period, aiming for stable body weight and a comparable diet composition among all participants. Hereafter, participants were randomised to a diet-induced weight loss group, an exercise-induced weight loss group, or a stable weight control group. The aim of both weight loss interventions was to lose 5-6 kilograms of body weight. Our reasoning was that an equivalent amount of weight loss induced by diet or mainly by exercise allows to study the possible additional effect of exercise on biomarkers for breast cancer risk.

The exercise intervention consisted of two hours of facility-based endurance and strength training, and two additional hours of Nordic walking. Furthermore, to ensure a substantial weight loss, a caloric intake restriction of -250 kcal/day was prescribed. Women in the diet group were prescribed a larger caloric restriction, of -500 kcal/day and asked to maintain their usual activity pattern. Adherence to the intervention programmes and body weight were closely monitored throughout the study by supervising physiotherapists

Table 1 Randomised controlled trials investigating effects of diet or exercise on serum sex hormones in postmenopausal women

	Population	Intervention	Results body composition		Results sex hormones	
			Body weight†	Body fat†	ITT analysis: intervention vs. control	Secondary analysis: intervention vs. control
EXERCISE TRIALS						
1. McTiernan 2004[33,34]	N=173, 50-75 years, BMI>25 kg/m^2 (mean 30.5), sedentary • Study duration: 1 year	(1) Exercise: 5*45 min/wk aerobic (2) Control	(1) -1.3 kg (2) +0.1 kg	(1) -1.4 kg (2) -0.1 kg	Oestrogens: — SHBG: — Androgens: —	• Subgroup: lost >2 % body fat: Oestrogens: (free) oestradiol ↓ SHBG: — Androgens: (free) testosterone ↓
2. SHAPE 2009[32]	N=189, age 50-69 years, BMI 22-40 kg/m^2 (mean 27), sedentary • Study duration: 1 year	(1) Exercise: 2*60 min/wk + 1*30 min/wk, aerobic (2) Control	(1) -0.66 kg (2) -0.34 kg	(1) -0.81 kg (2) -0.2 kg	Oestrogens: — SHBG: — Androgens: —	• Subgroup: lost >2 % body fat: Oestrogens: — SHBG: — Androgens: ↓
3. ALPHA 2010[35]	N=320, age 50-74 years, BMI 22-40 kg/m^2, (mean 29.1), sedentary • Study duration: 1 year	(1) Exercise: 5*45 min/wk aerobic (2) Control	(1) -2.3 kg (2) -0.5 kg	(1) -2.4 kg (2) -0.8 kg	Oestrogens: (free) oestradiol ↓ SHBG: ↑ Androgens: —	
DIETARY TRIALS						
4. Berrino 2001[37]	N=312, age 50-65 years, mean BMI 27 kg/m^2 • Study duration: 18 wk	(1) Diet: low fat & carbohydrates. (2) Control	(1) -4.1 kg (2) -0.5 kg		Oestradiol: — SHBG: ↑ Testosterone: ↓ • Only 3 hormones were measured	• After adjustment for weight: Oestradiol: — SHBG: — Testosterone: —
5. Bhargava 2006[38]	N=994, age 50-79 years, mean BMI 28.8 kg/m^2 • Study duration: 1 year	(1) Diet: low fat (-20%), high fruit/ vegetable/fibre (2) Control	(1) -2.1 kg (2) -0.4 kg		Oestradiol: — SHBG: ↑ • Only 2 hormones were measured	
WEIGHT LOSS AND EXERCISE TRIALS						
6. NEW 2012[39]	N=439, age 50-75 years, BMI >25 kg/m^2 (mean 30.9), inactive • Study duration: 1 year	(1) diet: 10% weight loss goal (2) Exercise: 5*45 min/wk aerobic (3) Diet + exercise: Combination of the above programmes (4) control	(1) -9.1 kg (2) -2.8 kg (3) -9.8 kg (4) -0.5 kg	(1) -5.0% (2) -1.8% (3) -6.4% (4) -0.3%	(1): Oestrogens: ↓, SHBG: ↓, Androgens: free testosterone ↓ (2): Oestrogens: oestrone ↓, SHBG: — Androgens: — (3): Oestrogens: ↓, SHBG: ↓, Androgens: free testosterone ↓ • Conclusion: greater weight loss produced greater effects	

Studies 1, 2, 3 and 6 assessed: (free) oestradiol, oestrone, (free) testosterone, androstenedione and sex hormone binding globulin (SHBG). Study 1 additionally assessed dehydro-epi-androstenedione (DHEAS) and dehydro-epi-androstenedione sulphate (DHEA-S); Study 2 additionally assessed oestrone sulphate. Study 4 assessed oestradiol, SHBG and testosterone. Study 5 assessed oestradiol and SHBG.

† All intervention effects on body weight and fat mass were significantly different from control. —= no significant change vs control, ↑ significant increase vs control, ↓ significant decrease vs control. A decrease in oestrogens and androgens and an increase in SHBG are associated with a decrease in breast cancer risk

and dietitians. At baseline and after 16 weeks, blood samples were collected for assessment of the primary outcome, i.e., serum sex hormone levels: (free) oestradiol, oestrone, (free) testosterone, androstenedione and SHBG. Other measurements included anthropometrics, body fat and lean mass (by dual-energy X-ray absorptiometry, DEXA), abdominal fat (by magnetic resonance imaging, MRI) and questionnaires on general health, dietary food intake and physical activity.

Aim and outline of the thesis

In this thesis, we aim to investigate the impact of lifestyle related risk factors on postmenopausal breast cancer risk. The emphasis is on the effects of weight loss, by diet or exercise, on serum sex hormones and other biomarkers associated with breast cancer risk, which we investigated in the SHAPE-2 study. The primary outcome are serum levels of sex hormones. Markers of inflammation and adipokines, which are also associated with breast cancer risk, were investigated as a secondary outcome.

In **Chapter 2**, we assess the fraction of postmenopausal breast cancer cases in the Netherlands that can be attributed to exercise and other lifestyle related risk factors, so called population attributable fractions. We included the risk factors physical inactivity, overweight/obesity, alcohol consumption, insufficient fibre consumption and smoking.

In **Chapter 3**, we investigate the effects of an exercise intervention on insulin sensitivity, another hypothesised mechanism whereby exercise influences breast cancer risk. We used data of the SHAPE(-1) trial.

In the second part of this thesis, we present the design (**Chapter 4**) and outcomes (**Chapters 5 to 8**) of the SHAPE-2 study. In **Chapter 5**, we investigate the effect of diet- or mainly exercise-induced comparable weight loss on serum sex hormone levels in postmenopausal women. In **Chapter 6,** we present results of the SHAPE-2 weight loss interventions on markers of inflammation and adipokines. In **Chapter 7**, we address the question whether a loss in general or (intra-)abdominal fat, assessed with MRI, is more important in affecting breast cancer risk biomarkers. In **Chapter 8,** we focus on the effects of the SHAPE-2 interventions on health related quality of life (HRQoL).

In **Chapter 9**, we will discuss outstanding questions and elaborate on the impact of our findings for prevention and future research. A summary of the main findings concludes this thesis.

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2

THE PROPORTION OF POSTMENOPAUSAL BREAST CANCER IN THE NETHERLANDS ATTRIBUTABLE TO LIFESTYLE-RELATED RISK FACTORS

Submitted

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ABSTRACT

Introduction

We aimed to estimate the proportion of Dutch postmenopausal breast cancer cases in 2010 that is attributable to lifestyle-related risk factors.

Methods

We calculated population attributable fractions (PAF) of potentially modifiable risk factors for postmenopausal breast cancer in Dutch women aged >50 in 2010. First, age-specific PAFs were calculated for each risk factor, based on the relative risks for postmenopausal breast cancer accompanying the risk factor (from meta-analyses) and age-specific prevalence in the population (from national surveys) around the year 2000, assuming a latency period of ten years. To obtain the overall PAF, age-specific PAFs were summed in a weighted manner, using the age-specific breast cancer incidence rates (2010) as weights. 95% Confidence intervals for PAF estimates were derived by Monte Carlo simulations.

Results

Of Dutch women >40 years, in 2000 51% were overweight/obese, 55% physically inactive (<5 days/wk 30 min activity), 75% regularly consumed alcohol, 42% ever smoked cigarettes, and 79% had a low fibre intake (<3.4 g/1,000 kJ/day).

These factors combined had a PAF of 25.7% (95% CI: 24.2 to 27.2), corresponding to 2,665 Dutch postmenopausal breast cancer cases in 2010. PAFs were 8.8% (95% CI: 6.3 to 11.3) for overweight/obesity, 6.6% (95% CI: 5.2 to 8.0) for alcohol consumption, 5.5% (95% CI: 4.0 to 7.0) for physical inactivity, 4.6% (95% CI: 3.3 to 6.0) for smoking and 3.2% (95% CI: 1.6 to 4.8) for low fibre intake.

Discussion

Our findings imply that modifiable risk factors are jointly responsible for approximately one out of four Dutch postmenopausal breast cancer cases. This suggests that incidence rates can be lowered substantially by living a more healthy lifestyle.

INTRODUCTION

Breast cancer, especially postmenopausal, is the most occurring cancer in women worldwide and the second leading cause of female cancer death[1]. In Western Europe, one in eight women develops breast cancer during her lifetime, of whom more than 75% after the age of 50[2]. The high burden of disease and associated treatment costs make post-menopausal breast cancer a major public health issue. Not only incidence rates differ according to menopausal status, effects of some risk factors are also modified by menopausal status. For example, overweight has no, or even a small protective effect in premenopausal women, whereas it increases risk after menopause[3].

Several of the established risk factors for postmenopausal breast cancer are not, or rather difficult to modify when the age of 40 has been reached, i.e., family history, age at menarche and menopause, parity, age at first child birth and duration of breastfeeding. As lifestyle is modifiable, it provides an opportunity for primary prevention. Overweight and obesity, physical inactivity, alcohol consumption, smoking and low dietary fibre intake are all associated with an increased breast cancer risk after menopause[4-7] and are still present and modifiable at a later age.

The potential impact of preventive measures can be assessed by computing the population attributable fraction (PAF). This fraction represents the proportion of cases in a population that could be prevented if exposure to a causal factor had not occurred[8].

We computed individual and combined PAF estimates for the above five lifestyle-related risk factors for the Netherlands, a country with one of the highest incidence rates of breast cancer worldwide[1].

METHODS

PAF calculations

The PAF was calculated for four age categories of each risk factor (50-60 years, 60-70 years, 70-80 years, >80 years) using the formula[9,10]: $PAF = 1 - 1/(P1*RR1 + \ldots + Pn*RRn)$. Where P is the prevalence of each exposure level of the risk factor (*1* to *n*), and RR is its accompanying relative risk of breast cancer.

We defined postmenopausal breast cancer as all invasive breast malignancies in women aged 50 years and older. Assuming a latency period of 10 years between exposure to the hazardous lifestyle and breast cancer occurrence, prevalence rates were taken from the years 2000 to 2001, and 1997 for dietary fibre consumption, of women aged 40 years and older. To estimate the PAF per risk factor, the absolute number of attributable cases per age category was calculated. This was done by using the age-specific PAFs. Age-specific PAFs were calculated by the RR accompanying a risk factor, multiplied by the prevalence of exposure in each age category. The age-specific PAF was then multiplied by

the age-specific number of incident invasive breast cancer cases in 2010. Next, the attributable cases were summed over all ages and divided by the total number of breast cancers diagnosed age 50 and over.

To estimate the PAF of postmenopausal breast cancer for five risk factors combined, summing of the five separate PAFs would lead to an overestimation of the attributable proportion of cases. A multiplicative model is therefore used which accounts for the overlap between risk factors [11]. The following formula was proposed:

PAF (joint risk factors) = 1-(1-PAF$_{x1}$)(1-PAF$_{x2}$)*...(1-PAF$_{xn}$)*. Where x_1,*n* refer to the different risk factors. The multiplicative formula assumes independence of the effects of different risk factors on breast cancer risk, which is considered correct by using relative risks (RR) adjusted for potential confounders, including other lifestyle factors.

We used a 20,000 fold Monte Carlo simulation to derive 95% confidence intervals (95%CI) for the PAF estimates for each risk factor and joint. RRs and prevalence rates were independently sampled in each Monte Carlo trial from a lognormal distribution (based on a literature-derived RR estimate with 95% CI) and a beta distribution, respectively. Analyses were performed using R statistics software, version 3.0.2.

Risk factors and relative risks

We considered lifestyle-related – thus potentially modifiable – risk factors for postmenopausal breast cancer with sufficient scientific proof for a causal association (i.e., judged by the World Cancer Research Fund as 'probable' or 'convincing' causally related [6], or with a large body of evidence based on other scientific literature[4,5]). Furthermore, we evaluated risk factors that are currently present in middle-aged women in the Netherlands and only those which can be modified at a later age.

We derived RRs adjusted for confounding factors from meta-analyses[4-7]. Reference levels of exposure were defined per risk factor as the theoretical optimum levels, meaning that the reference levels are zero where possible, or if physiologically impossible, the advised level by (inter)national health guidelines (Table 1).

For overweight/obesity (defined by body mass index (BMI)), physical activity, alcohol and fibre intake, a continuous RR was obtained from the literature, assuming a log-linear association between exposure and risk increase. To match these continuous RRs with categorised risk factor prevalence rates, we calculated new categorical RRs based on the literature-derived continuous RR. These categorical RRs were combined with the mean exposure level within each risk factor category, as observed from the population exposure rates (for an example see footnote Table 1).

The RRs for overweight/obesity and alcohol consumption were derived from meta-analyses by the World Cancer Research fund in 2010, based on 19 and nine cohort studies, respectively[6]. The estimated RR was 1.13 (95% CI: 1.08 to 1.18) per 5 kg/m^2 increase of body mass index (BMI), from the reference BMI category of 20 to 25 kg/m^2.

Table 1 Estimated relative risks for five lifestyle-related risk factor and breast cancer

Risk factor	RR (95% CI)†	Mean level within risk category	Comment	Source
BMI (kg/m^2)			Continuous RR of 1.13 (95% CI: 1.08-1.18) per 5 kg/m^2	World Cancer Research Fund 2010[6]
<25	Reference	21.9 kg/m^2		
25-30	1.15 (1.09-1.21)	27.6 kg/m^2		
>30	1.33 (1.19-1.49)	33.8 kg/m^2		
Physical inactivity (active days/wk)			Days/wk of at least 30 min moderate intensity physical activity.‡ Continuous RR of 1.05 (95% CI: 1.03-1.07) per 2h activity/wk.	Wu et al. 2013[7]
5	Reference	170 min/day§		
3-4	1.06 (1.03-1.08)	152 min/day§	The reference is based on (inter)national guidelines for physical activity[36].	
1-2	1.07 (1.04-1.10)	147 min/day§		
Inactive	1.34 (1.19-1.51)	73 min/day§		
Alcohol (glass/day)			Continuous RR of 1.08 (95% CI: 1.05-1.10) per glass/day	World Cancer Research Fund 2010[6]
Never drinker	Reference	0 glasses/day		
<1	1.05 (1.03-1.06)	0.5 glasses/day		
1-3	1.20 (1.12-1.28)	1.9 glasses/day		
4+	1.64 (1.35-1.97)	5.2 glasses/day		
Smoking			Categorical RR	Gaudet et al. 2013[5]
Never	Reference			
Past	1.09 (1.04-1.15)			
Current	1.12 (1.08-1.16)			
Dietary fibre (g/1000 kJ/day)			Continuous RR of 0.95 (95% CI: 0.91-0.98) per 10 g/day.	Aune et al. 2012[4]
>3.4	Reference	27 g/day	The reference is based on (inter)national recommendations for dietary fibre intake[37,38]	
2-3.4	1.03 (1.01-1.06)	21 g/day		
<2	1.07 (1.03-1.13)	14 g/day		

† Relative risk and 95% confidence interval. ‡ The questionnaire included both occupational and non-occupational activities. § Average number of min/wk were derived from activity diaries filled in by a subsample of participants. Reported activity in the diaries includes all types of physical activity, irrespective of intensity.

NB. For BMI, physical inactivity, alcohol and fibre intake, a continuous RR available from the literature was converted in an RR that matched the mean level of exposure in each risk factor category as observed from the population exposure rates. E.g., based on the literature-derived RR for overweight/obesity of 1.13 per 5 units of increase in BMI, and a mean BMI of 21.9 kg/m^2 in the reference category, 27.6 kg/m^2 in the overweight category, and 33.8 kg/m^2 in the obese category, the risk-category associated RRs compared to the reference are: $1.13^{(27.6-21.9)/5}$=1.15, and $1.13^{(33.8-21.9)/5}$=1.33 (outcome based on the calculation by using exact numbers).

The RR for physical inactivity was derived from a meta-analysis by Wu et al. based on eight studies evaluating moderate plus vigorous recreational activity[7]. A dose-response analysis indicated that the RR for breast cancer was 0.95 (95% CI: 0.93 to 0.97) for every two hours per week increase in moderate and vigorous recreational activity. We, therefore, used the inverse of this continuous RR (1.05, 95% CI: 1.03 to 1.07) to estimate the increase in breast cancer risk per increase of 2 hours of activity/week in contrast to the reference category (i.e., inactive). The RR of smoking was derived from a meta-analysis by Gaudet et al[5]. This meta-analysis was based on 15 cohort studies including 991,100 women, of which 31,198 breast cancer cases. RRs were 1.09 (95% CI: 1.04 to 1.15) and 1.12 (95% CI: 1.08 to 1.16) for past and current smoking, respectively. The RR for fibre intake was derived from a meta-analysis by Aune et al. evaluating 16 prospective studies[4]. The authors estimated a continuous RR of 0.95 (95% CI: 0.91 to 0.98) per increase of 10 grams of fibre intake per day. Studies that were included in the above meta-analyses provided estimates that were adjusted for multiple confounders, including other lifestyle-related risk factors.

Prevalence of exposure

Age-specific prevalence rates of risk exposure were derived from large national surveys or registration databases in 1997[12] and 2000 to 2001[13-15]. Detailed information about these surveys is available in the online supplement.

RESULTS

Prevalence rates

Age-specific prevalence rates of risk exposure, were derived from large national surveys or registration databases in the years 1997[12] and 2000 to 2001[13-15].

The permanent national survey on living conditions and welfare (Dutch acronym: *POLS*) by Statistics Netherlands (CBS) provided information on the exposure prevalence of alcohol use and BMI[13]. The ongoing questionnaire survey started in the year 1997. Data are collected through questionnaires sent out to a random sample of the Dutch population. In the year 2001 the response rate was 60%. BMI was derived from self-reported weight and height (kg/m^2) and classified in normal weight (BMI≤25 kg/m^2), overweight (BMI 25 to 30 kg/m^2) and obese (BMI≥30 kg/m^2), based on 2,136 available questionnaires for females aged >40 years. Alcohol use was estimated in average number of consumptions per week (reported on weekdays and weekends separately) and was based on 1,736 questionnaires. Data on physical activity was collected from 2,898 women via the *OBIN* investigation, a continuous online questionnaire and telephonic survey in a random sample of 11,000 Dutch inhabitants on accidents and physical activity in the Netherlands[14]. Information was collected on number of days per week that women adhered to the prescribed 30 minutes/day of at least moderate intense physical activity, including occupational and non-occupational

activity. Additionally, a sample of *OBIN* participants filled in more detailed activity diaries, from which the mean absolute number of minutes activity per day was estimated for each different level of risk factor exposure.

Prevalence rates of smoking were derived from a continuous national online questionnaire survey on smoking behaviour in 20,000 adults, including 6,590 women in the age category of interest[15]. The questionnaire explored current and past smoking behaviour. Dietary fibre intake was assessed by the Dutch National Food Consumption Survey from 1997 to 1998, which was performed in a random sample of 5,958 Dutch inhabitants. Participants kept a two-day food diary in which they recorded all foods and beverages they consumed. Data were available for 1,462 women above the age 40[12]. Dietary fibre intake was calculated using an application in SAS and the Dutch Food Composition Table (NEVO, 1996). Subsequently, age-specific prevalence rates of dietary fibre intake and the mean dietary fibre intake per defined risk category were determined.

Table 2 presents the prevalence rates of exposure to lifestyle-related risk factors in women >40 years in the Netherlands in 2000 to 2001 and 1997. Of these women, 51% were overweight/obese, which increased with age from 40% to 56% in the ages 40 to 50 and >70 years, respectively. 55% were estimated to be physically inactive (i.e., <5 days/wk 30 min of moderate intensity physical activity). Non-adherence to the national activity guideline also modestly increased with age (i.e., 53% in 40 to 50 years, and 58% in >70 years). Alcohol was regularly consumed by 75% of women. Consumption was less prevalent in older than younger women (61% in >70 years, versus 84% in 40 to 50 years). 42% of women reported to be currently smoking, or smoked in the past, which decreased with an increasing age (54% in women aged 40 to 50 and 28% in women aged >70 years). Dietary fibre intake was below the recommended level in 97% of women, being lowest in women aged 40 to 50 (85%).

Population attributable fraction of postmenopausal breast cancer

The estimated PAFs for the separate and combined risk factors are presented in Table 3. PAFs varied across age categories, as a result of the above described differences in prevalence rates. Overweight/obesity had the highest PAF of 8.8% (95% CI: 6.3 to 11.3) (on average for all age categories). The PAF increased with age, from 7.3% in the ages of 50 to 60, to maximum 10% in women >70 years. Alcohol consumption had the second highest overall PAF of 6.6% (95% CI: 5.2 to 8.0). This PAF decreased with age from 7.4% in 50 to 60 years to 3.9% in >80 years. Physical activity had an average PAF of 5.5% (95% CI: 4.0 to 7.0); ranging from 4.9% in ages 50 to 60, to 7.8% in women >80. Smoking had an average PAF of 4.6% (95% CI: 3.3 to 6.0), which was highest in younger women (i.e., 5.6% in ages 50 to 60), and decreased with age (2.9% in ages >80). Low fibre intake had a PAF of 3.2% (95% CI: 1.6 to 4.8) for all age categories, which was highest in younger women (i.e., 3.7%, ages 50 to 60).

Table 2 Prevalence rates of risk factor exposure among Dutch women per age category (in 2000-2001)

Risk factor	Prevalence (%)				Source
	40-50 y	50-60 y	60-70 y	>70 y	
BMI (kg/m²)					Ongoing national survey on living conditions and welfare
≤25	60	51	43	44	(Dutch acronym POLS)[13]
25-30	30	35	42	41	
≥30	10	14	15	15	
Number of people in the survey†	744	612	440	340	
Physical inactivity‡ (active days/wk)					National survey on accidents and physical activity
5	46	47	44	42	(Dutch acronym OBIN)[14]
3-4	27	28	28	23	
1-2	21	18	19	17	
Inactive	6	6	9	17	
Number of people in the survey	808	845	688	557	
Alcohol (glass/day)					Ongoing national survey on living conditions and welfare
Never drinker	17	18	28	39	(Dutch acronym POLS)[13]
<1	49	44	50	45	
1-3	32	36	36	16	
4+	3	2	2	0	
Number of people in the survey	569	534	368	265	
Smoking					STIVORO, national survey on adult smoking behaviour[15]
Never	46	51	65	72	
Past	18	19	16	13	
Current	36	30	20	15	
Number of people in the survey	2041	1407	1466	1676	
Dietary fibre (grams/day)§					Dutch National Food Consumption Survey
>3.4	15	21	28	23	(Dutch acronym VCP 1997/1998)[12]
2-3.4	54	60	56	64	
<2	31	20	16	14	
Number of people in the survey	579	369	265	249	

NB. The presented numbers are rounded, and may therefore not always add up to 100%.

† BMI: number of people in the survey were calculated by the reported standard error of the prevalence rates.

‡ Active is defined as at least 30 minutes of moderate to vigorous physical activity per day, including occupational and non-occupational activities.

§ prevalence rates of low fibre intake are based on the years 1997-1998.

Table 3 Population attributable fraction (PAF) for five lifestyle-related risk factors and postmenopausal breast cancer

Age at exposure	Age at outcome	Observed cases in 2010†	Risk factor									
			Overweight/obesity (BMI>25 kg/m²)		Physical inactivity		Alcohol consumption		Smoking		Low dietary fibre intake	
			PAF (95% CI)‡	Excess cases	PAF (95% CI)‡	Excess cases	PAF (95% CI)‡	Excess cases	PAF (95% CI)‡	Excess cases	PAF (95% CI)‡	Excess cases
40-50	50-60	3362	7.3%	246	4.9%	164	7.4%	250	5.6%	189	3.7%	124
50-60	60-70	3367	9.1%	305	4.8%	161	7.6%	256	5.0%	169	3.1%	105
60-70	70-80	2016	10.0%	202	5.7%	115	5.8%	116	3.6%	74	2.8%	56
>70	>80	1622	9.9%	161	7.8%	126	3.9%	64	2.9%	47	2.9%	47
	Total	10367	8.8% (6.3-11.3)	913	5.5% (4.0-7.0)	566	6.6% (5.2-8.0)	686	4.6% (3.3-6.0)	479	3.2% (1.6-4.8)	332
			PAF for all five risk factors combined: 25.7% (95% CI: 24.2-27.2)									

† Data from the Dutch national cancer registry[2]

‡ The 95% confidence intervals (95% CI) were derived from Monte Carlo simulations. The presented numbers are rounded, the calculations were performed with the use of exact numbers.

Combined, these risk factors accounted for an estimated 25.7% (95% CI: 24.2 to 27.2) of all 10,367 postmenopausal breast cancer cases in the Netherlands in 2010[2], amounting to 2,665 excess cases (Table 3).

DISCUSSION

We estimated that approximately one out of four postmenopausal breast cancer cases in women aged >50 years in 2010 were attributable to lifestyle factors as present at age 40 and older. Overweight/obesity (8.8%) contributed the most, followed by alcohol consumption (6.6%), physical inactivity (5.5%), smoking (4.6%) and suboptimal dietary fibre intake (3.2%). These estimates were based on comprehensive and up-to-date literature and matched with detailed prevalence rates of risk factor exposure in the Netherlands.

Strengths of our study include detailed data on prevalence of risk factor exposure, allowing us to use continuous RRs that ensured little loss of information. In addition we used RRs which were derived from recent meta-analyses evaluating multiple studies with risk estimates that were adjusted for several confounders, including lifestyle-related risk factors. Furthermore, Monte Carlo simulations were performed to compute 95% confidence intervals for the PAF estimates, incorporating imprecision in RRs and prevalence rates.

However, there are also some limitations. We cannot rule out possible residual confounding which could have influenced our PAF estimates. However, since the literature-derived RRs incorporated in the meta-analyses usually are adjusted for most important confounders, it is unlikely that remaining unmeasured confounders influenced the results considerably. Simulation studies show that estimates which are corrected for major confounders are affected minimally after additional correction for more possible confounders[16]. Nevertheless, measuring lifestyle habits in a valid way is difficult due to measurement errors in assessing the confounders.

Prevalence rates were based on self-reported exposure. Therefore, misclassification (most likely due to underreporting of exposure) may have led to an underestimation of our PAFs. Also, the prevalence rates were measured in a subsample of people wherein response rates were high (60%) but not 100%. Therefore, also participation bias may have affected the results. Furthermore, we included exposure to risk factors from age 40 on only, whereas it is also likely that not only short-term, but also life-long exposure to life-style-related risk factors, or exposure during a critical period of life (e.g., between menarche and first childbirth) contributes to a higher breast cancer risk[17]. However, there is still much uncertainty around the latency period and which period in life is most influential.

In comparable research, hormone replacement therapy (HRT) is often included as a risk factor. Although RRs of 1.10 to 1.66 have been described for current HRT use[18,19], we did not include this factor in our analysis. In 2001, the estimated prescription rate of HRT in women >40 years of age in the Netherlands was 5.6% and dropped to 2.4% in 2004[20].

Current prescription rates are close to zero[21]. As shown by the Million Women study, the increased risk of breast cancer caused by HRT almost disappears after five years of cessation[19], meaning that HRT use (past and current) barely influences breast cancer incidence in the Netherlands anymore.

Attributable fractions of modifiable risk factors for all age breast cancer have been estimated for several countries in Europe, reaching up to 25% in the UK and Germany[22,23]. However, different sets of risk factors were considered, making results difficult to compare. Regarding the whole of Europe, Soerjomataram et al[24], estimated the number of excess, i.e., avoidable breast cancer cases by comparing a countries all-ages incidence rate to the lowest incidence rate in a European country (the baseline incidence rate). For the Netherlands they estimated around 30% of all age breast cancer to be avoidable, which was comparable to their estimates for other Western and Northern European countries, but much higher than estimates for Eastern (i.e., Czech Republic, Romania, Lithuania: up to approximately 5%)) and Southern Europe (i.e., Spain, Portugal: up to approximately 15%)). The authors speculate that this higher incidence rate could be caused by over-diagnosis due to extensive screening programmes and higher exposure to reproduction-linked risk factors. Even though these estimates cannot be directly compared to our PAF numbers, as they used a different methodology, it gives us an idea about the Dutch situation in proportion to the rest of Europe with regard to avoidable cancer cases. And although their number refers to all age breast cancer, it will largely refer to postmenopausal breast cancer as most cases occur after age 50.

We included five lifestyle-related risk factors for postmenopausal breast cancer for which a large body of evidence is available and that occur with substantial prevalence rates in middle-aged women in the Netherlands. Fibre intake and smoking are not, or seldom, considered when estimating PAFs for breast cancer. Since there is emerging strong evidence that these factors increase breast cancer risk, we included these factors and recommend including them in future studies. A recent Canadian study that included smoking as a risk factor, reported a PAF of 3 to 4% based on prevalence rates of risk facture exposure in the years 1994 to 2006[25].

Overweight and obesity, alcohol consumption and physical inactivity are often included in other studies. Considering these three factors, we estimate a combined PAF of around 20%. Similar results were found for neighbouring countries. Parkin et al. estimated that 17% of all breast cancer cases, irrespective of age, in 2011 were attributable to these factors in the UK[22]. Barnes et al. estimated a PAF of 21% for Germany in 2010[23]. However, we observed some differences for the separate risk factors. PAF estimates for a BMI >25 kg/m^2 vary from 2.5% in Germany[23], to 5.6% in France[26] and 8.7% in the UK[27], the latter being comparable to our estimate (8.8%). The attribution of overweight/obesity has previously been computed for the Netherlands. Bergstrom et al estimated a PAF of 6.3% based on a 42% exposure rate in the years 1993 to 1996, and similar RRs as we used[28]. Since the prevalence of overweight/obesity is still increasing in the Western world, the PAF is doing so concordantly.

For alcohol consumption also similar PAFs, ranging from 6.4% to 9.4%, are described in adjacent countries[23,26,29]. But not in other world regions' developed countries as the US and Australia, where PAFs reach up to a maximum of 3%[25,30,31]. Consumption of alcohol by European women is rather high: 75% of Dutch women >40 years drink on a regular basis. For physical inactivity, mainly higher PAF estimates than ours (5.5%) were reported in Europe, of around 10% to 14%[23,26,32], except for the UK (3.4%)[33]. Numbers in the U.S. even rise up to 16%[34]. Differences in prevalence rates largely explain this variation, i.e., in the U.S., 78% of women were considered physically inactive, versus 56% in the Netherlands. Another explanation why estimates vary greatly, could lie in the fact that PAFs are sensitive to differences in risk category definitions with their accompanying RR[35]. Due to the great difficulty of measuring activity levels and determining proper risk categories, other definitions for physical inactivity and RRs are used in literature. Also, we did not incorporate intensity of activities.

Often, success rates of lifestyle modifying programmes are limited. Therefore, for the Netherlands a 25.7% reduction in postmenopausal breast cancer incidence would be the maximum to be achieved, rather than realistic. However, these estimates may help motivating women as well may they inform policy makers about which risk factors should be addressed first.

To conclude, we estimated that approximately one in four postmenopausal breast cancer cases in the Netherlands in 2010 is attributable to five lifestyle-related risk factors. These risk factors are: excess body weight, an inactive lifestyle, alcohol consumption, smoking and low dietary fibre intake.

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EFFECT OF EXERCISE ON INSULIN SENSITIVITY IN HEALTHY POSTMENOPAUSAL WOMEN: THE SHAPE STUDY

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ABSTRACT

Introduction

An inactive lifestyle is a risk factor for several types of cancer. A proposed pathway through which exercise influences cancer risk is via insulin. We aim to investigate the effect of a one-year exercise intervention on insulin sensitivity, and the role of body fat in this association, in healthy, normal to overweight/obese, postmenopausal women.

Methods

In the Sex Hormones And Physical Exercise (SHAPE) study, 189 healthy, inactive and postmenopausal women (ages, 50-69, body mass index (BMI) 22-40 kg/m^2) were randomly assigned to a one-year aerobic and strength exercise intervention (150 min/wk), or a control group. Between group differences in fasting insulin, glucose and homeostatic model assessment of insulin resistance (HOMA2) over time were estimated using linear mixed models.

Results

Follow-up measurements of insulin sensitivity were available for 181 (95.8%) and 182 (96.3%) women at 4 and 12 months, respectively. The intention-to-treat analysis showed no significant differences between the two study groups [treatment effect ratio of the exercise group versus control (β; 95% confidence interval)): insulin, β=1.07 (95% CI: 0.96 to 1.19); glucose, β=1.01 (95% CI: 0.99 to 1.02); HOMA2, β=1.07 (95% CI: 0.96 to 1.20)]. Similar results were found in a per protocol analysis in compliant women, and in a subgroup of women who lost >2% body fat (measured by dual-energy X-ray absorptiometry (DEXA)).

Conclusions

Participation in a one-year aerobic and strength exercise intervention programme did not result in changes in insulin sensitivity in healthy postmenopausal and inactive women.

INTRODUCTION

An inactive lifestyle is a recognised risk factor for several cancers, with the largest body of evidence for colorectal and postmenopausal breast cancer[1,2]. The mechanism through which a lack of physical activity affects cancer risk is not fully understood. A commonly proposed pathway is via a decrease in insulin sensitivity[3].

Physical activity, insulin and cancer are closely linked in a causal network, where energy balance plays a key role[4,5]. Chronic hyperinsulinaemia, as a result of a decreased insulin sensitivity, might influence cancer risk through different mechanisms, including activation of inflammatory mediators, as well as increase of bioavailable insulin-like growth factor-I (IGF-1) and increase of sex hormone levels[6].

Meta-analyses of cohort and case-control studies showed that women with diabetes mellitus have a significant 16% to 23% increase in postmenopausal breast cancer risk[7,8] and a 26% to 35% increased risk of colon cancer[8,9]. In addition, a large case-cohort and nested case-control study found that hyperinsulinaemia is an independent risk factor for breast cancer[10,11].

For long-term and chronic effects on insulin, exercise-induced reduction of body weight and body fat plays an important role. The way by which excess body weight influences cancer risk, mediated by insulin resistance, is complex and multifactorial[12]. Besides insulin mediated, obesity is also an independent risk factor for different cancer types[1,13].

Several exercise intervention studies have investigated the effect of six months to one year endurance and/or strength training on insulin resistance in healthy post-menopausal women[14-21]. Results show that aerobic exercise mainly results in improvements in insulin sensitivity, but not resistance training alone that was investigated in one study[19]. One of the above trials investigated three aerobic interventions varying in intensity and duration[17]. The researchers found that longer total endurance exercise duration resulted in a larger improvement in insulin sensitivity. However, in the above trials, exercisers lost more weight than their controls. After adjusting for weight loss, the exercise effect disappeared in some[15,18,19], but not all studies[16,17,22].

To gain more insight in the pathways whereby physical activity influences cancer risk, and the role of body fat in this association, we studied the effect of a one-year combined aerobic and strength exercise intervention on insulin sensitivity in healthy, normal to overweight/obese, postmenopausal women. We hypothesise that exercise improves insulin sensitivity, and that this association is mainly explained by concordant loss of body fat.

METHODS

The Sex Hormones and Physical Exercise (SHAPE) study is a randomised controlled trial executed in 2006, comparing a one-year exercise intervention to control. The detailed study design is described elsewhere[23]. Primary outcomes of the study were serum sex hormone levels and body composition. In short, the exercise programme resulted in significantly more loss of total and percentage body fat, and waist circumference versus controls, whereas lean mass increased[24]. Sex hormone levels decreased in women who lost more than 2% of body fat, where significant differences between groups were found for androgens, but not for oestrogens[25]. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht. All participants provided signed informed consent. Trial registration: ClinicalTrials.gov NCT00359060.

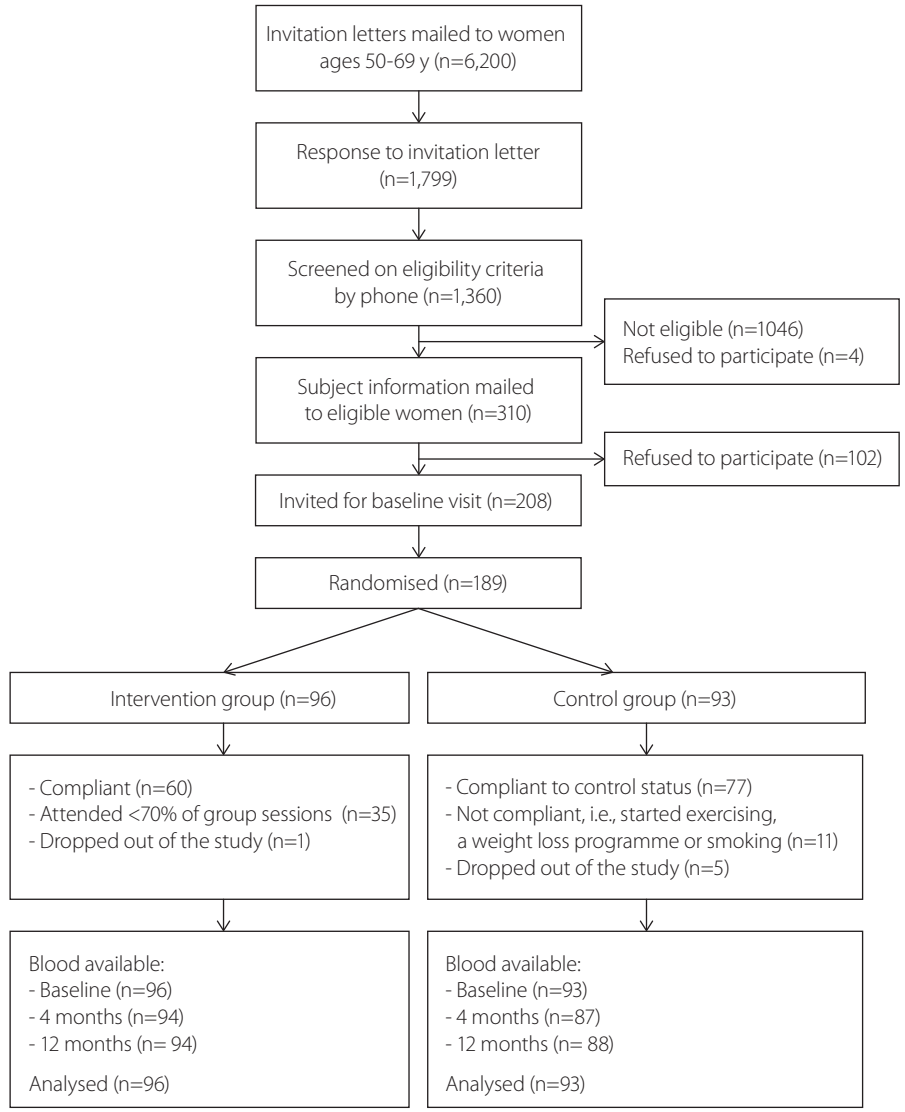
Study participants and randomisation

Study participants were recruited from the general population. Eligible women were of ages 50 to 69 years, postmenopausal and sedentary. Postmenopausal was defined as ≥12 months since last menses. Being sedentary was defined as being engaged in less than two h/wk of moderate or vigorous physical activities. Furthermore, women had to be non-smokers and had to have non-diabetic fasting glucose levels (<7 mmol/l). Main exclusion criteria were having diabetes (type 1 or 2), ever diagnosed with cancer in the 5 years preceding recruitment, and use of exogenous hormones. In total, 189 women were randomly assigned (Figure 1). Randomisation was blocked on two categories of waist circumference (< and ≥92 cm) and was performed by using a computer-generated sequence.

Exercise intervention

The one-year exercise programme comprised 2.5 hours of moderate to vigorous intensity physical activity (average metabolic equivalent (MET) of 7[26]) per week. Women were strictly advised to perform the 2.5 hours of exercise in addition to their usual physical activity pattern. Supervised group sessions of one hour combined aerobic and strength exercise were provided twice a week. In addition, participants were instructed to perform one 30-minute home-based session of individual aerobic exercise. The group sessions were provided in a nearby fitness centre by qualified sports instructors. Fifteen to 20 women were included in one group. Classes started with a 10-minute warming-up and ended with a 5-minute cooling down. Heart rate monitors were worn to ensure an intensity of 60% to 85% of the age-predicted maximum heart rate during the 30-minute aerobic exercise. The 25-minute strength training consisted of sets of eight to twelve repetitions of exercises for each major muscle group. The intensity and number of sets were gradually increased during the study period (See supplementary material). Compliance to the exercise programme was monitored by the sports instructors, who

Figure 1 Flow chart of the inclusion, randomisation, and retention of the Sex Hormone And Physical Exercise (SHAPE) study participants



registered attendance, and by study personnel who visited the exercise sites regularly. Women were asked to record their home based exercise activities (type, duration and average heart rate) in an exercise diary.

Controls were asked to maintain their habitual physical activity level. All participants were asked to maintain their usual diet.

Outcome measures

Study measures were obtained by research nurses at baseline, four and twelve months. Blood samples and anthropometric measurements of body weight, height and waist and hip circumference were taken. Body mass index (BMI) was calculated by weight divided by height squared. Total and percentage body fat were estimated from a total body dual-energy X-ray absorptiometry (DEXA). At each visit, habitual (last year) physical activity was measured by the modified Baecke questionnaire[27], dietary intake by a food frequency questionnaire[28] and medication use was assessed. Current physical activity level was assessed every four months by the Physical Activity Scale for the Elderly (PASE questionnaire, measuring activity pattern in the last 7 days)[29].

Information about socio-demographic characteristics, reproductive factors, medical history, smoking history and past physical activity levels was assessed by questionnaires at baseline only.

Blood sampling

Blood samples were drawn after an overnight fast between 9:00 and 11:00 a.m. and stored at -70 ^{0}C. Participating women were asked not to perform any moderate to vigorous physical activity in the 48 hours preceding the blood sampling. Serum insulin and glucose were determined in the laboratory "Stichting Huisartsenlaboratorium Oost", in Velp, the Netherlands. Laboratory technicians performing the analyses were blinded to the intervention status. All samples of one individual were analysed in the same batch.

Insulin was measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX (DSL-1600)), with a mean intra-assay coefficient of variation (CV) of 9%. Glucose was measured using the hexokinase method (Hitachi High Tech, clinical analyser 7600). Homeostatic model assessment for insulin resistance (HOMA2) is a surrogate measure for whole-body insulin sensitivity, based on insulin and glucose levels[30,31]. We calculated HOMA2 by computer software[32] according to an updated model[33,34]. A decrease in HOMA2 represents a beneficial increase in insulin sensitivity.

Statistical analyses

The SHAPE study was powered on serum oestrone levels that was the primary outcome[23]. For the outcome insulin we had a power of over 80% to detect a between-group difference of up to 2% on a significance level of α=0.05 with the current sample size, based on findings from previous trials[15,16]. Descriptive data are presented as means and standard deviations (SD), medians and range or frequencies and percentages. The primary analysis was performed according to the intention-to-treat principle. Metabolic variables were log-transformed to achieve a normal distribution. Mean changes in insulin, glucose and

calculated HOMA2 between exercisers and controls were computed. Intervention effects were evaluated using linear mixed models for repeated measurements. Mixed models are a suitable technique for repeated measurements over time while it considers within individual correlations.

Insulin, glucose and HOMA2 at four and twelve months were taken as dependent, and study group as key independent variable. Models were adjusted for baseline levels of metabolic factors. Adjustment for baseline leads to equal starting points for both groups, and therefore, the intervention effect over time (including changes from zero to four months and four to twelve months) is presented by the coefficient of study group[35].

Second, a per protocol analysis was performed. Exercisers were considered non-compliant if they missed 30% or more of all group sessions. Non-compliance in control women was defined as having started an exercise programme or a formal weight loss programme.

To investigate if body fat moderates the effect of exercise, we tested the interaction between body fat percentage and study group. Furthermore, we performed an analysis stratified by change in body fat percentage (i.e., <2% and >2% fat loss).

RESULTS

At baseline, women in intervention (n=96) and control (n=93) groups were comparable with respect to age, years since menopause, body composition and alcohol use (Table 1). Despite randomisation, slightly higher baseline levels were observed for BMI, body fat, and education in the control group. Baseline levels of glucose were similar in both study groups (Table 1). However, baseline levels of insulin and HOMA2 were slightly higher in the exercise group.

Six women did not complete the study (3.2%) (one in the intervention group and five controls). Insulin and glucose were available for all women at baseline (n=189), for 181 (95.8%) at four months, and 182 (96.3%) women at twelve months. Overall, 46 (24%) women who completed the trial were non-compliant

Eleven participants in the control group started an exercise or weight loss programme and 35 participants in the intervention group missed 30% or more of all group sessions. The median attendance rate of sports hours in the exercise group was 76%, which is comparable with other exercise intervention trials in older adults[36]. Physical activity level measured by the modified Baecke questionnaire increased in exercisers on average by 6.9 points and 15-MET hours, and by 1.5 points and 1.5-MET hours in control women[37]. Adverse events as a result of the exercise programme were not reported. Body weight did not change in both study groups (-0.66 kg and -0.34 kg in the exercise and control group, respectively). However, declines in body fat (both in kg as in percentage) were significantly greater in the exercise group versus control (0.33 kg, 95% confidence interval (CI), 0.66 to

Table 1 Baseline characteristics of the study population

	Exercise Group (n = 96)	Control Group (n = 93)
	Mean (standard deviation) / Median (range)	
Age (years)	58.9 (4.6)	58.4 (4.2)
Years since menopause	8.9 (6.3)	9.9 (6.2)
Weight (kg)	73.6 (8.2)	74.8 (10.8)
Height (cm)	165.5 (6.5)	166.4 (6.0)
Body mass index (kg/m2)	26.6 (2.9)	27.3 (3.6)
Fat mass (kg)	28.3 (5.7)	29.9 (8.0)
Body fat (%)	39.8 (4.5)	40.9 (5.8)
Alcohol (g/d)	7.5 (0.0-53.9)	5.3 (0.0-75.2)
Total energy intake (kJ/d)	7,818 (1,946)	8,096 (1,788)
Physical activity (MET h/wk)[a]	4.9 (0.0-120.0)	4.3 (0.0-70.7)
Education	*number (%)*	
Primary school	5 (5)	5 (5)
Technical/professional school	29 (30)	29 (31)
Secondary school	38 (40)	20 (22)
Academic degree	24 (25)	39 (42)
	Median (interquartile range)	
Insulin (µU/ml)	6.68 (4.78-9.60)	7.39 (5.25-10.23)
Glucose (mmol/l)	5.22 (4.94-5.48)	5.11 (4.88-5.44)
HOMA2	0.76 (0.54-1.11)	0.84 (0.58-1.17)

Insulin, n=189 (100%); glucose, n=181 (95.8%); HOMA2, n=181 (95.8%).
MET=metabolic equivalent. HOMA2=homeostatic model assessment of insulin resistance

0.005 and 0.43%, 95% CI 0.74 to 0.13[24]. Furthermore, lean mass increased significantly in the exercise group and was higher when compared with the control group that showed a slight decrease (difference in lean mass between exercise and control of +0.31 kg, 95% CI: 0.11–0.50)[24].

The intention-to-treat analysis (Table 2) showed no significant differences in changes in the one-year study period in insulin, glucose or HOMA2 levels between intervention and control participants (treatment effect ratio (β; 95% CI) of exercisers versus control of β=1.07 (95% CI: 0.96 to 1.19), β=1.01 (95% CI: 0.99 to 1.02) and β=1.07 (95% CI: 0.96 to 1.20), respectively; Table 2).

The per protocol analysis showed similar results and did not show significant differences between the study groups either (Table 3).

Table 2 Means and changes in insulin, glucose, and HOMA2 in exercisers versus controls

	Geometric mean			% change 0-4 months	% change 0-12 months	β[†] (95% CI)
	Baseline	4 months	12 months			
Insulin (µU/ml)						
Control	7.06	6.65	6.79	-5.75	-3.81	1.07 (0.96 to 1.19)
Exercise	6.74	7.05	6.76	4.66	0.39	
Glucose (mmol/l)						
Control	5.20	5.17	5.17	-0.39	-0.58	1.01 (0.99 to 1.02)
Exercise	5.20	2.53	5.18	0.58	-0.29	
HOMA2						
Control	0.80	0.75	0.77	-5.84	-3.97	1.07 (0.96 to 1.19)
Exercise	0.76	0.80	0.77	4.76	0.36	

NOTE: Measures were available for all women at baseline (n=189). Data were missing for 8 women at four months, and for 7 women at 12 months.
[†] The β (95% CI), is the treatment effect ratio representing the overall intervention effect on metabolic variable (adjusted for baseline), including changes from baseline to 4 months and 4 to 12 months. Because metabolic variables were log-transformed for the analysis, the regression coefficient is the antilogarithm of the original coefficient. Therefore, the antilogarithm of the coefficient is a ratio that indicates whether the hormone level is, on average, higher in the intervention group compared with controls (>1) or lower (<1); e.g., 1.01 indicates that the metabolic variable is on average 1% higher in the intervention group compared with controls.
HOMA2=homeostatic model assessment of insulin resistance

No significant interaction was found between body fat percentage and study group for insulin, glucose and HOMA2. In women who lost more than 2% of body fat, levels of all three metabolic factors declined in both study groups (Table 4), however, not different between the study groups (insulin (β=1.02, 95% CI: 0.87 to 1.19); glucose (β=1.02, 95% CI: 0.99 to 1.04); HOMA2 (β=1.02, 95% CI: 0.88-1.19)). In the group of women who did not lose body fat, also no significant effects were found.

DISCUSSION

In the SHAPE study, we did not observe favourable effects on insulin, glucose and HOMA2, known to be associated with cancer risk, of a one-year aerobic and strength exercise intervention in healthy and sedentary postmenopausal women. A per protocol analysis in women who adhered to the study programme, and a subgroup analysis in women who lost more than 2% of body fat, showed similar results.

Our null results on glucose are in line with other exercise intervention studies[16-18,38]. However, our results on insulin and HOMA2 are contradictory with our hypothesis that exercise would lower insulin levels and, thereby, decrease cancer risk. Several other studies

Table 3 Means and changes in insulin, glucose, and HOMA2 in women adherent to the study protocol

	Geometric mean			% change 0-4 months	% change 0-12 months	β† (95% CI)
	Baseline	4 months	12 months			
Insulin (μU/ml)						
Control	6.95	6.64	6.80	-4.51	-2.21	1.06 (0.93 to 1.20)
Exercise	6.70	7.18	6.64	7.12	-0.96	
Glucose (mmol/l)						
Control	5.19	5.18	1.15	-0.14	-0.64	1.01 (0.99 to 1.03)
Exercise	5.25	5.30	5.23	0.98	-0.35	
HOMA2						
Control	0.79	0.75	0.77	-4.57	-2.42	1.06 (0.93 to 1.21)
Exercise	0.76	0.82	0.76	7.33	-0.95	

In total, 137 women were considered in this analysis (n=60 in the intervention, and n=77 in the control group). Follow-up data of three women in the control group were missing.
Adherence is defined as >70% presence in sports classes for the exercise group, and not started a formal exercise or weight loss programme in the control group.
† The β (95% CI) is the treatment effect ratio representing the overall intervention effect on metabolic variable (adjusted for baseline), including changes from baseline to 4 months and 4 to 12 months. Because metabolic variables were log-transformed for the analysis, the regression coefficient is the antilogarithm of the original coefficient. Therefore, the antilogarithm of the coefficient is a ratio that indicates whether the hormone level is, on average, higher in the intervention group compared with controls (>1) or lower (<1); e.g., 1.01 indicates that the metabolic variable is on average 1% higher in the intervention group compared with controls.
HOMA2=homeostatic model assessment of insulin resistance

found statistically significant effects of exercise on insulin and HOMA levels. Possible explanations for these differences could be that the dose of exercise in the SHAPE study was not high enough and/or that the exercise effect on insulin sensitivity is dependent on concordant (substantial) weight loss, which was not aimed and achieved in our trial.

Comparable trials, where exercise programmes induced substantial weight loss in postmenopausal women, suggest that weight loss is important for favourable effects on insulin resistance[15,16,18,21,38-40]. For example, in the NEW trial, effects of weight loss induced by diet, exercise, or a combined programme versus controls were investigated in an overweight study population (n=439). The exercise-only group (225 min/wk vigorous aerobic exercise) failed to lose a significant amount of body fat (-3.3%) and weight (-2.4%) in contrast to the diet and combined intervention groups[18]. Subsequently, the exercise-only group showed no changes in insulin, glucose or HOMA whereas both other intervention groups did. Furthermore, compared with diet alone, the combined diet plus exercise group had no additional favourable effect on these measures of insulin sensitivity[18]. Another weight loss trial, the CALERIE study, induced substantial and equal weight loss

Table 4 Means and changes in insulin, glucose, and HOMA2 in women with >2% fat loss

	Geometric mean			% change 0-4 months	% change 0-12 months	β† (95% CI)
	Baseline	4 months	12 months			
Insulin (μU/ml)						
Control	6.83	6.38	6.67	-6.51	-2.36	1.02 (0.87-1.19)
Exercise	7.44	7.32	6.88	-1.55	-7.49	
Glucose (mmol/l)						
Control	5.26	5.15	5.14	-2.13	-2.35	1.02 (0.99-1.04)
Exercise	5.19	5.20	5.15	0.15	-0.71	
HOMA2						
Control	0.78	0.72	0.75	-6.91	-2.92	1.02 (0.88-1.19)
Exercise	0.84	0.83	0.78	-1.50	-7.57	

In total, 69 women were considered in this analysis (n=39 in the intervention and n=30 in the control group). Data were missing for 2 women (1 control, 1 intervention) at 4 months, and for 1 woman (intervention) at 12 months.
† The β (95% CI), is the treatment effect ratio representing the overall intervention effect on metabolic variable (adjusted for baseline), including changes from baseline to 4 months and 4 to 12 months. Because metabolic variables were log-transformed for the analysis, the regression coefficient is the antilogarithm of the original coefficient. Therefore, the antilogarithm of the coefficient is a ratio that indicates whether the hormone level is, on average, higher in the intervention group compared with controls (>1) or lower (<1); e.g., 1.01 indicates that the metabolic variable is on average 1% higher in the intervention group compared with controls.
HOMA2=homeostatic model assessment of insulin resistance

(approximately 9.5%) in both a caloric restriction and exercise group, in 48 older men and women during 12 months[21]. Women in the caloric restriction group were prescribed a diet with a deficit of 20% of total calories and women in the exercise group were prescribed 90 minutes of exercise daily, corresponding to an energy deficit of 20%. Insulin improved equally in both groups.

These, and our findings, suggest that changes in insulin sensitivity may seem to result largely from concurrent changes in body weight/fat instead of directly from physical activity. The fact that in our study insulin and glucose levels improved more, however not significantly, in the subgroup of women who lost more than 2% of body fat compared with women who did not lose fat mass, also supports this hypothesis that weight loss is necessary for long-term beneficial exercise effects on metabolic factors. However, also some trials found an exercise effect independent of weight loss[16,17,20,22].

So far, it is not evident which exercise dose is optimal for insulin improvement and cancer risk[3]. Although some epidemiological studies suggest that exercise intensity (i.e., vigorous versus light to moderate) is the determinant for causing effects on insulin[41,42], others provide evidence that total time spent in physical activity is most important[15-17,43-45]. Houmard and colleagues[17] investigated exercise effects on insulin sensitivity in 154 postmenopausal women who were randomised to three groups of exercise dosages,

differing in intensity, volume and duration. Groups included exercise programmes of a low-volume/moderate intensity/170 min/wk, a low-volume/high-intensity/115 min/wk and a high-volume/high-intensity/170 min/wk. The relative increases in insulin sensitivity were greatest in the exercise groups who spent the most time being physically active (i.e., 170 versus 115 min/wk). Hence, the authors concluded that the total exercise duration rather than intensity is the most important factor in influencing insulin levels. These effects were also seen when change in body mass was made equivalent across all exercise groups. In addition, a secondary analysis in a large exercise intervention study, the ALPHA trial, found a significant linear dose-response relationship between exercise duration and improvement of insulin and HOMA levels in women who exercised more than 150 min/wk[16]. Similarly, a comparable trial reported positive findings on insulin in women who exercised 130 to 190 min/wk, but not in women who spent less time exercising[15]. These results suggest that the effects on insulin resistance are dose-dependent. In the SHAPE study, women exercised 150 min/wk, of which 120 minutes were supervised. Therefore, the second explanation for our null results on insulin sensitivity could be that the total time of exercise in our study was too low to enhance effects.

More pathways whereby physical activity may influence cancer risk have been hypothesised, as via endogenous sex hormones, adipokines, and inflammatory markers[3]. Regarding breast cancer risk, in a previous analysis of the SHAPE study, we found effects on sex hormones in women who also lost >2% of body fat. Thus, even though no effect was observed on insulin sensitivity, we can conclude that the intervention influenced breast cancer risk via other biomarkers. Although it is likely that these pathways interact at a certain level, changes in some biomarkers can be observed irrespective of the lack of changes in other outcomes.

Strengths of this study include the relatively large study population and the substantial contrast in the level of physical activity between the intervention and control group that was achieved after 12 months[37]. The combined aerobic and strength training comprised an exercise level achievable by postmenopausal women. Furthermore, the comprehensive measurement of body composition using dual-energy X-ray absorptiometry allowed us to address the effect of changes in insulin sensitivity in relation to body fat.

There were also some limitations in our study. The exercise programme included home-based training sessions of 30 min/wk, making it more difficult to achieve adherence. Participants of our trial were postmenopausal women, the stage of life with the highest (breast) cancer risk. However, a rather healthy selection was recruited, including women with normal body weights and non-diabetic fasting glucose at baseline. Achieving substantial effects in these women in both body weight as insulin levels is rather difficult, because there is little room for improvements. Furthermore, women in the control group also lost a modest amount of weight (-0.34 kg) and their dietary intake decreased during the study compared with the exercise group (-445 kJ/day (-106 kcal) versus -27 kJ/day (-6 kcal))[24] that might have influenced our findings. Furthermore, insulin sensitivity was

estimated by glucose, insulin and HOMA2, which are alternatives for the reference test: hyperinsulinemic-euglycemic clamp[46]. However, HOMA2 is proven to be a good measure of insulin sensitivity in epidemiologic research[34] since the reference test is usually not feasible in these studies.

In conclusion, we did not find effects of a one-year combined aerobic and strength exercise intervention on insulin sensitivity and fasting glucose, in a population of healthy and sedentary, postmenopausal women. Possible explanations are that the exercise dosage was not high enough or the effect of exercise on insulin sensitivity depends on substantial concurrent weight loss. Future intervention studies are needed to give more insight in the optimal dosage of exercise and possible additional effects of exercise when weight loss is reached, on insulin sensitivity and subsequent cancer risk.

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Author contributions

Willemijn A.M. van Gemert carried out data analyses, interpreted data and drafted the manuscript. Evelyn M. Monninkhof participated in the design of the study, performed data collection, and critically revised the data analyses and manuscript. Anne M. May critically revised the manuscript. Petra H.M. Peeters and Albertine J. Schuit participated in the study design, were involved in data collection and critically revised the manuscript.

Exercise programme of the SHAPE study

The supervised group sessions (two hours per week) contain either aerobic training or spinning, combined with strength exercises. The 30-minutes home-based individual programme exists of aerobic exercise. Intensity is gradually increased during the one-year study period, which is outlined in Tables 1 to 4 below.

Supplementary Table 1 Schedule for supervised group sessions

	time (min)	Intensity (% HRmax)			
		Week 1-4	Week 5-10	Week 11-13	Week 14 +
Warming-up	10 min	50-65%	50-65%	50-65%	50-65%
Endurance training	20 min	65-70%	70-80%	20 min 70-85%	20 min 70-85%
Strength training	25 min	Run-in	See Table 3a-3d		
Cooling down	5 min				

HRmax: maximum heart rate, based on the age-predicted maximum heart rate: 220-age.
Run-in strength training: the focus of the strength training in the first four weeks is on instructions and proper technical execution of the exercises.

Supplementary Table 2 Home-based individual aerobic exercise sessions

	time (min)	Intensity (% HRmax)	
		Week 5-13	Week 14+
Warming-up	10 min	60-65%	60-65%
Endurance training	15 min	65-70%	70-80%
Cooling down	5 min	50-60%	50-60%

HRmax: maximum heart rate, based on the age-predicted maximum heart rate: 220-age.

Supplementary Table 3 a-d. Schedules for strength training exercises throughout different study phases

Supplementary Table 3a Strength training exercises (week 5-10)

Muscle groups	Exercise	Intensity
Back	Rowing	1x 20-25 reps, 60% 1RM
Legs (hips, glutes and thighs)	Lunges	1x 20-25 reps, 60% 1RM
Shoulder	side-raises	1x 20-25 reps, 60% 1RM
Legs (hips, glutes and thighs)	Squat	1x 20-25 reps, 60% 1RM
Arms (Biceps)	Biceps curl	1x 20-25 reps, 60% 1RM
Abdomen	Crunch	1x 30-40 reps, 50% 1RM

Supplementary Table 3b Strength training exercises (week 11–18)

	Exercise	Intensity
Chest	Bench press	1x 20-25 reps, 60% 1RM
Back	Rowing	1x 15-20 reps, 60% 1RM
Shoulder	Side raise	1x 15-20 reps, 60% 1RM
Back	Deadlift	1x 15-20 reps, 60% 1RM
Legs (hips, glutes and thighs)	Lunges	1x 20-25 reps, 60% 1RM
Legs (hips, glutes and thighs)	Squat	1x 20-25 reps, 60% 1RM
Arms (biceps)	Biceps curl	1x 20-25 reps, 60% 1RM
Arms (triceps)	Triceps extensie	1x 20-25 reps, 60% 1RM
Abdomen	Crunch (Mini) Hoover	1x 30-50 reps 1 to 2 minutes

Supplementary Table 3c Strength training exercises (week 19-26)

	Exercise	Intensity
Chest	Bench press	2x 15-20 reps, 70% 1RM
Back	Rowing	2x 15-20 reps, 60% 1RM
Shoulder	Shoulder press	1x 15-20 reps, 60% 1RM
Back	Deadlift	2x 15-20 reps, 60% 1RM
Legs (hips, glutes and thighs)	Lunges	2x 20-25 reps, 60% 1RM
Legs (calves)	Calf-raises	1x 20-25 reps, 60% 1RM
Legs (hips, glutes and thighs)	Squat	2x 20-25 reps, 60% 1RM
Arms (biceps)	Bicepscurl	1x 20-25 reps, 60% 1RM
Arms (triceps)	Triceps extensie	1x 20-25 reps, 60% 1RM
Abdomen	Crunch Hoover	1x 30-50 reps, 50% 1RM
Lowerback	Lowerback extension	1x 20-30, 60% 1RM

Supplementary Table 3d Strength training exercises (week 27+)

	Exercise	Intensity
Chest	Bench press	2x 10-15 reps, 80% 1RM
Legs (hips, glutes and thighs)	Lunges	2x 15-20 reps, 70% 1RM
Back	Rowing	2x 10-15 reps, 80% 1RM
Legs (calves)	Calf-raises	1x 15-20 reps, 70% 1RM
Shoulder	Shoulderpress	1x 10-15 reps, 80% 1RM
Legs (hips, glutes and thighs)	Squat	2x 15-20 reps, 70% 1RM
Back	Deadlift	2x 15-20 reps, 80% 1RM
Abdomen	Crunch	1x 30-50 reps, 50% 1RM
Arms (biceps)	Bicepscurl	1x 15-20 reps, 70% 1RM
Lower back	Lower back extension	1x 20-30 reps, 60% 1RM
Arms (triceps)	Triceps extension	1x 15-20 reps, 70% 1RM

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4

DESIGN OF THE SHAPE-2 STUDY: THE EFFECT OF PHYSICAL ACTIVITY, IN ADDITION TO WEIGHT LOSS, ON BIOMARKERS OF POSTMENOPAUSAL BREAST CANCER RISK

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ABSTRACT

Background

Physical inactivity and overweight are two known risk factors for postmenopausal breast cancer. Former exercise intervention studies showed that physical activity influences sex hormone levels, known to be related to postmenopausal breast cancer, mainly when concordant loss of body weight was achieved. The question remains whether there is an additional beneficial effect of physical activity when weight loss is reached.
The aim of this study is to investigate the effect attributable to exercise on postmenopausal breast cancer risk biomarkers, when equivalent weight loss is achieved by exercise compared to diet-induced weight loss.

Design

The Sex Hormones And Physical Exercise (SHAPE)-2 study is a three-armed, multicentre trial. 243 inactive, postmenopausal women who are overweight or obese (BMI 25-35 kg/m^2) are enrolled. After a four to six-week run-in period, wherein a standardised diet is prescribed, women are randomly allocated to (1) a diet group, (2) an exercise group or (3) a control group. The aim of both intervention groups is to lose an amount of 5 to 6 kg body weight in 10-14 weeks. The diet group follows an energy restricted diet and maintains the habitual physical activity level. The exercise group participates in a 16-week endurance and strength training programme of four hours per week. Furthermore, they are prescribed a moderate caloric restriction. The control group is asked to maintain body weight and continue the run-in standardised diet.
Measurements include blood sampling, questionnaires, anthropometrics (weight, height, waist and hip circumference), maximal cycle exercise test (VO_{2peak}), DEXA-scan (body composition) and abdominal MRI (subcutaneous and visceral fat). Primary outcomes are serum levels of oestradiol, oestrone, testosterone and sex hormone binding globulin (SHBG).

Discussion

This study will give insight in the potential attributable effect of physical activity on breast cancer risk biomarkers and whether this effect is mediated by changes in body composition, in postmenopausal women. Eventually this may lead to the design of specific lifestyle guidelines for prevention of breast cancer.

Trial registration: clinicaltrials.gov, NCT01511276

BACKGROUND

There is strong evidence that physical inactivity is associated with a higher postmenopausal breast cancer risk[1,2]. In contrast to most other risk factors, physical activity provides an opportunity for primary prevention.

The causal pathway through which exercise influences breast cancer risk is hypothesised to be predominantly hormone mediated, i.e., metabolic and sex hormones[3]. The evidence that oestrogens (endogenous as well as exogenous) contribute to breast cancer risk is strong and widely accepted[4-6]. Postmenopausal women with elevated levels of androgens also showed increased risk of developing breast cancer, even after adjustment for oestrogens[5-7]. A low level of physical activity has been associated with higher serum concentrations of sex hormones in postmenopausal women in many cross-sectional observational studies[7-14], but not in all[15,16]. Physical activity might also influence postmenopausal sex hormones by increasing levels of sex hormone binding globulin (SHBG), resulting in lower amounts of unbound (free) active oestrogens and androgens in the circulation[10,11,14,17].

The beneficial effect of exercise on breast cancer related biomarkers might be partly explained by exercise-induced fat loss and prevention of becoming overweight or obese. Observational studies show associations between body mass index (BMI) and oestrogen levels in postmenopausal women[8-11]. Compared with normal-weight women, obese postmenopausal women have higher blood concentrations of oestrogens[13] and lower concentrations of SHBG resulting in increased levels of free oestradiol[14-16,18]. The association between BMI and androgens is less clear, i.e., cross-sectional studies reported conflicting results[10,19-21].

In our previous SHAPE trial we found that in the exercise group, reductions of sex hormone levels mainly occur when concordant loss of body fat was achieved[22]. These findings were in line with results from a comparable exercise intervention study[23,24]. Another exercise intervention study[25], however, found an overall intervention effect of exercise on sex hormones, which might be explained by the fact that in this study the overall difference in weight reduction between the intervention and control group was much greater compared to the earlier trials. A fourth trial investigating the effects of dietary, exercise and combined weight loss interventions, found that greater weight loss produced stronger effects on oestrogens and SHBG[26].

The question remains whether the beneficial effect of physical activity on breast cancer risk is fully explained by the accompanied weight loss, or whether physical activity has an additional positive effect on hormones.

Therefore, we set out to study the effect of weight loss mainly driven by exercise compared to equivalent weight loss driven by a diet only, on breast cancer risk biomarkers. Furthermore, we are specifically interested whether weight loss due to physical exercise induces greater amounts of fat loss (total and abdominal) and subsequently results in

stronger favourable effects on relevant hormones compared with equivalent diet-induced weight loss.

METHODS AND STUDY DESIGN

The aim of the Sex Hormones And Physical Exercise (SHAPE)-2 study is to investigate the effect attributable to exercise on postmenopausal breast cancer risk biomarkers, when equivalent weight loss is achieved by exercise compared to diet-induced weight loss. The secondary aim is to study the effects of equivalent weight loss achieved with or without exercise, on body composition and fat distribution and whether this mediates effects on sex hormone levels.

The SHAPE-2 study is designed as a three-armed, randomised controlled trial. The study programme runs in eight municipalities surrounding two research centres in the middle (Utrecht) and the east (Enschede) of the Netherlands. The total study duration for each study participant is about 21 weeks. After a four to six-week run-in period, eligible women are randomly allocated to (1) a diet group; (2) an exercise group or (3) a waiting list control group. Both intervention groups have the aim to lose an amount of 5 to 6 kg of bodyweight in 10 to 14 weeks. The intervention period is followed by a weight maintenance period lasting at least two weeks.

The study protocol is approved by the Medical Ethics Committee of University Medical Center Utrecht, in accordance with the Helsinki declaration, before the start of data collection.

Study population

In total 243 postmenopausal women, aged 50-69, were included. Eligible women are overweight or obese (BMI 25-35 m/kg^2), have an inactive lifestyle and live in the middle or east of the Netherlands.

Postmenopausal state is defined as natural cessation of menses for at least 12 months, or in case of hysterectomy: aged 55+ and likely to be postmenopausal based on their medical history. Inactive is defined as less than two hours of moderate-to-vigorous physical activity per week (≥4 metabolic equivalents (MET))[27]. Energy expenditure from occupational activity (except for highly active jobs e.g., courier, sports instructor), walking at moderate pace and cycling as a transport medium (<16 km/hour) are not considered. In case of doubt, individuals are discussed in the study team. Exclusion criteria are factors that either interfere with endogenous sex hormone levels or successful completion of the diet or exercise intervention (Table 1).

Recruitment and screening

Study participants are mainly recruited by invitation letters explaining the study goals and inclusion criteria. These letters are sent to a random selection of female inhabitants (aged

Table 1 SHAPE-2 study in- and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Female	Presently using sex hormones
50 to 69 years of age	Use of beta-blockers or oral corticosteroids
Postmenopausal (last menses >12 months)	Smoking,
BMI 25-35 m/kg^2	Alcohol or drug abuse
Inactive lifestyle (<2 h/wk of at least moderately intensive activities (>4 MET))	Diagnosed breast cancer (present or history) , or other cancer types (present or <5 years of history), except non-melanoma skin cancer
Willing to be randomly assigned to one of the three study arms	Diabetes mellitus or other (unstable) endocrine related diseases
Informed consent for all screening and study activities	Any disorder that might impede participation in the exercise programme
	Following, or intention to follow, a structured weight loss programme elsewhere
	Investigators opinion (successful fulfilling of the programme is highly unlikely)

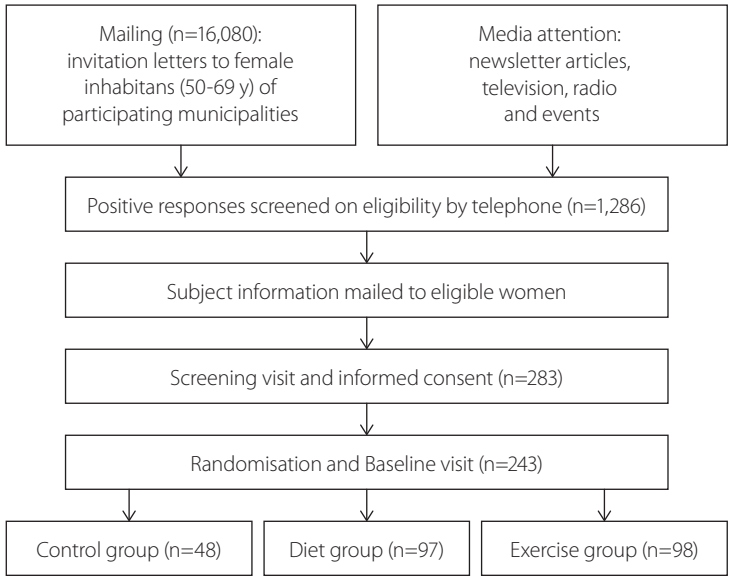
BMI=Body mass index. MET=metabolic equivalent.

50-69, Dutch nationality) of the participating municipalities: Zeist, Bilthoven, Utrecht, Nieuwegein, Houten, IJsselstein, Enschede, and Oldenzaal. Furthermore, we aim to publish articles in local newspapers including calls for participants. Responding women are contacted by phone by a study nurse to provide more information and to further assess eligibility. Potential candidates are invited for a screening visit at the research unit in their region where informed consent is signed and BMI and glucose (ACCU-CHEK® Aviva) are checked. Additionally, motivation and physical ability to perform the exercise programme are discussed. If eligible, the participant is scheduled for the study dietitian and starts with the run-in period. See Figure 1 for the flow chart of the recruitment and inclusion procedure. All participating women gave informed consent before start of the study.

Standardised diet during run-in period

During the four to six-week run-in period, a standardised diet is prescribed which resembles the habitual intake of the participant and is in accordance with the Dutch Guidelines for a Healthy Diet[28] (50-60% carbohydrate, 15-20% protein and 20-35% fat). The energy content of the standardised diet is determined using the individual's habitual energy intake (dietary history), body weight history and a calculated estimate using the Harris & Benedict formula[29] multiplied by an estimate of their Physical Activity Level (PAL). Special attention is paid to alcohol intake (maximum of one drink per day) and fibre intake (minimum of 25 grams per day) since these may influence sex hormone levels[30-32]. The

Figure 1 Flow-chart recruitment and inclusion procedure



run-in period and the standardised diet aim to normalise the dietary pattern, stabilise body weight, check the estimated energy requirements and evaluate protocol adherence. During the run-in, adherence to the programme is monitored by filling out a three-day food diary, weekly self-weighing and telephone contacts with the dietitian (Table 2).

Randomisation and Intervention

After successful completion of the run-in period, subjects are randomised to (1) a diet group; (2) an exercise group or (3) a waiting list control group. Randomisation is performed via a computer-generated sequence, stratified per municipality, in block sizes of five (ratio interventions vs. control; 2:2:1).

The goal of both intervention programmes is to lose an equivalent amount of 5 to 6 kg of body weight, in 10-14 weeks' time. The weight loss interventions are supervised by dietitians and physiotherapists, established in each participating municipality.

Body weight is closely monitored in both intervention groups by continuation of weekly self-weighing. Supervised weighing, by the dietitian (at every visit) and physiotherapist (weekly), is performed in addition. Participants, whose weekly weight loss do not meet or exceed the 0.5 kg/wk loss for 3 consecutive weeks, receive extra coaching to adapt their diet or exercise level.

If the weight loss goal is reached, or after a maximum of 14 weeks, a weight maintenance period (two to six weeks) starts in which energy intake and energy expenditure is balanced

by dietary adaptations. The goal of this maintenance period is to establish stable weight in order to obtain stable levels of sex hormones.

Diet group: weight loss induced by diet only

The weight loss intervention is delivered by registered dietitians, experienced in treatment of overweight and motivational interviewing. Motivational interviewing is a client-centred counselling approach which is a proven effective method used to increase motivation and to establish behaviour change[33,34].

Frequent contacts with the dietitian are scheduled (see Table 2 for an overview of the study programme). After randomisation, women individually meet their dietitian for the prescription of a calorie restricted diet. The diet has a deficit of 500 kcal/day as compared to the individuals energy requirements estimated at the run-in period. The diet is composed of the same proportions of macronutrients as the standardised diet conform National Guidelines for a Healthy Diet[28]. Additionally, five one-hour interactive group sessions are planned (maximum 12 women/group). The programme for these sessions is based on principles of cognitive behavioural therapy[35] and motivational interviewing[34] and consists of nutrition education, behaviour change techniques and self-management training. Adherence to the programme is monitored by completing a three-day food diaries and frequent telephone contacts with the dietitian (Table 2). Women in the diet group are requested to maintain their habitual physical activity level.

Exercise group: weight loss mainly induced by exercise

Women randomised to the exercise group are enrolled in a 16-week exercise training programme, delivered by physiotherapists. Additionally, a moderate caloric restriction of 250 kcal/day is prescribed by a dietitian in an individual session. From recent literature, we know that achieving and maintaining a body weight reduction by exercising in untrained and obese women is a long-term process and a goal hard to attain[36,37]. Compensatory mechanisms both physically and mentally, and behavioural reasons withhold the person from losing weight adequately[38,39]. We, therefore, decided to study the effect of exercise in combination with a slight diet energy deficit.

The prescribed diet is monitored by regular telephone contacts with the dietitian. In this group, main emphasis is placed on the exercise programme, which contains four hours of moderate-to-vigorous exercise per week in group- and individual sessions. The estimated energy expenditure of the exercise programme is approximately 350 kcal/day, based on corresponding MET rates[27]. For our specific study population, we corrected METs for age[40,41].

The exercise protocol contains both endurance and strength training. Levels of exercise intensity are gradually increased during the study programme. Intensity of strength training is determined by pragmatic 20- and 15-repetition maximum (RM) tests, performed several times throughout the 16 weeks to adapt the resistance.

Table 2 Overview study programme, contact moments and measurements

	RUN-IN PHASE						INTERVENTION PHASE														MAINTENANCE	
week	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diet group																						
- diet composition	STANDARDISED DIET						ENERGY RESTRICTED DIET (-500 kcal/day)															
- dietary counselling†	F		T		T		F	G	T	G	T	G	T		T	G	T			F, G		T
Exercise group																						
- diet composition	STANDARDISED DIET						ENERGY RESTRICTED DIET (-250 kcal/day)															
- dietary counselling†	F		T		T		F		T		T		T		T		T			F		T
- exercise programme‡							AEROBIC AND STRENGTH TRAINING (4 hours/week)															
							E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
Control group																						
- diet composition	STANDARDISED DIET						STANDARDISED DIET															
- dietary counselling†	F		T		T		T				T											T
MEASUREMENTS§																						
- Habitual dietary intake (dietary history)	X																					
- Actual dietary intake (3-day food records)				X						X											X	
- Habitual physical activity (questionnaires)							X															X
- Actual physical activity (accelerometer)	X																				X	
- Anthropometrics (weight, waist, hip circumference); body composition (DEXA); visceral and subcutaneous abdominal fat (MRI); fitness (maximal exercise test); blood pressure; sex hormones (blood sampling)							X															X

† dietary counselling: F= face-to-face individual counselling; T= individual counselling by telephone; G= group counselling session

‡ exercise programme: E= group fitness and Nordic walking, 4 hours/week.

§ measurements: X=all participants

Intensity of endurance training is determined by the heart rate reserve based on the guidelines of the American College of Sports Medicine, adapted for older women[42]. Target heart rates are based on results of a maximal exercise test and calculated by the formula: [intensity%*(maximum heart rate – resting heart rate)] + resting heart rate. Study participants wear heart rate monitors while exercising and receive a badge with the individuals' calculated target heart rate zones for a range of training intensities. During every training session, subjects fill in an exercise log which is used as a tool to adhere to the protocol and for monitoring by the physiotherapist.

Group Exercise

Twice a week, subjects participate in a standardised one-hour group session, facilitated by a physiotherapist. Groups consist of five to six women. The group exercise sessions include 20-25 minutes high intensity endurance training combined with 25 minutes strength training. Classes start and end with a 5-10 minute warming up and cooling down, respectively.

Endurance training is performed in circuits on several exercise machines, e.g., a treadmill, cycle or cross-trainer. Intensity is gradually increased (Table 3).

The standardised strength training protocol includes exercises for the major muscle groups which is also performed in circuits (Table 3).

Individual exercise

For feasibility reasons, individual home-based exercise is also included. It comprises two hours of Nordic walking at 60-65% of the heart rate reserve. All participants receive Nordic walking poles and instructions. If due to medical reasons Nordic walking is not desirable, a proper alternative is sought, e.g., swimming laps or cycling (vigorous effort). Supervised

Table 3 SHAPE-2 study in- and exclusion criteria

Week	Endurance	Strength
1-3	40% to 60% HRR	1 circuit of 20 to 25 repetitions. Weights based on 20-RM Exercises: legs (squat, lunges, calve raises), arms (biceps curl, triceps extension), shoulder (shoulder press), thorax (Barbell bench press), back (rowing). Abdomen: crunch 30 to 40 repetitions.
4-8	60% to 70% HRR 15 to 20 min, plus 70% to 89% HRR 5 to 10 min	
9-12	Interval training: 10 x 30 sec. vigorous to maximum intensity exercise, alternated with 1 min active rest, plus 10 min 60% to 75% HRR endurance	2 circuits of 15 to 20 repetitions. Weights based on 15-RM Exercises: legs (squat), arms (biceps curl, triceps extension), shoulder (shoulder press), thorax (Barbell bench press), back (rowing). Abdomen: crunch 30 to 40 repetitions; hoover 2x 45 seconds. If in time, calve raises and lunges can be added.
13-16	Interval training: 2 circuits of 8 x 30 sec vigorous to maximum, alternated with 1 min active rest, plus 5 min 60% to 75% HRR endurance	

HRR=Heart rate reserve. RM=epetition maximum.

lessons of Nordic walking by instructors are organised to increase motivation and compliance. These sessions can be attended voluntarily, however, women are strongly encouraged to join.

Home-based Nordic walking sessions are registered in an exercise-log, which are checked regularly by the physiotherapist. Group sessions at the physiotherapists centre and Nordic walking lessons are regularly monitored by the researchers.

Control group: stable weight

Participants in the control group are requested to keep their weight stable by adhering to the standardised diet, and maintaining their habitual exercise pattern. They are offered an alternative weight loss programme after the study period, consisting of 4 dietary group sessions and several exercise classes such as Nordic walking and/or fitness.

Outcomes and measurements

Study participants visit the research centre for measurements twice: at baseline (i.e., the end of the run-in period) and at the end of the study (Table 2). Measurements include blood sampling, anthropometrics: height (at baseline), weight, waist- and hip circumference), a total body DEXA scan, abdominal MRI, blood pressure and cardio-respiratory fitness. At every visit, information on medication use is assessed. Furthermore, we assess information on socio-demographic variables, general health, medical history, reproductive history and smoking history at baseline by a self-constructed questionnaire.

Blood samples

Blood samples (30 m**L**) are drawn in order to determine serum concentrations of oestradiol (total and free), oestrone, testosterone and SHBG. After centrifugation, samples are directly stored at -20 °C and at -80 °C within one week. All samples from one individual will be analysed in the same batch since the batch-to-batch variation can be higher than any woman's likely change in hormones over the year[43]. Serum oestrogens and testosterone will be determined by use of the LC-MS method, in the UHSM laboratory, Manchester[44,45]. SHBG will be measured by commercially available double-antibody radioimmunoassay kits (Roche Cobas: SHBG-03052001), performed in the laboratory "Stichting Huisartsenlaboratorium Oost" in Velp[46]. Technicians are blinded to study allocation.

Anthropometrics and body composition

Body weight and height are measured while the subjects wear light clothes without shoes. To measure body weight, we use calibrated analogue balance and digital balance scales (SECA®), depending on study centre. Subjects are always measured on the same balance scale. Analogue values are rounded to the nearest 0.5 kg. Height is measured using a wall mounted tape measure and rounded to the nearest 0.5 cm.

Waist circumference (to the nearest 0.5 cm) is measured standing at the midway between lower ribs and iliac crest. Hip circumference (to the nearest 0.5 cm) is measured standing over the buttocks. All measurements are taken in duplicate and averaged.

Total body fat (kg) and fat% are assessed by a total body DEXA-scan (Lunar, Prodigy™). The DEXA scan measures body composition according to a three-compartment model: fat mass, lean tissue, and bone mineral content. The standard soft tissue analysis is performed using software supplied by the manufacturer.

Visceral abdominal fat and subcutaneous abdominal fat are measured by MRI (Philips, Ingenia 1.5 T) with the use of the three-point IDEAL method, described by Dixon [47].

Blood pressure

Blood pressure is measured by an automatic tonometer (OMRON M4 +) after a minimum of 5 minutes rest. Measurements are taken twice, with a 2-minute time interval.

Cardiorespiratory fitness

A maximal cycle exercise test with respiratory gas analysis is performed to measure cardio-respiratory fitness, defined by the highest oxygen uptake during the test (VO_{2peak}).

All testing is conducted according to the ATS guidelines[48]. Subjects are tested on a bicycle ergometer (Ergoline, type Ergoselect 200 P, CareFusion, Houten, the Netherlands and Jaeger ER800®, Würtzburg, Germany). Seat height is adjusted so that subject's legs are near full extension during each pedal revolution.

The ramp cycle test protocol starts with 2 minutes of rest and 3 minutes of active rest (cycling without workload). The test phase consists of 24-second stages of graded exercise.

Workload increases with 12.5 Watt or 15 Watt at every step, depending on the predicted maximum Watt per subject. Pedalling speed is kept around 65 revolutions per minute (RPM). If participants fail to keep up or drop below 40 RPM, the test is ended and followed by a 2-minute recovery phase. The maximal exercise test is performed under medical supervision. During the test, a 12-lead electrocardiogram (ECG) and respiratory data through breath-by-breath analysis (Oxycon Pro®, Jaeger, by Care Fusion, Houten, The Netherlands) are continuously measured. Heart rate is determined from the ECG. Cuff blood pressure is monitored before and throughout the exercise and recovery phase. VO_{2peak} is defined as the highest 15-second average of VO_2 obtained at the end of the test and is expressed as ml/min and ml/kg/min.

The goal of the maximal cycle exercise test is threefold. In addition to measuring cardiorespiratory fitness/VO_{2peak}, it also serves as a medical evaluation, and maximum heart rate is used to estimate training intensity for women participating in the exercise programme.

Physical activity

Physical activity level is assessed with an activity monitor (GT3X+ Tri-Axis Actigraphy Monitor, ActiGraph®). This non-invasive device provides information on individual activity including energy expenditure, sedentary behaviour, activity intensity levels, and METs. It is worn in 7 consecutive days during the run-in and maintenance period.

Furthermore, validated physical activity questionnaires are used to measure habitual activity level (PASE questionnaire)[49] and short-term physical activity level (SQUASH questionnaire)[50] at baseline and at the end of study.

Dietary intake

Habitual dietary intake is assessed at baseline using the dietary history method. Actual dietary intake and adherence to the diet plan are assessed using three-day food records (including 1 weekend day) during every study period (run-in, intervention and maintenance, see Table 2). Participants are instructed by the dietitian how to complete the records. Filled-in records are checked by the dietitian for completeness and can be discussed with participants in the next telephone contact. Energy intake and nutrient composition are calculated using the Dutch Food Composition Database[51].

Sample size

Sample size calculations are based on the effect of the interventions on the primary outcome, i.e., serum oestradiol levels. The following comparisons will be made: 1) diet-induced weight loss versus exercise-induced weight loss. 2) diet-induced weight loss versus control. 3) exercise-induced weight loss versus control. First, we calculated the sample size for the first comparison, i.e., diet-induced weight loss (n=85) versus exercise-induced weight loss (n=85), since the difference in oestradiol levels between these groups

is expected to be the smallest (8%). Based on the estimated sample sizes, we calculated the number of subjects needed in the control group (n=36). The sample size of the control group can be much smaller, since the expected difference with the interventions groups is large (12% and 20%, respectively). The sample size calculations resulted in the following estimated numbers per group, taking into account 5% drop out and 15% non-compliance: control group n=45, diet group n=104 and exercise group n=104

Statistical analysis

Descriptive statistics will be used to characterise the study population at baseline per study arm. Baseline and end of study values of sex hormone levels, total body fat and intra-abdominal body fat will be tabulated by treatment group. Sex hormones will be log-transformed and geometric means will be presented if not normally distributed.

The main analysis will be performed according to the intention-to-treat principle, by linear regression analysis, where outcomes for patients are analysed by assigned treatment, regardless of the level of adherence. As a secondary analysis, adherence will be examined as a potential modifier of the intervention effects.

A per-protocol analysis will be performed analysing women who reached the weight loss goal only. Whether changes in body fat (total, abdominal) mediate or moderate intervention effects on sex hormone levels will be explored.

DISCUSSION

In the SHAPE-2 study, we aim to investigate the potential effect of physical activity on biomarkers of breast cancer risk (sex hormones), additional to weight loss. We hypothesise that exercise-induced weight loss results in a stronger decrease in serum sex hormones compared to equivalent diet-induced weight loss and compared to controls.

The goal of the intervention, and challenge in this trial, is for subjects to lose an equivalent amount of 5 to 6 kg of body weight in both intervention groups. Success of the study depends heavily on subjects' adherence and motivation.

We implemented several strategies to increase adherence and motivation of the study subjects. First, the weight loss interventions are delivered by experienced dietitians and physiotherapists, who are situated in the municipality and easily accessible. For participants in the intervention groups, we scheduled a high contact frequency with the dietitian and physiotherapists which is proven to be a relevant success factor for weight reduction[52]. Second, group sessions are implemented in both intervention programmes which provide a combination of social support, a healthy dose of competition and increase self-efficacy[53]. It is also suggested that dietary group sessions produce greater weight loss effects than individual counselling alone[54]. Groups are kept small to secure enough room for interactions and tailoring of the programme to the specific needs of the

participants. Third, to strengthen motivation of the participants and for monitoring purposes, the researchers visit diet and exercise group sessions regularly. Furthermore, participants receive newsletters about the study or related topics and the study website is updated frequently.

Adherence of the control group is also a challenge. We expect women allocated to the control group to be disappointed since they have their mind set on losing weight. This might lead to a change in lifestyle, either conscious or unconscious, resulting in slight weight loss. To anticipate, we repeatedly stress the importance of the control group. Moreover, we offer an evenly attractive alternative weight loss programme starting at the end of the study.

This trial has a strict time schedule. We aim to include 25-30 participants per municipality in a small time window, since the participants have to start simultaneously with the group interventions. The first group starts at the central research site in the Utrecht region. Inclusion in the second participating region (Enschede) starts in parallel. Consecutive groups within one region start after a minimum time interval of one month.
Due to a consecutive inclusion of municipalities, the winter season, summer holidays and national holidays (e.g., Christmas) might affect the adherence of some study groups. To retain compliance in order to achieve the same weight loss goal, dietitians and physiotherapists will anticipate these circumstances. We pay extra attention to the Nordic walking programme in winter since the colder climate and shorter days may threat compliance. We therefore provide more options for supervised Nordic walking hours and reflective clothing in winter.

In our study, we will measure visceral and subcutaneous abdominal fat by using MRI, which is a highly sensitive technique to measure changes in (intra-)abdominal fat. A side effect of the use of cross sectional imaging in healthy subjects is that there is a risk of incidental findings. Rates of over 30% have been described whereas the proportion of subjects that might benefit from these findings is likely to be much lower than the proportion that will have no or even an adverse effect[55,56]. Outweighing the risk-benefit ratio and ethical considerations[57], we decided on the following procedure. The non-contrast enhanced T1-weighted IDEAL scans are considered non-diagnostic and therefore will not be routinely reviewed by a radiologist. If researchers encounter an apparent finding that strikes them as a possible abnormality, the radiologist will be consulted. When the finding is of potential clinical relevance, the participant and their general practitioner will be informed and advised on further work up.

Our trial is the first study especially designed to assess the additional effect of exercise on sex hormone levels when equivalent weight loss is achieved. Four former exercise intervention studies in the field suggested an interplay between sex hormones and body weight/fat mass[22-26]. Greater weight loss produced greater effects on serum sex hormone levels and SHBG.

The question remains whether there is an additional effect of exercise on serum sex hormones, as breast cancer risk biomarkers, when equivalent weight loss is reached. In the SHAPE-2 study, we aim to investigate the potential effect attributable to physical activity on postmenopausal breast cancer risk biomarkers, in addition to weight loss.

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5

EFFECT OF WEIGHT LOSS, WITH OR WITHOUT EXERCISE, ON BODY COMPOSITION AND SEX HORMONES IN POSTMENOPAUSAL WOMEN: THE SHAPE-2 TRIAL

Submitted

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ABSTRACT

Introduction

We studied the effect of equivalent weight loss, with or without exercise, on sex hormones associated with breast cancer risk in postmenopausal women, and the potential mediating role of body fat.

Methods

243 overweight and inactive women were randomised to a diet (n=97), exercise (n=98) or control group (n=48). Goal of both interventions was to achieve 5 to 6 kilograms of weight loss by a diet, or an intensive exercise programme with a small caloric intake restriction. Primary outcomes after 16 weeks were serum sex hormones and sex hormone binding globulin (SHBG). Body fat and lean mass were measured by DEXA.

Results

Both the diet (-4.9 kg) and exercise group (-5.5 kg) achieved the target weight loss. Loss of body fat was significantly greater with respectively exercise versus diet (difference -1.4 kg, 95% CI: -2.0 to -0.8). Compared with control, beneficial effects were seen with diet and exercise on oestradiol (treatment effect ratio (TER)=0.86, 95% CI: 0.75 to 0.98; TER=0.83, 95% CI: 0.73 to 0.95), free oestradiol (TER=0.80, 95% CI: 0.70 to 0.92; TER=0.77, 95% CI: 0.67 to 0.88), SHBG (TER=1.14, 95% CI: 1.07 to 1.23; TER=1.21, 95% CI: 1.12 to 1.30) and free testosterone (TER=0.91, 95% CI: 0.83 to 1.01; TER=0.84, 95% CI: 0.76 to 0.93). Exercise reduced free testosterone significantly greater (TER=0.92, 95% CI: 0.85 to 0.99), and androstenedione (TER=0.90, 95% CI: 0.80 to 1.01) and SHBG (TER=1.05, 95% CI: 1.00 to 1.12) suggestively greater, compared to diet. After adjustment for changes in body fat percentage, intervention effects attenuated or disappeared.

Conclusions

Weight loss, either by diet or exercise, resulted in significant favorable effects on serum sex hormones, indicating a decrease in postmenopausal breast cancer risk. Exercise-induced weight loss resulted in a significantly larger fat loss, maintenance of lean mass and a higher fitness level. Body fat seems to largely mediate the effects on sex hormones.

INTRODUCTION

Overweight or obesity and physical inactivity are convincing risk factors for post-menopausal breast cancer, according to the World Cancer Research Fund[1]. More important, these are two of the few known lifestyle related risk factors and, therefore, exposure is modifiable. It has been estimated that for every five units increase in body mass index (BMI), breast cancer risk increases with 13%[1]. The effect of weight loss, also after menopause, remains unclear[2,3]. For physical inactivity, when comparing the highest versus lowest levels of activity, risk reductions ranging from 20-40% have been described[4] which were still present after adjustment for change in BMI.

One of the pathways whereby overweight and obesity and an inactive lifestyle might influence postmenopausal breast cancer risk, is through serum sex hormones i.e., oestrogens, androgens and sex hormone binding globulin (SHBG)[5]. Observational studies showed consistent increases in postmenopausal breast cancer risk of up to two-fold in women with endogenous sex hormone levels in the highest versus the lowest quintile[6,7].

Two exercise trials in postmenopausal women found small but significant differences in oestrogens[8,8] and androgens[9,10] between exercisers and controls, but only in those women who lost concordant fat mass. A third trial found an overall effect on oestradiol and SHBG, however, women in the exercise group also lost substantially more weight than their control counterparts[11]. A recent weight loss and exercise RCT, concluded that greater weight loss resulted in greater changes in sex hormones[12].

The purpose of the Sex Hormones And Physical Exercise (SHAPE)-2 trial was to investigate the additional beneficial effect of exercise when comparable weight loss is achieved by diet or exercise[13]. We hypothesised that exercise-induced weight loss produces greater favourable changes than equivalent diet-induced weight loss in serum sex hormone levels and that these effects are mediated by changes in body fat.

METHODS

Design and study population

The SHAPE-2 study is a three-armed randomised controlled trial allocating postmenopausal women either to a diet-induced or exercise-induced weight loss intervention or control. The study ran from February 2012 until June 2013, in eight municipalities in the Netherlands. The study was approved by the ethical committee of the University Medical Center Utrecht. Written informed consent was obtained from all participants. Details of the trial design were reported previously (chapter 4 of this thesis)[13]. The trial was registered in ClinicalTrials.gov, identifier: NCT01511276.

Women, aged 50-69, were recruited via mass mailings and media attention. Women were eligible if they were postmenopausal (>12 months since last menses), overweight or

obese (BMI 25-35 kg/m^2), and inactive (<2 h/wk of ≥4 metabolic equivalent (MET) activity). Main exclusion criteria were smoking, use of exogenous (sex) hormones, diabetes, or ever diagnosed with breast cancer.

Run-in period, standardised diet and randomisation

All women started with a four to six-week run-in period during which a standardised diet was prescribed, in order to remain their weight stable and to achieve a comparable diet composition among all study participants (50-60% carbohydrates, 15-20% proteins, 20-35% fat, min. 25 grams fibre, max. 1 alcoholic consumption/day)[14].

After baseline measurements, women were randomised by computer, stratified for municipality to a diet (n=97), exercise (n=98), or control group (n=48). The programme contained an automatically generated random sequence with block sises of five (ratio interventions versus control; 2:2:1).

Interventions

Both weight loss intervention programmes aimed for 5 to 6 kg weight loss were delivered by physiotherapists and/or dietitians, who also closely monitored body weight by supervised weighing. When participants reached the target weight loss, or after a maximum of 14 weeks, they entered a period of weight maintenance (of two to six weeks) wherein diet was adapted to stabilise body weight.

Diet group

Women in the diet group were prescribed a caloric restriction of -500 kcal/day as compared to their estimated needs and habitual intake. They were asked to maintain their habitual physical activity level. Contacts with the dietitian included two half-hour individual consultations and five one-hour group sessions (maximum 12 women/group) wherein the diet was adjusted and nutritional education, self-management training and behaviour change techniques were provided. Furthermore, telephone consultations were scheduled every other week for monitoring and motivation[15].

Exercise group

The exercise programme included two one-hour group sessions at the physiotherapist center and two one-hour individual sessions of moderate-to-vigorous exercise per week, resulting in an estimated mean energy expenditure of 350 kcal/day. Group sessions included 20-25 minutes of endurance training, 25 minutes of strength training and 5-10 minute warm ups and cool downs. Heart rate monitors were worn while exercising. Training intensity was gradually increased during the study (for endurance training: from 60% to 90% of the heart rate reserve (HRR)), for strength training: based on repetition maximum tests). Individual sessions included Nordic Walking at 60-65% HRR. Supervised classes were also organised and women were strongly encouraged to join these.

Participants kept an exercise-log that was regularly checked by the physiotherapist.

In order to ensure substantial weight loss, a dietitian-prescribed caloric restriction of -250 kcal/day was added to the programme[16].

Control group

Women in the control group were asked to maintain a stable weight by continuing the standardised diet and their habitual physical activity pattern.

Outcome measurements

At baseline (i.e., before randomisation) and after 16 weeks measurements were taken.

Body weight, height and waist and hip circumference were obtained according to standard procedures[13]. Fat and lean mass were assessed by dual energy X-ray absorptiometry (DEXA) whole-body scan (Lunar, prodigy). Cardiorespiratory fitness (VO_{2peak}) was measured by a maximal cycle exercise test with respiratory gas analysis using a ramp protocol. Physical activity was measured by the SQUASH questionnaire[17] and objectively during seven consecutive days by the GT3X+ ActiGraph activity monitor[18,19].

Serum sex hormone analyses

Participants were asked not to perform moderate to vigorous physical activity in the 48 hours preceding the blood sampling. Serum was collected and stored at −80 °C. After trial completion, all samples were sent, frozen, to the laboratory for analyses. Multiple samples from each individual were analysed in the same batch. Oestradiol, oestrone, androstenedione and testosterone were determined by liquid chromatography-mass spectrometry (LC-MS)[20], in the UHSM Manchester laboratory[21]. Free fractions of oestradiol and testosterone were calculated by using the total hormone levels, SHBG and a constant for albumin[22]. SHBG was measured by commercially available double-antibody radioimmunoassay kits (Roche Cobas: SHBG-03052001), performed in the SHO Velp laboratory[23]. Inter- and intra-coefficients of variation (CV) were <10% for androgens[24], <7% for oestrogens[25] and <2% for SHBG. Technicians were blinded to study allocation.

Hormone values below the lower limit of detection were assigned the value of this limit, i.e. 1.4 pg/ml for oestrone (n=16), 86 pg/ml for testosterone (n=24) and androstenedione (n=1). Six oestradiol measures outside acceptable postmenopausal values (>42 pg/ml) and accompanying oestrone levels were excluded for analyses (five at baseline, one at follow-up).

Statistical analyses

We calculated that 104 women in both intervention groups and 45 women in the control group were required to detect a difference of at least 8% in oestradiol levels between the two intervention groups (alpha 0.05), and a 12-20% decrease versus control (alpha 0.025), with 80% power.

The primary analysis is according to the intention-to-treat principle. Outcomes are based on complete cases[26], i.e., both baseline and follow-up measurements. Between-group differences in outcomes, adjusted for baseline hormone level were computed by linear regression. Hormones were log-transformed, therefore, their coefficients with 95% confidence interval (95% CI) represent a treatment effect ratio (TER) that indicates how many times the level in one group is higher (TER>1) or lower (TER<1) compared with the reference group.

Secondary analyses were performed in: (a) women who reached >2 kg weight loss in the intervention groups and stable weight (±2 kg) in controls and, (b) women who adhered to the exercise goal (i.e., for the exercise group, >80% attendance; for diet and control, <60 minutes increase in leisure time activities of ≥4 MET/wk according to the SQUASH questionnaire or, if missing, ActiGraph). SPSS (version 20) was used for the analyses.

RESULTS

Of all the 243 participating women, 232 (95.5%) completed the trial and 11 women dropped out of the study (three in both the control and diet group and five in the exercise group) (see Figure 1). Women in the intervention and control groups were comparable in baseline characteristics (Table 1). Study participants had a mean age of 60 years, a BMI of 29.2 kg/m^2, a body fat percentage of 44% and a mean VO_{2peak} of 21.9 ml/kg/min, indicating poor physical fitness.

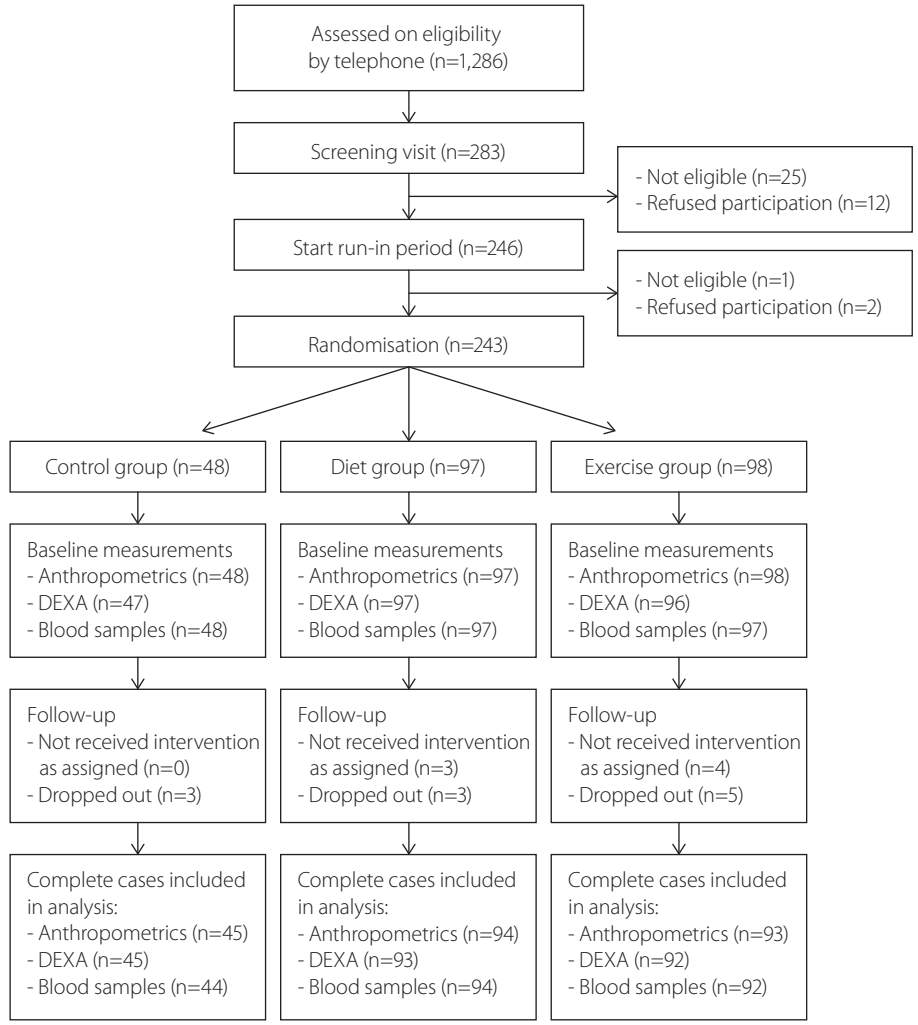
The median number of group sessions attended by women in the diet group was four (out of five offered). In the exercise group, the median attendance rate of all exercise sessions was 84%. Musculoskeletal injuries were reported by 9% in the control group, 5% in the diet group and 15% in the exercise group. No serious adverse events occurred.

Body composition and fitness outcomes

After 16 weeks, both the diet and exercise group accomplished an average weight loss of -4.9 kg (-6.1%) and -5.5 kg (-6.9%), respectively. The control group remained weight stable (0.06 kg, 0.1%) (Table 2). All anthropometric factors and body fat (kg and %) decreased significantly in both intervention groups versus control (Table 2).

Compared with the diet group, the exercise group showed a larger decrease in waist and hip circumference, however, the differences were not significant (-0.86 cm, 95% CI: -1.84 to 0.13, and -0.36 cm, 95% CI: -1.15 to 0.44, respectively). Decreases in body fat were significantly larger in the exercise versus the diet group (difference of -1.43 kg, 95% CI: -2.02 to -0.84 and -1.56%, 95% CI: -2.14 to -0.98). Lean mass was preserved in the exercise group compared with control (0.02 kg, 95% CI: -0.42 to 0.46), whereas the diet group had significantly lost lean mass (-0.71, 95% CI: -1.14 to -0.23). VO_{2peak} increased significantly in women in the exercise group only by 198 ml/min (95% CI: 137 to 260) versus control, and by 32.0 ml/min (95% CI: -29.9 to 93.8) versus diet.

Figure 1 Flow-chart of the inclusion, random assignment, and follow-up of the SHAPE-2 study participants



DEXA=Dual energy X-ray absorptiometry.

Table 1 Baseline characteristics of the SHAPE-2 study population

	Control group (n=48)	Diet group (n=97)	Exercise group (n=98)
	Mean (standard deviation)		
Age (years)	60.0 (4.9)	60.5 (4.6)	59.5 (4.9)
Time since last menses (years)	11.4 (7.8)	10.7 (6.1)	10.9 (7.7)
Education[†], number (%)			
Low	15 (31.3%)	27 (27.8%)	33 (33.6%)
Middle	15 (31.3%)	27 (27.8%)	20 (20.4%)
High	18 (37.5%)	42 (43.3%)	44 (44.9%)
First degree family member with breast cancer, number (%)	9 (18.8%)	23 (23.7%)	24 (24.5%)
Anthropometrics			
Weight (kg)	80.9 (10.0)	80.0 (8.6)	80.4 (9.0)
BMI (kg/m^2)	29.5 (2.6)	29.3 (2.5)	29.0 (2.9)
Waist circumference (cm)	99.0 (8.7)	97.8 (7.5)	97.5 (8.3)
Hip circumference (cm)	110 (7.7)	110 (6.8)	109 (6.7)
Body composition by DEXA			
Body fat percentage (%)	43.6 (5.0)	44.1 (3.8)	43.8 (4.0)
Total body fat (kg)	34.2 (7.4)	33.9 (5.7)	33.9 (6.2)
Lean mass (kg)	43.4 (3.9)	42.7 (4.0)	43.1 (4.1)
Physical fitness and activity			
VO_{2peak}, relative (ml/kg/min)	22.1 (4.7)	21.9 (4.0)	21.8 (3.7)
VO_{2peak} (ml/min)	1751 (363)	1742 (310)	1749 (293)
Physical activity monitor[‡] (min/day)	*Median (interquartile range)*		
Sedentary	652 (600 - 691)	637 (606 - 685)	630 (593 - 678)
Light	179 (164 - 226)	194 (175 - 214)	197 (157 - 229)
Moderate	35 (25 - 39)	35 (22 - 46)	33 (27 - 46)
Vigorous	0.33 (0.17 - 0.61)	0.35 (0.17 - 0.53)	0.29 (0.14 - 0.47)
SQUASH moderate and vigorous activity[§] (min/wk)	270 (120 - 495)	184 (115 - 420)	248 (90 - 465)
Alcohol (g/day)	3.7 (0.0 - 11.7)	5.7 (0.0 – 10.0)	4.3 (0.0 – 10.0)
	Geometric mean (95% confidence interval)		
Oestradiol (pg/ml)	4.10 (3.51 - 4.79)	4.15 (3.67 - 4.70)	3.70 (3.33 - 4.12)
Oestrone (pg/ml)	21.0 (18.4 - 24.0)	20.4 (18.9 - 22.0)	19.3 (17.7 - 21.1)
Free oestradiol (pg/ml)	0.10 (0.08- 0.12)	0.10 (0.08 - 0.11)	0.09 (0.08 - 0.10)
Testosterone (pg/ml)	201 (174 - 233)	196 (178 - 215)	183 (167 - 200)
Androstenedione (pg/ml)	593 (508 - 692)	561 (508 - 620)	556 (497 - 622)

Table 1 Continued

	Control group (n=48)	Diet group (n=97)	Exercise group (n=98)
	Geometric mean (95% confidence interval)		
Free testosterone (pg/ml)	2.78 (2.36 - 3.28)	2.54 (2.31 - 2.79)	2.41 (2.21 - 2.63)
SHBG (nmol/l)	45.1 (39.7 - 51.3)	50.1 (45.7 - 55.0)	48.8 (44.7 - 53.3)

Data on family history of breast cancer were available for 241 (99.2%) women; DEXA scan, n=240 (98.8%); VO_{2peak}, n=237 (97.5%); alcohol intake, n=226 (93.0%); accelerometer data, n=161 of 215 women in total (74.9%); SQUASH questionnaire, n=236 (97.1%). All hormone levels were missing for 1 woman, (free) oestradiol, (free) testosterone and androstenedione were also missing for 1 woman. 5 baseline values for (free) oestradiol and oestrone were excluded (>42 pg/ml). All other data were available for all women (n=243).

[†] Education, low: primary and technical/professional school. Middle: college degree. High: university degree.

[‡] GT3X+ ActiGraph activity monitor. Minutes/day of activity spent in each activity category. Activity categories are based on Freedson 1998 cut-off points.

[§] Activities performed ≥4 metabolic equivalents (MET).

BMI=body mass index. SHBG=Sex hormone binding globulin

Sex hormone outcomes

All hormones decreased and SHBG increased (beneficial) in both intervention groups versus control, except for testosterone in the diet group (Table 3). Changes were significant for oestradiol (bound and free) and SHBG in both the diet and exercise group, and for free testosterone in the exercise group. Free testosterone decreased borderline significantly in the diet group versus control.

Moreover, when compared to the diet group, the exercise group consistently showed larger treatment effects compared to control. A significant effect was found for free testosterone (TER= 0.92, 95% CI: 0.85 to 0.99) and borderline significant effects for androstenedione (TER=0.90, 95% CI: 0.80 to 1.01) and SHBG (TER=1.05, 95% CI: 1.00 to 1.12).

When adjusted for changes in body fat percentage (Table 4), the observed intervention effects of (free) oestradiol, oestrone, free testosterone and SHBG attenuated or disappeared. The additional effect of exercise versus diet on androstenedione did not substantially change.

In a secondary analysis in women who lost more than two kilogrammes (intervention groups) or remained weight stable (control) (n=206), results on sex hormones and SHBG were comparable to the intention-to-treat analysis (supplement Table 1). A per protocol analysis in women adherent to the exercise prescription (n=168) showed larger intervention effects on all hormones. The additional effect of exercise on SHBG when compared to diet increased to a significant level, whereas the additional effects of androstenedione and free testosterone disappeared (supplement Table 2).

Table 2 Baseline and 16-week differences in body composition and fitness between study groups

	Baseline mean	16 weeks mean	change 16 weeks	% change 16 weeks	Treatment effect† (95% CI): Intervention vs. Control	P-value‡	Treatment effect† (95% CI): Exercise vs. Diet	P-value§
Body weight, (kg)								
Control	80.4	80.4	0.06	0.07				
Diet	80.3	75.4	-4.89	-6.09	-4.95 (-5.69 to -4.21)	<0.001		
Exercise	80.4	74.9	-5.52	-6.87	-5.58 (-6.32 to -4.84)	<0.001	-0.63 (-1.23 to -0.04)	0.037
BMI (kg/m^2)								
Control	29.3	29.4	0.02	0.08				
Diet	29.2	27.5	-1.78	-6.07	-1.80 (-2.06 to -1.53)	<0.001		
Exercise	29.0	27.0	-2.00	-6.88	-2.02 (-2.29 to -1.75)	<0.001	-0.22 (-0.44 to -0.006)	0.044
Waist circumference (cm)								
Control	98.6	97.9	-0.66	-0.67				
Diet	97.9	92.7	-5.14	-5.25	-4.54 (-5.76 to -3.33)	<0.001		
Exercise	97.5	91.6	-5.97	-6.12	-5.40 (-6.62 to 4.18)	<0.001	-0.86 (-1.84 to 0.13)	0.087
Hip circumference (cm)								
Control	109.2	109.6	0.35	0.32				
Diet	109.9	105.9	-3.99	-3.63	-4.31 (-5.30 to -3.32)	<0.001		
Exercise	109.1	104.8	-4.31	-3.95	-4.67 (-5.65 to -3.68)	<0.001	-0.36 (-1.15 to 0.44)	0.377
Body fat percentage (%)								
Control	43.5	43.7	0.22	0.50				
Diet	44.0	41.5	-2.54	-5.76	-2.82 (-3.54 to -2.11)	<0.001		
Exercise	43.9	39.8	-4.11	-9.38	-4.38 (-5.10 to -3.67)	<0.001	-1.56 (-2.14 to -0.98)	<0.001
Total body fat (kg)								
Control	33.8	34.0	0.17	0.49				
Diet	34.0	30.3	-3.70	-10.89	-3.87 (-4.60 to -3.14)	<0.001		
Exercise	34.0	28.8	-5.13	-15.11	-5.30 (-6.03 to -4.56)	<0.001	-1.43 (-2.02 to -0.84)	<0.001
Lean mass (kg)								
Control	43.3	43.2	-0.10	-0.22				
Diet	42.9	42.1	-0.78	-1.82	-0.71 (-1.14 to -0.23)	<0.001		
Exercise	43.0	43.0	-0.06	-0.14	0.02 (-0.42 to 0.46)	0.930	0.73 (0.38 to 1.08)	<0.001
VO_{2peak} (ml/min)								
Control	1761	1682	-78.6	-4.46				
Diet	1752	1707	-44.9	-2.56	32.0 (-29.9 to 93.8)	0.310		
Exercise	1766	1885	119	6.72	198 (137 to 260)	<0.001	166 (117 to 216)	<0.001

Complete cases, i.e., women with both baseline and follow-up measurements, are presented. Therefore, baseline values may differ from the values as presented in Table 1. Weight, BMI, waist and hip circumference: n=232 (95.5%); body fat and lean mass, n=230 (94.7%); $VO_{2peak,}$ n=219 (90.1%).

† Treatment effect (95% confidence interval): the regression coefficient of a linear regression analysis. ‡ p<0.025 was considered significant for the comparison of both intervention groups vs. control. § p<0.05 was considered significant for the comparison exercise vs. diet.

Table 3 Baseline and 16-week differences in serum sex hormones and treatment effects between study groups

	Baseline Geometric mean	16 weeks Geometric mean	% change 16 weeks	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
Oestradiol (pg/ml)							
Control	3.89	4.01	3.11				
Diet	4.20	3.62	-13.8	0.86 (0.75 to 0.98)	0.025		
Exercise	3.69	3.22	-12.7	0.83 (0.73 to 0.95)	0.007	0.97 (0.87 to 1.08)	0.562
Oestrone (pg/ml)							
Control	20.1	20.4	1.51				
Diet	20.4	20.1	-1.26	0.98 (0.88 to 1.08)	0.650		
Exercise	19.9	18.5	-6.67	0.92 (0.82 to 1.02)	0.109	0.94 (0.86 to 1.02)	0.154
Free oestradiol (pg/ml)							
Control	0.09	0.10	3.23				
Diet	0.10	0.08	-17.7	0.80 (0.70 to 0.92)	0.002		
Exercise	0.09	0.07	-19.1	0.77 (0.67 to 0.88)	<0.001	0.96 (0.85 to 1.07)	0.425
Testosterone (pg/ml)							
Control	194	186	-4.07				
Diet	197	189	-3.76	1.01 (0.92 to 1.10)	0.886		
Exercise	186	172	-7.63	0.96 (0.87 to 1.05)	0.332	0.95 (0.88 to 1.02)	0.166
Androstenedione (pg/ml)							
Control	575	560	-2.60				
Diet	562	537	-4.50	0.97 (0.85 to 1.12)	0.684		
Exercise	573	488	-14.7	0.87 (0.76 to 1.00)	0.059	0.90 (0.80 to 1.01)	0.064
Free testosterone (pg/ml)							
Control	2.71	2.61	-3.90				
Diet	2.53	2.25	-11.2	0.91 (0.83 to 1.01)	0.069		
Exercise	2.44	2.01	-17.7	0.84 (0.76 to 0.93)	0.001	0.92 (0.85 to 0.99)	0.043
SHBG (nmol/l)							
Control	44.2	44.0	-0.30				
Diet	50.7	57.1	12.6	1.14 (1.07 to 1.23)	<0.001		
Exercise	49.3	58.6	19.0	1.21 (1.12 to 1.30)	<0.001	1.05 (1.00 to 1.12)	0.070

Complete cases, i.e., women with both baseline and follow-up measurements, are presented. Therefore, baseline values may differ from the values as presented in Table 1. Complete case data of oestradiol were available for 223 women; oestrone, n=221; free oestradiol, n=222; testosterone and androstenedione, n=229; free testosterone, n=228; SHBG, n=230.

† TER=Treatment effect ratio (95% confidence interval), which represents the overall intervention effect on hormone change (adjusted for baseline), estimated by linear regression analysis. Because the linear regression models were based on log-transformed hormone data, the presented treatment effect is the antilogarithm of the original estimate. Therefore, the TER is a ratio that indicates how many times the level in one group is higher (TER>1) or lower (TER<1) compared with a reference group. For example, TER intervention versus control=0.9 indicates that the hormone level in the intervention group is on average 10% lower compared with the control group.

‡ $p<0.025$ was considered significant for the comparison of both intervention groups vs. control.

§ $p<0.05$ was considered significant for the comparison exercise vs. diet.

SHBG=Sex hormone binding globulin

Table 4 Treatment effects on sex hormones, adjusted for changes in fat percentage

	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
Oestradiol (pg/ml)				
Diet	0.96 (0.83 to 1.10)	0.530		
Exercise	0.98 (0.83 to 1.16)	0.814	1.03 (0.92 to 1.15)	0.650
Oestrone (pg/ml)				
Diet	1.04 (0.93 to 1.16)	0.500		
Exercise	0.99 (0.87 to 1.13)	0.925	0.96 (0.88 to 0.96)	0.320
Free oestradiol (pg/ml)				
Diet	0.92 (0.79 to 1.06)	0.238		
Exercise	0.94 (0.79 to 1.12)	0.490	1.03 (0.92 to 1.16)	0.631
Testosterone (pg/ml)				
Diet	1.01 (0.91 to 1.12)	0.810		
Exercise	0.96 (0.85 to 1.08)	0.464	0.94 (0.87 to 1.02)	0.166
Androstenedione (pg/ml)				
Diet	1.00 (0.85 to 1.16)	0.949		
Exercise	0.89 (0.75 to 1.06)	0.198	0.90 (0.79 to 1.01)	0.071
Free testosterone (pg/ml)				
Diet	0.96 (0.87 to 1.07)	0.511		
Exercise	0.91 (0.81 to 1.03)	0.150	0.95 (0.87 to 1.09)	0.201
SHBG (nmol/l)				
Diet	1.07 (0.99 to 1.16)	0.069		
Exercise	1.08 (0.99 to 1.18)	0.070	1.01 (0.95 to 1.07)	0.747

† TER=Treatment effect ratio (95% confidence interval), which represents the overall intervention effect on hormone change (adjusted for baseline), estimated by linear regression analysis. Because the linear regression models were based on log-transformed hormone data, the presented treatment effect is the antilogarithm of the original estimate. Therefore, the TER is a ratio that indicates how many times the level in one group is higher (TER>1) or lower (TER<1) compared with a reference group. For example, TER intervention versus control=0.9 indicates that the hormone level in the intervention group is on average 10% lower compared with the control group.

‡ p<0.025 was considered significant for the comparison of both intervention groups vs. control.

§ p<0.05 was considered significant for the comparison exercise vs. diet.

SHBG=Sex hormone binding globulin

DISCUSSION

We found that modest weight loss of 6-7% either induced by diet or exercise, resulted in significantly favourable effects on oestradiol, free oestradiol, androstenedione (exercise only), free testosterone and SHBG compared to control. Exercise-induced weight loss resulted in a more favourable body composition (i.e., larger loss of body fat and preservation of lean mass), physical fitness and larger effects on free testosterone as well as suggestive effects for androstenedione and SHBG compared to diet. After adjustment for changes in body fat, most intervention effects attenuated or disappeared, supporting the hypothesis that body fat largely mediates the effects of physical activity on sex hormones as breast cancer risk biomarkers.

Our results confirm findings of previous exercise trials[8,9,11,27] showing that reduction in weight, and more specifically fat, is an important factor in inducing changes in sex hormones. This can biologically be explained because after menopause, fat tissue is the most important source of oestrogens, since the enzyme aromatase, present in adipose tissue, converts androgens to oestrogens[28]. Furthermore, abdominal fat is associated with higher levels of insulin, inhibiting SHBG production[29,30]. In our study, both intervention groups experienced a decrease in fat tissue which induced a decrease in oestradiol and an increase in SHBG, resulting in less unbound and biologically active oestradiol and testosterone.

Two randomised low-fat dietary intervention studies that resulted in small weight loss (3-6%) also showed significant improvements in SHBG[31,32] and testosterone[31], but not oestradiol.

The study that is most comparable to our study is the NEW trial, a 12-month trial in postmenopausal US women investigating the combined and individual effects of a diet and exercise intervention on sex hormones[12]. Unlike SHAPE-2, this trial was not aiming for comparable weight loss in the intervention arms. The combined exercise and diet group (most comparable to our exercise group) lost the most weight (-9.8 kg) and body fat (-6.4%), whereas diet alone resulted in -9.1 kg weight loss and -5.0% fat loss[12]. Beneficial changes in all oestrogens and SHBG were larger in these two groups compared with our weight loss arms. Regarding androgens, effects in the SHAPE-2 study were slightly stronger. In the NEW study, none of the differences between the two study groups reached statistical significance, whereas we found additional effects of exercise on androgens and SHBG. However, despite the significantly larger loss of fat mass in the exercise group, we observed no significant differences in oestrogen levels between the exercise and diet group. The observed 3-4% difference might not have been detectable in our study because we powered the study to detect an arbitrary clinical meaningful difference of 8%.

Most observational studies show an independent effect of physical activity after adjusting for body weight[1,33,34]. This may either reflect residual confounding, since adjustment for weight not fully covers the adjustment for fat, the real relevant tissue.

Another explanation is that exercise affects other breast cancer risk related mechanisms, which are not (fully) fat dependent such as insulin sensitivity or the immune system and inflammation[5].

Our findings support current recommendations on lifestyle behaviour by both diet and exercise to reduce obesity[35,36]. A modest and sustained body weight reduction of 3-5% has been shown to result in clinically meaningful improvements in health, and the degree of weight loss is directly proportional to health benefits[37]. Losing weight mainly by exercise, instead of diet alone results in a larger loss of fat and lean body preservation. It is known that sarcopenia, characterised by a loss of lean mass, often affects elderly and is responsible for higher morbidity and mortality[38].

The direct impact on breast cancer risk of our study results remains speculative. Here, we use aromatase inhibitor and BMI studies to estimate the clinical impact. Aromatase inhibitors reduce oestradiol by 83-89% in breast cancer patients[39-41] and two randomised trials in healthy high risk women observed a 53-65% breast cancer risk reduction of these drugs during five years of follow-up[42,43]. Extrapolating this to our study wherein we observed a 13% decrease in oestradiol, would reveal a 8-10% reduction in breast cancer risk. Taking the observed two units reduction in BMI as a starting point would reduce breast cancer risk by approximately 5%; since every five units gain in BMI shows a relative risk of 1.13[1]. Although these different estimation methods indicate a 5-10% risk reduction, the direct and long-term impact on breast cancer risk of weight loss is still unclear, leaving a challenge for future research.

There are several strengths of our study. First, we used a strong design with the unique aim of reaching comparable weight loss between the two intervention groups, which was largely accomplished in both groups. In addition, our study design incorporated a run-in period, during which all women were prescribed a standardised diet. Therefore, food components that might potentially influence sex hormones, as alcohol and dietary fibre, are unlikely to have affected the results. Another strength is the high adherence to the study protocol in all three groups. Adherence of the control group is often challenging in lifestyle trials[44], therefore, we offered an alternative weight loss programme was offered after trial completion. Finally, we used the LC-MS method which is the reference standard since it is a highly sensitive technique to measure hormone levels because it is suffering less from cross reactions[45,46].

There are also some limitations which we need to acknowledge. Despite the fact that both intervention groups achieved the weight loss target, there was a difference of 0.6 kg in favour of the exercise group. Although this is a clinically small difference, it may have affected the outcomes related to the exercise-diet comparison slightly. However, the difference in fat loss, which we observed to be of most influence on sex hormones, was much larger between the two groups. Furthermore, as weight loss mainly represents fat loss, additional adjustment for weight change has no added value.

To conclude, we found that a modest reduction in body weight by either diet or exercise led to beneficial effects on sex hormones and SHBG. Moreover, exercise-induced weight loss led to a more favourable body composition (less fat and preservation of lean mass) and free testosterone, androstenedione (lower) and SHBG (higher), than comparable diet-induced weight loss. Body fat largely mediated the effects of exercise on these hormones, suggesting that weight loss, and in particular fat loss, is most important in influencing sex hormone levels which are associated with postmenopausal breast cancer risk.

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Supplement Table 1 Effects on sex hormones in women who lost >2 kg body weight (intervention groups), or remained weight stable (±2 kg, control group)

	Baseline Geometric mean	16 weeks Geometric mean	% change 16 weeks	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
Oestradiol (pg/ml)							
Control	3.80	4.00	5.18				
Diet	4.33	3.69	-14.6	0.85 (0.73 to 0.98)	0.030		
Exercise	3.73	3.23	-13.3	0.82 (0.71 to 0.95)	0.008	0.96 (0.86 to 1.08)	0.532
Oestrone (pg/ml)							
Control	20.1	20.6	2.65				
Diet	20.6	20.3	-1.56	0.97 (0.87 to 1.07)	0.507		
Exercise	20.2	18.9	-6.20	0.91 (0.82 to 1.02)	0.095	0.95 (0.87 to 1.03)	0.192
Free oestradiol (pg/ml)							
Control	0.09	0.10	4.31				
Diet	0.10	0.08	-19.4	0.79 (0.68 to 0.92)	0.002		
Exercise	0.09	0.07	-20.2	0.75 (0.65 to 0.88)	<0.001	0.95 (0.85 to 1.07)	0.432
Testosterone (pg/ml)							
Control	193	189	-2.33				
Diet	198	190	-4.17	0.99 (0.89 to 1.09)	0.780		
Exercise	186	174	-6.91	0.95 (0.86 to 1.04)	0.262	0.96 (0.89 to 1.03)	0.272
Androstenedione (pg/ml)							
Control	582	554	-4.67				
Diet	567	538	-5.27	0.98 (0.85 to 1.14)	0.834		
Exercise	576	505	-12.3	0.92 (0.79 to 1.06)	0.249	0.93 (0.83 to 1.04)	0.228
Free testosterone (pg/ml)							
Control	2.78	2.67	-3.70				
Diet	2.58	2.24	-13.3	0.89 (0.80 to 0.99)	0.024		
Exercise	2.42	1.99	-17.8	0.83 (0.75 to 0.92)	0.001	0.94 (0.87 to 1.01)	0.108
SHBG (nmol/l)							
Control	42.4	43.2	1.95				
Diet	50.1	58.2	16.1	1.16 (1.08 to 1.24)	<0.001		
Exercise	50.0	60.4	20.8	1.20 (1.13 to 1.29)	<0.001	1.04 (0.99 to 1.10)	0.128

Women included in this subgroup analysis: n=206 (84.7% of total study population). Control, n=35 (72.9% of the total control group); Diet, n=85 (87.6% of the total diet group); Exercise, n=86 (87.8% of the total exercise group).

† TER=Treatment effect ratio (95% confidence interval), which represents the overall intervention effect on hormone change (adjusted for baseline), estimated by linear regression analysis. Because the linear regression models were based on log-transformed hormone data, the presented treatment effect is the antilogarithm of the original estimate. Therefore, the TER is a ratio that indicates how many times the level in one group is higher (TER>1) or lower (TER<1) compared with a reference group. For example, TER intervention versus control = 0.9 indicates that the hormone level in the intervention group is on average 10% lower compared with the control group.

‡ p<0.025 was considered significant for the comparison of both intervention groups vs. control.

§ p<0.05 was considered significant for the comparison exercise vs. diet.

Supplement Table 2 Effects on sex hormones in women who were adherent to the prescribed physical activity

	Baseline Geometric mean	16 weeks Geometric mean	% change 16 weeks	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
Oestradiol (pg/ml)							
Control	4.49	4.76	6.08				
Diet	4.28	3.60	-15.8	0.78 (0.66 to 0.91)	0.002		
Exercise	3.73	3.19	-14.5	0.75 (0.64 to 0.88)	<0.001	0.96 (0.85 to 1.09)	0.548
Oestrone (pg/ml)							
Control	22.9	24.7	7.83				
Diet	20.5	20.1	-1.70	0.88 (0.79 to 0.98)	0.022		
Exercise	19.9	18.5	-7.18	0.82 (0.74 to 0.92)	0.001	0.94 (0.86 to 1.02)	0.134
Free oestradiol (pg/ml)							
Control	0.11	0.12	6.54				
Diet	0.10	0.08	-19.1	0.74 (0.62 to 0.87)	<0.001		
Exercise	0.09	0.07	-21.4	0.69 (0.58 to 0.81)	<0.001	0.93 (0.82 to 1.06)	0.294
Testosterone (pg/ml)							
Control	211	208	-1.31				
Diet	200	184	-7.56	0.93 (0.84 to 1.02)	0.132		
Exercise	184	171	-7.01	0.91 (0.83 to 1.01)	0.083	0.99 (0.91 to 1.07)	0.775
Androstenedione (pg/ml)							
Control	654	658	0.53				
Diet	596	536	-10.1	0.86 (0.73 to 1.01)	0.063		
Exercise	563	495	-12.0	0.82 (0.70 to 0.96)	0.016	0.96 (0.85 to 1.08)	0.473
Free testosterone (pg/ml)							
Control	3.05	3.03	-0.65				
Diet	2.64	2.27	-13.8	0.85 (0.76 to 0.95)	0.003		
Exercise	2.40	1.96	-18.3	0.79 (0.71 to 0.88)	<0.001	0.93 (0.86 to 1.02)	0.114
SHBG (nmol/l)							
Control	42.1	41.7	-0.94				
Diet	48.3	53.5	10.7	1.14 (1.05 to 1.24)	0.003		
Exercise	49.8	60.6	21.8	1.26 (1.16 to 1.37)	<0.001	1.10 (1.03 to 1.18)	0.003

NB. Adherence is defined as: for the exercise group, >80% attendance of all exercise classes. For diet and control, <60 min/wk increase in moderate to vigorous activities (≥4MET) based on the SQUASH questionnaire (questions on sports, or transportation, i.e., cycling moderate and high intensity/walking high intensity, or if missing, the Actigraph activity monitor. Women included in this subgroup analysis: n=168 (69.1% of the total study population). Control, n=30 (62.5% of the total control group); Diet, n=67 (69.1% of the total diet group); Exercise, n=71 (72.4% of the total exercise group).

† TER=Treatment effect ratio (95% confidence interval), which represents the overall intervention effect on hormone change (adjusted for baseline), estimated by linear regression analysis. Because the linear regression models were based on log-transformed hormone data, the presented treatment effect is the antilogarithm of the original estimate. Therefore, the TER is a ratio that indicates how many times the level in one group is higher (TER>1) or lower (TER<1) compared with a reference group. For example, TER intervention versus control = 0.9 indicates that the hormone level in the intervention group is on average 10% lower compared with the control group.

‡ p<0.025 was considered significant for the comparison of both intervention groups vs. control.

§ p<0.05 was considered significant for the comparison exercise vs. diet.

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6

EFFECT OF WEIGHT LOSS, WITH OR WITHOUT EXERCISE, ON INFLAMMATORY MARKERS AND ADIPOKINES: THE SHAPE-2 TRIAL

Submitted

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ABSTRACT

Introduction

Overweight and inactivity are lifestyle related risk factors for postmenopausal breast cancer. A proposed biological mechanism is via inflammatory markers and adipokines. We investigated the effect of weight loss, with or without exercise, on these markers associated with postmenopausal breast cancer risk.

Methods

243 inactive postmenopausal women, BMI 25-35 kg/m^2, were randomly allocated to a diet (n=97), exercise (n= 98) or stable weight control group (n=48). Both intervention groups had the goal to lose 5-6 kilograms of weight. The exercise group followed a 16-week endurance and strength training programme (4 hrs/wk) and was prescribed a dietary intake restriction (-250 kcal/day). The diet group was prescribed an intake restriction of -500 kcal/day. Outcomes at baseline and after 16 weeks included serum high-sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6), adiponectin and leptin.

Results

Both the diet and exercise group achieved the target weight loss (-4.9 kg and -5.5 kg, respectively). The control group remained weight stable (+0.06 kg). Compared with control, hsCRP was reduced with exercise (treatment effect ratio (TER)= 0.64, 95%CI 0.50;0.83) and borderline significant with diet (TER=0.77, 95%CI 0.60;0.99). There was a suggestively larger effect of exercise, directly compared to diet (TER=0.83, 95%CI 0.69;1.01). Leptin decreased with both interventions: exercise (TER=0.55, 95%CI 0.47;0.66) and diet (TER=0.59, 95%CI 0.54;0.70), versus control. No effects were seen on IL-6 and adiponectin. Adjustment for change in fat or fitness attenuated effects on hsCRP. For leptin, adjustment for fat loss attenuated the effect of exercise and -to a lesser extent- of diet.

Conclusions

A 16-week intervention that induced comparable weight loss by diet or exercise, resulted in favourable effects on serum hsCRP and leptin.

INTRODUCTION

Postmenopausal women who are overweight or obese and have an inactive lifestyle are at increased risk of breast cancer[1-3]. Evidence suggests that hormone pathways, as sex hormones and insulin, inflammation markers and adipokines play a key role in the link between these lifestyle related factors and breast cancer risk[4,5].

Obesity is strongly associated with a chronic inflammatory state. Fat tissue can be seen as an endocrine organ, that secretes multiple inflammatory factors and adipokines[5]. High levels of interleukin-6 (IL-6), C-reactive protein (CRP) and leptin have been associated with a higher risk of several cancers, including potentially postmenopausal breast cancer[6-10]. Whereas adiponectin, an adipokine inversely associated with obesity, seems to be protective for breast cancer development[11,12]. Leptin is an adipokine involved in the regulation of hunger and satiety and acts pro-inflammatory. Levels are increased in obese individuals[12]. IL-6 is mainly produced in adipose tissue, but also by leukocytes and skeletal muscle[13]. CRP is an acute phase protein which is produced by the liver in reaction to inflammation and production is up-regulated in direct response to IL-6.

Weight loss in overweight and obese women, by diet or exercise, may normalise levels of the above mentioned inflammatory markers and adipokines[14,15]. It has been argued that exercise may have beneficial effects on these markers, irrespective of concurrent weight loss[4,16]. But, empirical data for this hypothesis is still scarce.

The aim of the current study is to determine the effect of equivalent weight loss, with or without exercise, on markers of inflammation and adipokines in postmenopausal women. We hypothesise that weight loss induces favourable effects on these biomarkers, and that effects are more pronounced in the exercise group as compared to the diet-induced weight loss group.

METHODS

Design overview

The Sex Hormones And Physical Exercise (SHAPE)-2 study is a randomised controlled trial designed to investigate the effects of a comparable amount of weight loss reached with or without exercise, on markers of postmenopausal breast cancer risk, in healthy, inactive and overweight/obese postmenopausal women. The primary outcome was defined as change in serum sex hormone levels and sex hormone binding globulin (SHBG). Here, we report on inflammatory markers (hsCRP, IL-6) and adipokines (leptin and adiponectin). The study design and protocol are described elsewhere (chapter 4 of this thesis)[17]. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht and all participants signed informed consent.

Setting and participants

The SHAPE-2 study was conducted in eight municipalities in the Netherlands, surrounding two research sites. Eligible women were inactive (<2 h/wk of at least moderate intensive activities (≥4 metabolic equivalent, MET), overweight-to-obese (body mass index (BMI): 25-35 kg/m^2) and postmenopausal (>12 months cessation of menses). Main exclusion criteria were: use of sex hormones, smoking, diabetes mellitus, diagnosed with breast cancer (past or present) or other cancers in the past five years. The recruitment and inclusion procedure is depicted in Figure 1.

Run-in period

All participating women started with a four to six-week run-in period wherein a standardised diet was prescribed (50-60% carbohydrate, 15-20% protein and 20-35% fat, and maximum one unit of alcohol/day) based on the National Guidelines for Healthy Nutrition[18]. The goal of this diet was to keep their weight stable and achieve a comparable diet composition among study participants. The diet was prescribed by a study dietitian, after exploring the individuals' dietary history, body weight and physical activity level to assess energy needs[19].

Randomisation and interventions

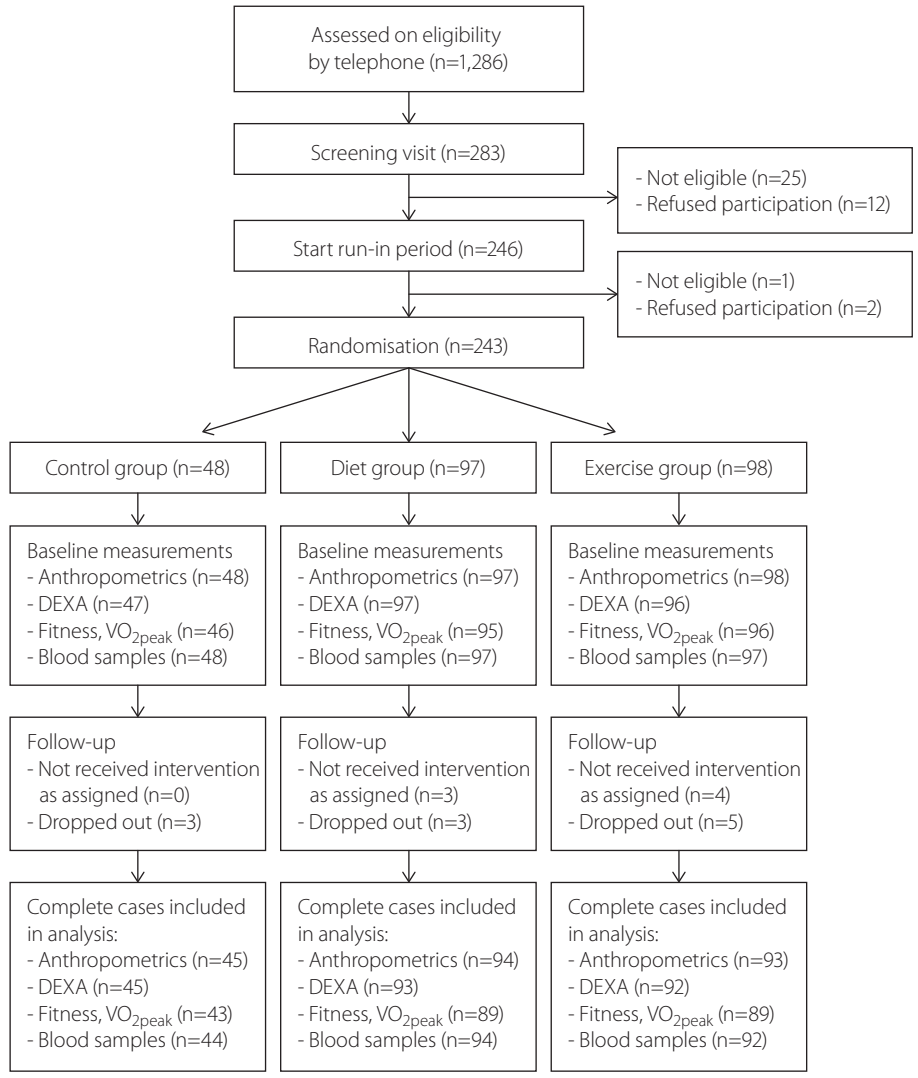
After the run-in period, women were randomly allocated to a diet (n=97) or exercise (n=98) weight loss group, or a stable weight control group (n=48). Randomisation was stratified for municipality and performed by computer with an automatically generated random sequence with block sizes of five (ratio interventions versus control; 2:2:1).

The exercise and diet interventions were both aiming for a 5-6 kg of weight loss and were delivered by physiotherapists and/or dietitians. Body weight was closely monitored by regular supervised weighing (weekly by the physiotherapist and every two weeks by the dietitian). When the target weight loss was achieved, or after a maximum of 14 weeks, the diet was adapted to stabilise body weight (±2 kg) until the end of the study (i.e., 16 weeks) so that end of study measurements would not be influenced by weight changes.

Diet group

Women allocated to the diet group were prescribed a caloric deficit of -500 kcal/day, compared to the estimated individuals' caloric needs (i.e., the standardised run-in diet). Participants were asked to maintain their habitual physical activity level. Two half-hour individual consultations and five one-hour group sessions were scheduled at the dietitian's office. Nutritional education, self-management training and behaviour change techniques were provided. Furthermore, telephone consultations were scheduled biweekly for monitoring and motivation.

Figure 1 Flow chart of the inclusion, random assignment, and follow-up of the SHAPE-2 study participants



DEXA=Dual energy X-ray absorptiometry.

Exercise group

The 16-week exercise programme, supervised by physiotherapists, prescribed four hours/week of moderate-to-vigorous exercise. One-hour group sessions consisted of endurance and strength training and were performed twice a week at the physiotherapist centre. The intensity of the endurance training was gradually increased from 60%-90% of the heart rate reserve (HRR) [Intensity%*(maximum heart rate-resting heart rate)+ resting heart rate][20], based on results from baseline cardiopulmonary exercise testing. Strength training was performed in circuits of 20-25 repetitions per exercise and comprised all major muscle groups. Additionally, two hours/week of Nordic walking at 60-65% HRR were performed individually or, preferably, in supervised classes.

To ensure substantial weight loss, a caloric restriction of -250 kcal/day compared to the run-in diet was prescribed by the dietitian. Since reduction of body weight by exercise only is shown to be unfeasible in a short period of time[21,22].

Control group

The control group was requested to maintain their habitual physical activity level and continue the standardised run-in diet. Participants in the control group were offered an alternative weight loss programme after study completion, which consisted of four one-hour dietary group sessions and several supervised exercise training sessions.

Outcome measurements

The primary outcomes of the current analysis were serum hsCRP, IL-6, adiponectin and leptin. Blood samples were collected at baseline and after 16 weeks, women were instructed not to exercise in the 48 hours preceding the blood sampling. Samples were directly centrifuged and stored at -80 °C. After the trial completion, the samples were sent to the laboratory for analysis all at once. Multiple samples of each individual were analysed in the same batch, in order to minimise random error due to batch-to-batch variation. Laboratory assays were performed at "Labor Nord-West" in Nordhorn, Germany[23]. High sensitivity CRP was measured by an immunoturbidimetric assay (CRP Gen.3, Cobas® Roche). Enzyme-linked immunosorbent assays (ELISA) were used to measure IL-6 (HS-600B, R&D systems, Inc.), leptin (ME E-0300, LDN®) and adiponectin (RD195023100, BioVendor). Intra-assay coefficients of variation were 3.3% for hsCRP, 2.4% for IL-6, 2.5% for leptin and 3.6% for adiponectin.

Secondary outcomes included anthropometrics, measured according to standard procedures[17] and lean and fat mass by dual-energy X-ray absorptiometry (DEXA, Lunar, Prodigy™). Cardio respiratory fitness (VO_{2peak}) was measured by a maximal cycle exercise test with respiratory gas analysis[17]. Physical activity was objectively measured during 7 consecutive days by the ActiGraph activity monitor (GT3X+ Tri-Axis). In addition, habitual physical activity was measured by the SQUASH questionnaire[24].

Statistical analysis

Sample size calculations were based on the primary outcome of the SHAPE-2 trial, i.e., serum oestradiol. The current sample size provides >80% power to detect a minimal difference of 0.4 mg/l between two study groups on hsCRP. Considering multiple testing, p-values <0.025 and <0.05 were considered significant for the comparisons of both interventions versus control, and exercise versus diet, respectively.

The primary analysis was performed according to the intention-to-treat principle. Levels of all four biomarkers were log-transformed to obtain normal distributions. Between-group differences in the markers were assessed by ANCOVA models with correction for the baseline biomarker level. As the biomarkers were log-transformed, their coefficients with 95% confidence interval (95%CI) from the ANCOVA models represent a treatment effect ratio (TER). The TER indicates how many times the level in one group is higher (TER>1) or lower (TER<1) compared with the reference group.

If an intervention effect was found, we explored whether change in body fat percentage or fitness (VO_{2peak}) mediated these effects, by adding each, separately, as a covariate to the model. All analyses were performed using SPSS version 21.

RESULTS

A total of 243 women were included in the SHAPE-2 study (control, n=48; diet, n=97; exercise, n=98) (Figure 1). Table 1 shows the baseline characteristics of the study population. The three groups were comparable in main patient characteristics. On average, participants were aged 60 years, had a BMI of 29 kg/m^2, a body fat percentage of 44% and a VO_{2peak} of 1747 ml/min. At 16 weeks' follow-up, blood samples were available for 231 participants (95%). In five samples, IL-6 and hsCRP values were below the limit of detection, i.e., <0.11 pg/ml, n=2 and <0.2 mg/l, n=3, respectively. The value of the lower limit of detection was therefore assigned to these samples. hsCRP values >25 mg/l were excluded from analysis (n=1 at follow-up) as these may indicate a clinical inflammation or infection.

Women in the diet group had a median attendance of 4 out of 5 group sessions. The exercise group showed a median attendance of 84% of all offered exercise hours.

Body composition and fitness

The results of the SHAPE-2 trial on body composition and fitness are reported in chapter 5 of this thesis. To summarise, both groups attained the weight loss goal. Women in the diet group lost -4.9 kg (-6.1%) and women in the exercise group -5.5 kg (-6.9%) (Table 2). Compared with control, total body fat (mass and percentage) decreased significantly in both intervention groups, with a significant larger loss with exercise compared with diet. Lean mass was preserved with exercise (+0.02 kg, 95%CI -0.42;0.46), and lost with diet (-0.71 kg, 95%CI -1.14;-0.23), compared to control. VO_{2peak} increased with exercise only, versus both control (+198 ml/min, 95%CI 137;260) and diet (+166 ml/min, 95%CI 117;216).

Table 1 Baseline characteristics of the SHAPE-2 study population

	Control group (n=48)	Diet group (n=97)	Exercise group (n=98)
	Mean (standard deviation)		
Age (years)	60.0 (4.9)	60.5 (4.6)	59.5 (4.9)
Time since last menses (years)	11.4 (7.8)	10.7 (6.1)	10.9 (7.7)
Education†, number (%)			
Low	15 (31.3%)	27 (27.8%)	33 (33.6%)
Middle	15 (31.3%)	27 (27.8%)	20 (20.4%)
High	18 (37.5%)	42 (43.3%)	44 (44.9%)
First degree family member with breast cancer, number (%)	9 (18.8%)	23 (23.7%)	24 (24.5%)
Weight (kg)	80.9 (10.0)	80.0 (8.6)	80.4 (9.0)
BMI (kg/m^2)	29.5 (2.6)	29.3 (2.5)	29.0 (2.9)
Body fat percentage (%)	43.6 (5.0)	44.1 (3.8)	43.8 (4.0)
Total body fat (kg)	34.2 (7.4)	33.9 (5.7)	33.9 (6.2)
Lean mass (kg)	43.4 (3.9)	42.7 (4.0)	43.1 (4.1)
Fitness, VO_{2peak} (ml/min)	1751 (363)	1742 (310)	1749 (293)
Physical activity, activity monitor‡ (min/day)	*Median (interquartile range)*		
Sedentary	652 (600 - 691)	637 (606 - 685)	630 (593 - 678)
Light	179 (164 - 226)	194 (175 - 214)	197 (157 - 229)
Moderate	35 (25 - 39)	35 (22 - 46)	33 (27 - 46)
Vigorous	0.33 (0.17 - 0.61)	0.35 (0.17 - 0.53)	0.29 (0.14 - 0.47)
SQUASH questionnaire, moderate and vigorous activity§ (min/wk)	270 (120 - 495)	184 (115 – 420)	248 (90 – 465)
Alcohol (g/day)	3.7 (0.0 - 11.7)	5.7 (0.0 – 10.0)	4.3 (0.0 – 10.0)
	Geometric mean (95% confidence interval)		
hsCRP (mg/l)	1.83 (1.40 - 2.40)	2.04 (1.67 - 2.49)	1.81 (1.49 - 2.19)
IL-6 (pg/ml)	1.31 (1.12 - 1.52)	1.41 (1.27 - 1.55)	1.41 (1.25 - 1.59)
Adiponectin (ng/ml)	9.34 (8.38 - 10.41)	9.68 (9.06 - 10.34)	9.53 (9.10 - 9.99)
Leptin (ng/ml)	27.4 (23.1 - 32.5)	28.5 (25.5 - 32.0)	31.4 (28.1 - 35.2)

Data on family history of breast cancer were available for 241 (99.2%) women; DEXA scan, n=240 (98.8%); VO_{2peak}, n=237 (97.5%); alcohol intake, n=226 (93.0%); accelerometer data, n=161 of 215 women in total (74.9%); SQUASH questionnaire, n=236 (97.1%); blood samples for risk markers, n=242. All other data were available for all 243 women.

† Education, low: primary school and technical/professional school. Middle: college degree. High: university degree

‡ GT3X+ ActiGraph activity monitor. Min/day of activity spent in each activity category. Activity categories are based on Freedson 1998 cut-off points.

§ Activities performed ≥4 metabolic equivalents (MET).

BMI=body mass index. hsCRP=high sensitivity C-reactive protein. IL-6=interleukine 6.

Markers of inflammation and adipokines

Compared with controls, circulating levels of hsCRP reduced significantly in the exercise group (TER=0.64, 95%CI 0.50;0.83) (Table 3). A borderline significant reduction in hsCRP was observed in the diet group versus control (TER=0.77, 95%CI 0.60;0.99). Also leptin decreased compared to controls in both the exercise group (TER=0.55, 95%CI 0.47;0.66) and diet group (TER=0.59, 95%CI 0.54;0.70). Although in favour of the intervention groups, no statistically significant effects were found for circulating levels of IL-6 and adiponectin. When directly comparing exercise with diet, CRP showed a TER suggestive for a larger effect in the exercise group, but just failed to reach significance (TER=0.83, 95%CI 0.69;1.01). For leptin, no statistically significant differences were observed between the two intervention groups.

After adjusting for change in body fat percentage, intervention effects on hsCRP attenuated and lost significance. Effects on leptin also attenuated, but remained significant in the diet group (Table 4). Adjustment for VO_{2peak} showed that effects in the exercise group attenuated for hsCRP but not for leptin (Table 5).

DISCUSSION

A weight reduction of 6-7% in healthy postmenopausal women, achieved by a 16-week diet or exercise intervention, resulted in favourable serum levels of hsCRP and leptin. For hsCRP, an indication was found for an effect of exercise beyond weight loss. Although effects on adiponectin and IL-6 were in favour of the intervention groups, the differences did not reach significance. The observed effects on hsCRP were mediated by change in body fat and physical fitness. The effect on leptin was mediated by body fat, mainly in the exercise group and to a lesser extent in the diet group, and not by fitness.

Our results on hsCRP are in line with several previous studies, which show that intended weight loss, either by diet or exercise, improves circulating levels of inflammatory markers in healthy postmenopausal women[14,25-30]. Results of exercise-only interventions in a comparable population are mixed. Some trials observed beneficial effects on CRP and IL-6 by exercise[31,32]. However, other trials reported no effect[33,34] or they observed beneficial effects that seem to be mainly explained by accompanied weight loss[26,35,36]. There are few trials that studied the effects of comparing diet- and exercise-induced weight loss in postmenopausal women[26,29,37]. Of these, only the NEW trial[26], is of a comparable size and scope. In this 12-month trial, 406 inactive and overweight-to-obese postmenopausal women were randomised to a reduced calorie diet, exercise, a combined diet and exercise intervention or control[26]. The NEW trial did not aim for equal weight losses across the intervention groups and there was no run-in period to standardise diet. Both interventions that included diet, resulted in more loss of body weight than the interventions in our study (-10.8% and -8.5% vs -6%). In these groups of the NEW trial, CRP

Table 2 Baseline and 16-week differences in body composition measures between study groups

	Baseline mean	16 weeks mean	change 16 weeks	% change 16 weeks	Treatment effect† (95% CI): Intervention vs. Control	P-value‡	Treatment effect† (95% CI): Exercise vs. Diet	P-value§
Body weight, (kg)								
Control	80.4	80.4	0.06	0.07				
Diet	80.3	75.4	-4.89	-6.09	-4.95 (-5.69 to -4.21)	<0.001		
Exercise	80.4	74.9	-5.52	-6.87	-5.58 (-6.32 to -4.84)	<0.001	-0.63 (-1.23 to -0.04)	0.037
Body fat percentage (%)								
Control	43.5	43.7	0.22	0.50				
Diet	44.0	41.5	-2.54	-5.76	-2.82 (-3.54 to -2.11)	<0.001		
Exercise	43.9	39.8	-4.11	-9.38	-4.38 (-5.10 to -3.67)	<0.001	-1.56 (-2.14 to -0.98)	<0.001
VO_{2peak} (ml/min)								
Control	1761	1682	-78.6	-4.46				
Diet	1752	1707	-44.9	-2.56	32.0 (-29.9 to 93.8)	0.310		
Exercise	1766	1885	119	6.72	198 (137 to 260)	<0.001	166 (117 to 216)	<0.001

Complete cases, i.e., women with both baseline and follow-up measurements, are presented. Therefore, baseline values may differ from the values as presented in Table 1. Complete case data of weight were available for 232 (95.5%) women; body fat and lean mass, n=230 (94.7%); $VO_{2peak,}$ n=219 (90.1%).
NB the results on body composition in this table are similar to the results presented in chapter 5, Table 2 of this thesis.

† Treatment effect: the regression coefficient of a linear regression analysis with a 95% confidence interval (95% CI).
‡ p<0.025 was considered significant for the comparison of both intervention groups vs. control. § p<0.05 was considered significant for the comparison exercise vs. diet.

and IL-6 both decreased to an extent comparable to our study. They did not find significant decreases in CRP or IL-6 for the exercise only group, wherein weight loss was -2.4%. Their results implicate that greater weight loss has greater effects on CRP and IL-6, and that exercise only, without concurrent substantial weight loss, does not show effects on inflammatory markers.

Another trial by You et al., randomised 34 obese but healthy women to a hypocaloric diet with and without exercise[29]. Larger decreases in both CRP as IL-6 occurred in the diet plus exercise group versus diet alone. This may be explained by the greater weight loss in the combined group (-8.5% vs. -5%).

We found an indication for a beneficial effect of exercise on hsCRP beyond weight loss. This effect is partly mediated by the larger amount of fat loss that was experienced in the exercise group and probably also by other exercise-induced pathways.

In contrast to the above mentioned studies[26,29], we did not observe significant reductions of IL-6. Effects have been shown to be less robust for IL-6 than for CRP[14]. This could possibly be explained by the amount of weight loss. A review suggested that a minimum weight loss of 8% is required to establish notable effects[38]. This was not the target in our study. We defined our target based on expected change in sex hormone levels, i.e., the primary endpoint of the SHAPE-2 trial. Another explanation for why we did not find effects on IL-6 could be that in our healthy population, substantial effects are more difficult to reach. This is supported by the findings of a weight loss trial by Camhi et al. where CRP and IL-6 only improved in women with metabolic syndrome at baseline[37].

Both our interventions show beneficial and comparable effects on leptin (-41% and -45%). This is in line with two smaller RCT's wherein weight loss after 12 weeks led to decreases in leptin, but exercise did not add to this effect[39,40]. Also the NEW trial reported reductions in leptin[41]: up to -40% in both intervention groups including diet. They furthermore observed a statistically significant reduction of -13% in the exercise only group. It has been hypothesised that exercise may decrease leptin levels irrespective of weight or fat loss. Insulin stimulates leptin secretion[42], and since exercise may lower insulin this can subsequently reduce leptin levels[43,44]. It may also be that longer exercise duration is needed than our 16 weeks to show effects.

Table 3 Baseline and 16-week differences in risk markers between study groups and treatment effects

	Baseline Geometric mean	16 weeks Geometric mean	% change 16 weeks	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
hsCRP (mg/l)							
Control	1.72	1.99	16.2				
Diet	1.99	1.75	-12.3	0.77 (0.60 to 0.99)	0.042		
Exercise	1.85	1.37	-26.1	0.64 (0.50 to 0.83)	<0.001	0.83 (0.69 to 1.01)	0.064
IL-6 (pg/ml)							
Control	1.32	1.53	15.9				
Diet	1.44	1.40	-2.80	0.88 (0.73 to 1.07)	0.192		
Exercise	1.42	1.34	-5.74	0.85 (0.70 to 1.03)	0.092	0.96 (0.83 to 1.12)	0.631
Adiponectin (ng/ml)							
Control	9.16	8.95	-2.29				
Diet	9.81	9.78	-0.32	1.03 (0.98 to 1.07)	0.241		
Exercise	9.59	9.76	1.79	1.05 (1.00 to 1.10)	0.049	1.02 (0.98 to 1.06)	0.313
Leptin (ng/ml)							
Control	26.2	26.5	1.13				
Diet	29.3	17.3	-41.0	0.59 (0.50 to 0.70)	<0.001		
Exercise	31.1	17.10	-45.1	0.55 (0.47 to 0.66)	<0.001	0.94 (0.82 to 1.07)	0.351

Complete cases, i.e., women with both baseline and follow-up measurements, are presented. Therefore, baseline values may differ from the values as presented in Table 1. Complete case data of CRP were available for 229 (94.2%) women, 1 woman was excluded because of an abnormal CRP value at follow-up (>25 mg/l). IL-6, Adiponectin, Leptin, n=231 (control, n=44 (91.7%); diet, n=94 (96.9%); exercise, n=93 (94.9%))

† TER: the Treatment Effect Ratio (95% confidence interval) represents the overall intervention effect on change in biomarker (adjusted for baseline biomarker level), estimated by linear regression analysis. The linear regression models were based on log-transformed biomarker data, therefore, the treatment effect is a ratio that indicates how many times the biomarker level is, on average, higher (TER>1) or lower (TER<1) in (i) the intervention group compared with the control group, or (ii) exercise compared with the diet group. For example, TER=0.9 indicates that the biomarker level in the intervention group is on average 10% lower compared with the control group).

‡ p<0.025 was considered significant for the comparison of both intervention groups vs. control.

§ p<0.05 for the comparison exercise vs. diet.

hsCRP=high sensitivity C-reactive protein. IL-6=interleukine 6.

In our analyses, after adjustment for change in body fat the effect on leptin remained in the diet group. Therefore, it seems that an effect beyond fat loss was induced on leptin by diet. We speculate that diet-induced weight loss, more than exercise-induced weight loss, results in a hunger feeling as a signal of the body to retain or gain weight[45]. As low levels of leptin induce a hunger-feeling, it could be that less leptin is produced in response to diet in order to regain body weight.

In line with our results, most trials aiming for weight loss do not observe significant improvements in adiponectin. Reviews suggest that at least 7.5%-10% weight loss is needed to show effects[38,46]. The NEW trial indeed found significantly improved adiponectin levels compared to controls in both diet-induced interventions (-8% and -10% weight loss), but not in the exercise only group (-2.5% loss).

Recently more insight was gained in the importance of the adiponectin:leptin-ratio[47]. This ratio has shown to be a possible surrogate measure for insulin resistance[48], which is also linked to breast cancer development[4,49]. In our study, the adiponectin:leptin ratio improved which was mainly driven by the observed decreases in leptin.

Markers of inflammation and adipokines seem to be able to increase cancer risk through several pathological mechanisms[12,47,50]. Large cohort and cross-sectional studies observed associations with higher levels of inflammatory markers or adipokines and an increased cancer risk[7,10,51]. Two meta-analyses on the effects of CRP on all-cancer risk found significant hazard ratios of 1.10 per log unit increase in CRP[6,9]. The evidence was weaker but also suggestive for an increase in breast cancer risk[6,9]. Another meta-analysis found that postmenopausal breast cancer risk was significantly increased with elevated

Table 4 Treatment effects on risk markers adjusted for change in fat percentage

	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
hsCRP (mg/l)				
Diet	0.87 (0.66 to 1.13)	0.296		
Exercise	0.77 (0.57 to 1.04)	0.091	0.89 (0.72 to 1.09)	0.243
IL-6 (pg/ml)				
Diet	0.94 (0.76 to 1.17)	0.604		
Exercise	0.93 (0.73 to 1.20)	0.601	0.99 (0.84 to 1.17)	0.919
Adiponectin (ng/ml)				
Diet	1.02 (0.97 to 1.07)	0.367		
Exercise	1.04 (0.99 to 1.10)	0.149	1.02 (0.98 to 1.06)	0.335
Leptin (ng/ml)				
Diet	0.83 (0.70 to 0.97)	0.023		
Exercise	0.93 (0.77 to 1.13)	0.469	1.13 (0.99 to 1.28)	0.061

† TER: the Treatment Effect Ratio (95% confidence interval) represents the overall intervention effect on change in biomarker (adjusted for baseline biomarker level), estimated by linear regression analysis. The linear regression models were based on log-transformed biomarker data, therefore, the treatment effect is a ratio that indicates how many times the biomarker level is, on average, higher (TER>1) or lower (TER<1) in (i) the intervention group compared with the control group, or (ii) exercise compared with the diet group. For example, TER=0.9 indicates that the biomarker level in the intervention group is on average 10% lower compared with the control group).
‡ p<0.025 is considered significant for the comparison of both intervention groups vs. control.
§ p<0.05 is considered significant for the comparison exercise vs. diet.
hsCRP=high sensitivity C-reactive protein. IL-6=interleukine 6.

levels of leptin[8]. Our decreases in CRP and leptin, therefore, imply that cancer risk can be positively influenced by a modest amount of weight loss.

Strengths of the SHAPE-2 study include the unique design with the aim for a comparable weight loss by either diet or exercise. Another strength of the design is the run-in period with the standardised diet which minimises inequalities in dietary intake of different food components that may influence the outcome. In addition, adherence to the study protocol was high and the drop-out rate was low (5%).

A limitation to our study is that despite the fact that the weight loss target was achieved by both intervention groups, the exercise group lost 0.6 kg more than the diet group. Although, this is a clinically small difference, it might have affected our results slightly. Another limitation is that only one blood sample was taken at baseline and follow-up. Validity of the outcomes could be improved by repeated blood sampling as natural day-to-day variability and extraneous effects can affect inflammatory markers and adipokines[52-55]. Therefore, random misclassification might have diluted our effects. To reduce influences of extraneous effects, we instructed women not to exercise in the 48

Table 5 Treatment effects on inflammatory markers adjusted for change in VO_{2peak} (ml/min)

	TER† (95% CI): Intervention vs. Control	P-value‡	TER† (95% CI): Exercise vs. Diet	P-value§
hsCRP (mg/l)				
Diet	0.78 (0.61 to 1.01)	0.057		
Exercise	0.71 (0.54 to 0.93)	0.014	0.91 (0.73 to 1.13)	0.384
IL-6 (pg/ml)				
Diet	0.92 (0.75 to 1.12)	0.385		
Exercise	0.87 (0.70 to 1.07)	0.183	0.95 (0.80 to 1.12)	0.514
Adiponectin (ng/ml)				
Diet	1.02 (0.97 to 1.07)	0.473		
Exercise	1.04 (0.99 to 1.09)	0.103	1.02 (0.99 to 1.07)	0.230
Leptin (ng/ml)				
Diet	0.58 (0.49 to 0.69)	<0.001		
Exercise	0.56 (0.46 to 0.67)	<0.001	0.96 (0.83 to 1.11)	0.574

† TER: the Treatment Effect Ratio (95% confidence interval) represents the overall intervention effect on change in biomarker (adjusted for baseline biomarker level), estimated by linear regression analysis. The linear regression models were based on log-transformed biomarker data, therefore, the treatment effect is a ratio that indicates how many times the biomarker level is, on average, higher (TER>1) or lower (TER<1) in (i) the intervention group compared with the control group, or (ii) exercise compared with the diet group. For example, TER=0.9 indicates that the biomarker level in the intervention group is on average 10% lower compared with the control group).
‡ p<0.025 is considered significant for the comparison of both intervention groups vs. control.
§ p<0.05 is considered significant for the comparison exercise vs. diet.
hsCRP=high sensitivity C-reactive protein. IL-6=interleukine 6.

hours prior to the blood sampling. Moreover, our observed effects on hsCRP and leptin were larger than reported intra-individual variations[52,54].

In conclusion, a 16-week intervention by diet or exercise induced the target weight loss of 6%-7%, and compared to stable weight controls showed a significant reduction in circulating levels of the inflammatory marker hsCRP and the adipokine leptin. An effect of exercise irrespective of weight loss was only suggested for hsCRP. Body fat and fitness appeared to be mediators in this association.

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7

THE ROLE OF TOTAL BODY FAT AND ABDOMINAL FAT IN RELATION TO BIOMARKERS FOR BREAST CANCER RISK: THE SHAPE-2 TRIAL

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ABSTRACT

Introduction

We assessed the effect of diet- or exercise-induced weight loss interventions on (intra) abdominal fat. Furthermore, we aimed to elucidate the role of total body fat and abdominal fat in relation to breast cancer risk biomarkers, including sex hormones and inflammatory markers.

Methods

The SHAPE-2 study is a three-armed randomised trial in 243 overweight women: diet (n=97), exercise (n=98) or control group (n=48). Both intervention groups aimed for equivalent weight loss (5 to 6 kg). Outcomes after 16 weeks were levels of sex hormones, inflammatory markers and sources of body fat. Abdominal subcutaneous and intra-abdominal fat were measured by magnetic resonance imaging (MRI) and total body fat by dual energy X-ray absorptiometry (DEXA). We applied linear regression analyses to investigate the associations between total body fat or abdominal fat and breast cancer risk biomarkers.

Results

The diet group lost -4.9 kg (-6.1%) and the exercise group -5.5 kg (-6.9%). Subcutaneous abdominal and intra-abdominal fat reduced significantly with both diet (-12.5% and -12%) and exercise (-16.0% and -14.6%). The reduction in subcutaneous fat was significantly larger with exercise. Change in total body fat contributed to the effects on most of the biomarkers: free oestradiol, free testosterone, sex hormone binding globulin, leptin and high sensitive C-reactive protein (hsCRP). Abdominal fat contributed to the effects on changes in hsCRP and leptin in addition to total body fat. Changes in IL-6 were driven by changes in abdominal fat only.

Conclusions

Weight loss of 6-7%, by diet or exercise, reduced abdominal subcutaneous and intra-abdominal fat. Subcutaneous fat reduced to a larger extent with exercise. Furthermore, we found that total body fat and not (intra-)abdominal fat in particular, plays an important role in influencing sex hormones. Whereas in affecting markers of inflammation and adipokines, abdominal fat plays an additional role.

INTRODUCTION

Postmenopausal women who are overweight or obese and physically inactive have an increased risk of several types of cancer, including postmenopausal breast cancer[1-3]. Intervention studies suggest that body fat is an important mediator in this association[4-7], as adipose tissue produces sex hormones, markers of inflammation and adipokines associated with an increased cancer risk. Weight loss, either by diet or exercise, reduces fat tissue and may, therefore, be an option for primary prevention.

The distribution of fat tissue varies between individuals. Abdominal fat has been associated with several chronic diseases such as cardiovascular diseases and diabetes[8-10] and a higher all-cause mortality[11], also independent of total body fat. Abdominal fat consists of subcutaneous adipose tissue and intra-abdominal adipose tissue. Intra-abdominal fat is hypothesised to be most harmful[12-14]. For postmenopausal breast cancer, the role of abdominal fat, and intra-abdominal fat in particular, is less clear. The World Cancer Research Fund judged that overall body fatness is "convincingly", and abdominal fatness is "probably" associated with an increased risk[15]. A limitation to previous research is that proxies were generally used for total and abdominal fat, such as body mass index (BMI) and waist circumference, respectively, or that a cross-sectional design was used, which fails to investigate causal pathways. We are aware of two trials in postmenopausal women that used computed tomography (CT) to investigate the effects of exercise-induced changes in abdominal fat on sex hormones and other breast cancer risk biomarkers. The effects of weight loss on sex hormones and adipokines were moderated[5,16] or mediated[17] by changes in total fat mass, but not by changes in abdominal fat.

The Sex Hormones And Physical Exercise (SHAPE)-2 study is a randomised controlled trial in 243 healthy overweight to obese postmenopausal women. It was designed primarily to investigate the effect of equivalent weight loss, induced with or without exercise, on biomarkers of postmenopausal breast cancer risk. Results on serum sex hormones and inflammatory factors and adipokines are reported in chapters 5 and 6 of this thesis. In short, a 6-7% loss of body weight by both interventions resulted in favourable changes in oestrogens, androgens, sex hormone binding globulin (SHBG), C-reactive protein (CRP) and leptin, but not interleukine (IL)-6 and adiponectin. Effects on free testosterone, SHBG and CRP were most pronounced in the exercise group, compared with diet only, mainly due to a greater amount of total body fat loss in the exercise group (chapter 5 of this thesis).

In the current study, we aim to address the following two aims. First, we investigate whether exercise-induced weight loss reduces (intra-)abdominal fat to a larger extent than comparable diet-induced weight loss. Second, we use the longitudinal data of the SHAPE-2 study to investigate the role of total body fat and abdominal fat in relation to several breast cancer risk biomarkers.

METHODS

Design and study population

The SHAPE-2 study is a three-armed randomised controlled trial conducted from February 2012 to May 2013 in the vicinities of two major cities (Utrecht and Enschede) in the Netherlands. Postmenopausal women (n=243) were randomised to either a diet-induced (n=97) or mainly exercise-induced (n=98) weight loss intervention, or stable weight control group (n=48).

Details of the study design are reported elsewhere[18]. The study was approved by the ethical committee of the University Medical Center Utrecht. All participants provided informed consent.

A random selection of women, aged 50-69 years, was recruited via mass mailings and media attention (Figure 1). Eligible women were postmenopausal (>12 months since last menses); overweight or obese (BMI 25-35 kg/m^2); inactive (<2 h/wk of ≥4 metabolic equivalent (MET) activity); non-smoking; not using exogenous (sex) hormones; and cancer-free and non-diabetic.

Interventions

All women started with a four to six-week run-in period wherein a standardised diet was prescribed, aiming for a stable weight and comparable macronutrient intake among all participants. In the intervention phase, both the diet and exercise intervention groups had the goal of losing 5 to 6 kg body weight in 14 weeks' time. After 14 weeks, or when the target weight loss was reached, women entered a weight maintenance period (two to six weeks) wherein diet was adapted in order to stabilise body weight. Women in the control group were asked to maintain a stable weight by continuing the standardised diet and their habitual physical activity pattern.

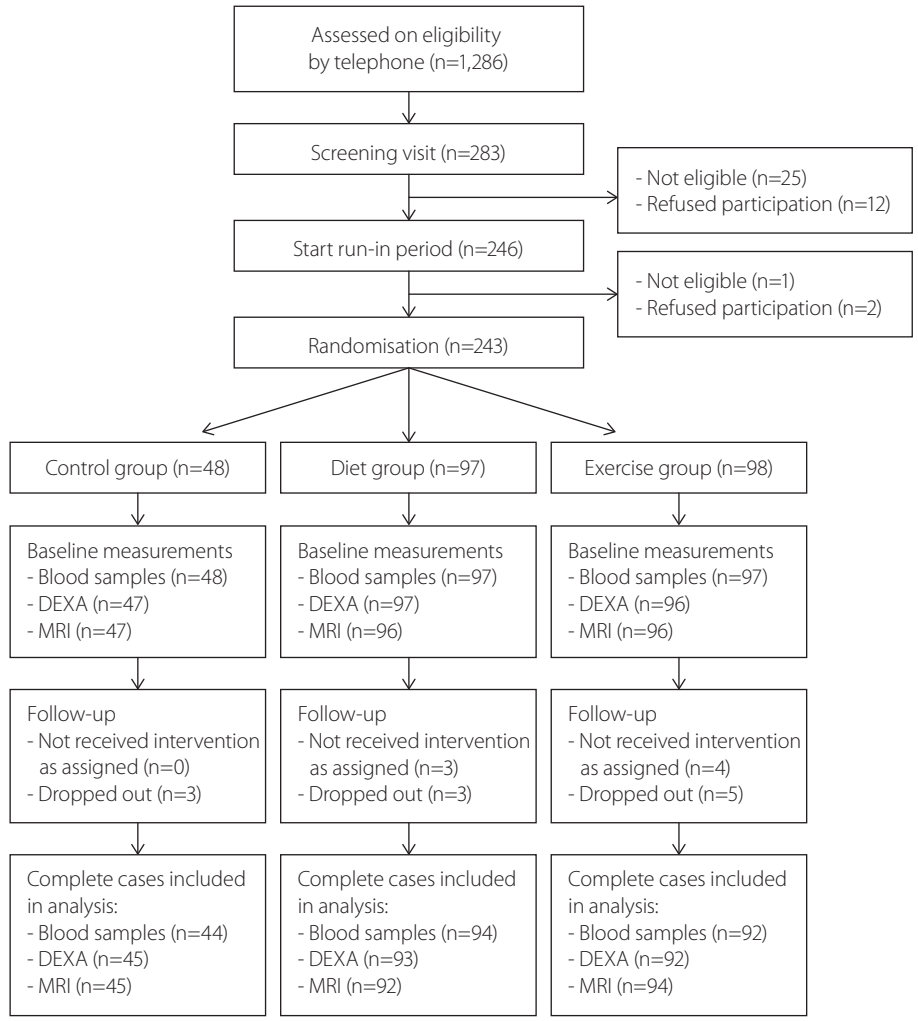
Diet group intervention

Women in the diet group were prescribed a caloric restriction of -500 kcal/day as compared to their estimated needs and habitual intake. They were asked to maintain their habitual physical activity level. Contacts with the dietitian included two 30-min individual consultations and five one-hour group sessions for nutritional education, behaviour change and dietary adaptations if needed. Also, telephone consultations were scheduled every other week for monitoring and motivation[19].

Exercise group intervention

The exercise intervention consisted of two one-hour group sessions at the physiotherapist centre and two one-hour individual sessions of moderate-to-vigorous exercise per week, resulting in an estimated mean energy expenditure of 350 kcal/day. Group sessions included 20-25 minutes of endurance training, 25 minutes of strength training and 5-10

Figure 1 Flow chart of the inclusion, random assignment, and follow-up of the SHAPE-2 study participants



DEXA: Dual energy X-ray absorptiometry. MRI: Magnetic resonance imaging.

minute warm ups and cool downs. Heart rate monitors were worn while exercising. Training intensity was gradually increased during the study (for endurance training: from 60% to 90% of the heart rate reserve (HRR)); for strength training: based on repetition maximum tests). Individual sessions included Nordic Walking at 60-65% HRR. Supervised classes were also organised and recommended. In order to ensure substantial weight loss, a dietitian-prescribed a caloric restriction of 250 kcal/day was added to the programme[20].

Outcome measurements

At baseline (i.e., before randomisation) and after 16 weeks, study participants visited the medical research unit where measurements were taken. Anthropometrics were taken according to standard operating procedures[18]. Total body fat and lean mass were assessed by dual energy X-ray absorptiometry (DEXA) whole-body scan (Lunar Prodigy). Abdominal fat, including subcutaneous abdominal adipose tissue (SAAT) and intra-abdominal adipose tissue (IAAT), was measured by magnetic resonance imaging (MRI, Philips, Ingenia 1.5 T) in breath-hold, with the use of the three-point IDEAL method described by Dixon[21]. For every scan, three slices at the intervertebral levels L2-L3, L3-L4 and L4-L5 were analysed using Hippo Fat software[22] to obtain quantitative fat measures. The measured cm^2 of SAAT and IAAT were averaged for the 3 slices. SAAT and IAAT were summed to obtain total abdominal adipose tissue (TAAT). Fat quantification was performed by two trained researchers (WvG and MS). Baseline and follow-up images of different intervertebral levels of one study participant were analysed by the same researcher. Intra- and inter-class correlation coefficients for both researchers were >0.99 for SAAT and >0.97 for IAAT.

Serum biomarker analyses

Serum was collected and stored at −80°C. After trial completion, all samples were sent, frozen, to the laboratory for analyses. Samples from one individual were analysed in the same batch.

Sex hormones

Oestradiol and testosterone were determined by liquid chromatography-mass spectrometry (LC-MS), the reference standard for measuring sex steroids[23], in the UHSM Manchester laboratory[24]. Free fractions of oestradiol and testosterone were calculated by using the total hormone levels, SHBG and a constant for albumin[25,25]. SHBG was measured by commercially available double-antibody radioimmunoassay kits (Roche Cobas: SHBG-03052001), performed in the SHO Velp laboratory[26]. Inter- and intra-coefficients of variation (CV) were <10% for androgens[27], <7%[28] for oestrogens and <2% for SHBG. Technicians were blinded to study allocation.

Twenty-four samples of testosterone were below the lower limit of detection and were, therefore, assigned the value of this limit: 86 pg/ml. Six oestradiol measures were outside acceptable postmenopausal values (>42 pg/ml) and were excluded (five at baseline, one at follow-up).

Markers of inflammation and adipokines

Enzyme-linked immunosorbent assays (ELISA) were used to measure IL-6 (HS-600B, R&D systems, Inc.), leptin (ME E-0300, LDN®) and adiponectin (RD195023100, BioVendor). High sensitivity CRP (hsCRP) was measured by an immunoturbidimetric assay (CRP Gen.3, Cobas® Roche). Analyses of these four biomarkers were performed at Labor Nord-West in Nordhorn, Germany[29]. Intra-assay coefficients of variation were 2.4% for IL-6, 3.3% for CRP, 3.6% for adiponectin and 2.5% for leptin. IL-6 and hsCRP values were below the limit of detection in five samples, i.e., <0.11 pg/ml (n=2) and <0.2 mg/l (n=3), respectively. These were assigned this value of the lower limit of detection. One hsCRP measurement at follow-up that was >25 mg/l was excluded from analysis as this may indicate a clinical inflammation or infection.

Statistical analyses

Descriptive data of the trial population are presented per study group, as means ±standard deviations (SD), medians and interquartile range (IQR) or geometric means and 95% confidence intervals (95% CI) for the risk markers. Pearson correlation coefficients were computed for change in the different fat measures and each of the biomarkers.

For the first study aim – to investigate the effects of weight loss, with or without exercise on abdominal fat – linear regression was used to estimate the between-group differences for TAAT, SAAT and IAAT, adjusted for baseline fat measure. Outcomes of complete cases (women with measurements at both baseline and follow-up) are presented.

To address the second research aim – which fat depot is most strongly associated with the studied breast cancer risk biomarkers – the study population is analysed as a cohort, regardless of intervention groups in order to make maximum use of the distribution of changes in fat measures. To examine the separate effects of total body fat and abdominal fat linear regression models were built for change in each biomarker (dependent) containing change in one of the fat measures (TAAT or IAAT) and the baseline biomarker level as a covariate (independent variables). We did not separately assess the effects of SAAT because of its high correlation with TAAT (r=0.92). Because we examine several biomarkers, we chose a significance level of <0.01 and corresponding 99% confidence intervals (99% CI) are presented.

To determine which fat depot is more strongly related to a biomarker, regression models were estimated for each biomarker. Stepwise forward selection was applied and the variables 'change in total body fat', 'change in TAAT', and 'change in IAAT' were allowed to enter the model if the corresponding p-value was ≤0.10. The variable 'baseline biomarker level' was entered as a covariate. Changes in biomarkers and changes in fat measures were all normally distributed, thus no transformation was applied. Results are presented as standardised regression coefficients (St-b) which represent the effect of a change in one standard deviation of the fat measure, on the change in standard deviations of the biomarker level. Standardised regression coefficients allow a better comparison

between the effects of different units of measurement. We tested for interactions between the study group and changes in fat measures to see whether or not we should account for the delivered intervention in the models. Furthermore, since we analysed the results in a within-woman design, time-invariant covariates do not confound the results and adjustment for these covariates was deemed unnecessary. All statistical procedures were performed using SPSS software version 20.

RESULTS

At baseline, the three study groups were comparable on main characteristics (Table 1). On average women were 60 years of age, had a BMI of 29.2 kg/m^2 and a body fat percentage of 44%. In total, 232 (95.5%) of the 243 women completed the study (Figure 1). In the diet group, the median number of group sessions attended by women was four (out of five offered). In the exercise group, the median attendance rate of all exercise sessions was 84%.

As reported in chapter 5 of this thesis, body weight decreased by -4.9 kg (-6.1%) and -5.5 kg (-6.9%) in the diet and exercise group, respectively, compared to control (+0.06 kg, 0.1%). Body fat percentage decreased by -2.8% (95% CI: -3.5 to -2.1) with diet, and by -4.4% (95% CI: -5.1 to -3.7) with exercise, versus control. The decrease in fat percentage was significantly greater in the exercise group, compared with diet (difference of -1.56%, 95% CI: -2.14 to -0.98). Lean mass was preserved with exercise (+0.02 kg, 95% CI: -0.42 to0.46), and decreased with diet (-0.71 kg, 95% CI: -1.14 to -0.23), versus control.

Intervention effects on abdominal fat

Figure 2 shows the baseline and end of study (i.e., 16 weeks) means and standard errors of total, subcutaneous and intra-abdominal fat in the three study groups. The control group showed small non-significant changes in TAAT (+1.1%, absolute: +5 cm^2, 95% CI: -4 to 14), SAAT (+1.2%, absolute: +4 cm^2, 95% CI: -4 to 11) and IAAT (+1.6%, absolute: +2 cm^2, 95% CI: -4 to 14) from baseline to end of study.

TAAT, SAAT and IAAT reduced significantly from baseline to end-of-study with both diet and exercise. TAAT reduced by -12.3% with diet and by -15.7% with exercise (absolute -56 cm^2 95% CI: -64 to -48, and -70 cm^2, 95% CI: -79 to 62, respectively). SAAT reduced by -12.5% with diet and by -16% with exercise (absolute -39 cm^2, 95% CI: -14 to 11 cm^2, and -49 cm^2, 95% CI: -18 to -14, respectively). IAAT reduced by -12% with diet, and by -14.6% with exercise (absolute: -17 cm^2, 95% CI: -21 to -13, and -21 cm^2, 95% CI: -24 to -17, respectively). Between-group changes showed that compared with control, TAAT, SAAT and IAAT decreased significantly by both diet and exercise. TAAT, diet: -61 cm^2, 95% CI: -74 to -47, and exercise: -75 cm^2, 95% CI: -89 to -62. SAAT, diet: -42 cm^2, 95% CI: -53 to -32, and exercise: -53 cm^2, 95% CI: -63 to -43. The decreases in TAAT and SAAT were significantly greater in the

Table 1 Baseline characteristics of the SHAPE-2 study population

	Control group (n=48)	Diet group (n=97)	Exercise group (n=98)
	Mean (standard deviation)		
Age (years)	60.0 (4.9)	60.5 (4.6)	59.5 (4.9)
Time since last menses (years)	11.4 (7.8)	10.7 (6.1)	10.9 (7.7)
Education†, number (%)			
Low	15 (31.3%)	27 (27.8%)	33 (33.6%)
Middle	15 (31.3%)	27 (27.8%)	20 (20.4%)
High	18 (37.5%)	42 (43.3%)	44 (44.9%)
First degree family member with breast cancer, number (%)	9 (18.8%)	23 (23.7%)	24 (24.5%)
VO_{2peak} (ml/min)	1751 (363)	1742 (310)	1749 (293)
Weight (kg)	80.9 (10.0)	80.0 (8.6)	80.4 (9.0)
BMI (kg/m^2)	29.5 (2.6)	29.3 (2.5)	29.0 (2.9)
Body composition by DEXA			
Body fat percentage (%)	43.6 (5.0)	44.1 (3.8)	43.8 (4.0)
Total body fat (kg)	34.2 (7.4)	33.9 (5.7)	33.9 (6.2)
Lean mass (kg)	43.4 (3.9)	42.7 (4.0)	43.1 (4.1)
Abdominal fat by MRI			
TAAT (cm^2)	467 (108)	452 (87)	450 (83)
SAAT (cm^2)	316 (82)	310 (68)	307 (59)
IAAT (cm^2)	151 (52)	142 (47)	143 (46)
	Geometric mean (95% confidence interval)		
Free oestradiol (pg/ml)	0.10 (0.08- 0.12)	0.10 (0.08 – 0.11)	0.09 (0.08 – 0.10)
Free testosterone (pg/ml)	2.78 (2.36 - 3.28)	2.54 (2.31 - 2.79)	2.41 (2.21 - 2.63)
SHBG (nmol/l)	45.1 (39.7 - 51.3)	50.1 (45.7 – 55.0)	48.8 (44.7 - 53.3)
hsCRP (mg/l)	1.83 (1.40 - 2.40)	2.04 (1.67 - 2.49)	1.81 (1.49 - 2.19)
IL-6 (pg/ml)	1.31 (1.12 - 1.52)	1.41 (1.27 - 1.55)	1.41 (1.25 - 1.59)
Leptin (ng/ml)	9.3 (8.4 - 10.4)	9.7 (9.1 - 10.3)	9.5 (9.1 - 10.0)
Adiponectin (ng/ml)	27.4 (23.1 - 32.5)	28.5 (25.5 - 32.0)	31.4 (28.1 - 35.2)

Available baseline data: family history of breast cancer, n=241 (99.2%); DEXA scan n=240 (98.8%); MRI scan n=239 (98.4%); VO_{2peak} n=237 (97.5%); alcohol n=226 (93.0%); SQUASH questionnaire n=236 (97.1%); accelerometer n=161 (out of 215 (74.9%)). SHBG, n=242; free oestradiol, n=236 (five baseline values >42 pg/ml were excluded); free testosterone, n=241; CRP, n=241 (one follow-up value>25 mg/l was excluded); IL-6, leptin, adiponectin, n=242. All other data were available for all women (n=243).

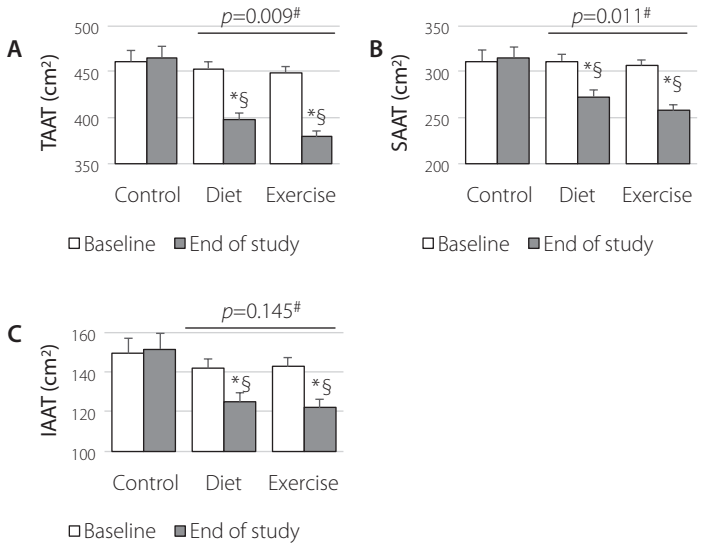
† Education, low: primary school and technical/professional school. Middle: college degree. High: university degree

‡ GT3X+ ActiGraph activity monitor. Minutes/day of activity spent in each activity category. Activity categories are based on Freedson 1998 cut-off points.

§ Activities performed ≥4 metabolic equivalents (MET).

BMI=Body mass index. TAAT=Total abdominal adipose tissue. SAAT=Subcutaneous abdominal adipose tissue. IAAT=Intra-abdominal adipose tissue. SHBG=Sex hormone binding globulin. hsCRP=High sensitive C-reactive protein. IL-6=Interleukine 6.

Figure 2A-C Changes in total abdominal fat (A), subcutaneous abdominal fat (B) and intra-abdominal fat (C)



Baseline and end-of-study means and standard errors (error bars) of (**A**) total abdominal adipose tissue (TAAT, cm^2), (**B**) subcutaneous abdominal adipose tissue (SAAT, cm^2), (**C**) intra-abdominal adipose tissue (IAAT, cm^2).
* p<0.001 compared to the baseline measurement (within-group change).
§ p<0.001 for the change in the intervention group vs. control (between-group change).
# p-value for the comparison in change from baseline to end-of-study between the diet and exercise group (between-group change).

exercise group compared with diet: differences of -15 cm^2 (95% CI: -26 to -4) for TAAT and -11 cm^2 (95% CI: -19 to -2) for SAAT. IAAT decreased to a similar extent in both the diet (-20 cm^2, 95% CI: -27 to -14) and exercise group (-24 cm^2, 95% CI: -30 to -18), compared with control.

Changes in fat measures and biomarkers in the total study population

In the total study population, regardless of intervention group, the mean change (SD) between baseline and 16 weeks' follow-up was for: body weight, -4.2 kg (2.9); for total body fat, -3.5 kg (2.8); for TAAT, -49.9 cm^2 (46.6); for SAAT, -34.8 cm^2 (34.3) and for IAAT, -14.8 cm^2 (20.3). The correlation between change in SAAT and change in IAAT was 0.43 (p<0.001). The mean change (SD) in biomarker levels between baseline and 16 weeks was for free oestradiol, -0.02 pg/ml (0.07); for free testosterone, -0.35 pg/ml (0.87); for SHBG, +6.7

Table 2 Associations between change in fat measure (independent) and change in biomarker level (dependent variable)

	St-b	99% CI	R^2
Δ Free oestradiol			
Δ Body fat (kg)	0.21**	0.07;0.34	0.44
Δ TAAT (cm^2)	0.15*	0.30;0.28	0.42
Δ IAAT (cm^2)	0.07	-0.07;0.20	0.40
Δ Free testosterone			
Δ Body fat (kg)	0.28**	0.13;0.12	0.34
Δ TAAT (cm^2)	0.26**	0.12;0.40	0.33
Δ IAAT (cm^2)	0.19**	0.05;0.33	0.30
Δ SHBG			
Δ Body fat (kg)	-0.41**	-0.56;-0.25	0.20
Δ TAAT (cm^2)	-0.32**	-0.48;-0.16	0.14
Δ IAAT (cm^2)	-0.28**	-0.44;-0.12	0.12
Δ CRP			
Δ Body fat (kg)	0.18*	0.04;0.32	0.34
Δ TAAT (cm^2)	0.17*	0.03;0.31	0.34
Δ IAAT (cm^2)	0.18*	0.04;0.32	0.34
Δ IL-6			
Δ Body fat (kg)	0.09	-0.07;0.25	0.18
Δ TAAT (cm^2)	0.18*	0.02;0.34	0.20
Δ IAAT (cm^2)	0.14	-0.02;0.29	0.19
Δ Leptin			
Δ Body fat (kg)	0.50**	0.39;0.62	0.53
Δ TAAT (cm^2)	0.43**	0.30;0.55	0.46
Δ IAAT (cm^2)	0.31**	0.78;0.44	0.37
Δ Adiponectin			
Δ Body fat (kg)	-0.15	-0.32;0.01	0.06
Δ TAAT (cm^2)	-0.15	-0.31;0.02	0.06
Δ IAAT (cm^2)	-0.10	-0.27;0.06	0.05

*p<0.01, **p<0.001. Models are adjusted for the baseline biomarker level.
A significance level of <0.01 was chosen considering multiple testing. Therefore, corresponding 99% confidence intervals (99% CI) are presented.
Δ: change, defined as follow-up minus baseline measurement.
St-b is the standardised regression coefficient from a linear regression model that represents the standard deviations (SD) change in biomarker level per 1 SD change in fat measure. E.g., as change in total body fat increases with 1 SD, the change in free oestradiol increases with 0.22 SDs.
R^2: percentage of variance in the dependent variable that is explained by the independent variable(s).
TAAT=Total abdominal adipose tissue. SAAT=Subcutaneous abdominal adipose tissue. IAAT=Intra-abdominal adipose tissue. SHBG=Sex hormone binding globulin. hsCRP=High sensitive C-reactive protein. IL-6=Interleukine 6.

nmol/l (12.4); for hsCRP, -0.01 mg/l (4.29); for IL-6, +0.09 pg/ml (1.74); for leptin, -10.6 mg/ml (13.9) and for adiponectin, +0.01 ng/ml (1.23).

The correlation coefficients for changes in the different fat measures and biomarkers ranged between 0.15 and 0.35 (supplemental Table 1A). The changes in different fat measures all correlated significantly with each other ($p<0.001$) (supplemental Table 1B).

Associations between different fat depots and biomarkers

There were no significant interactions between change in fat measures and study group for any of the biomarkers, therefore, group and the interactions were left out of the models. Results of the regression models, with 'change in a single fat measure' and 'baseline biomarker level' as determinants and biomarker as the outcome are presented in Table 2.

Both change in total body fat and abdominal fat were strongly and significantly associated with change in free oestradiol, free testosterone, SHBG, leptin and hsCRP. Leptin had the largest change per SD change in total body fat, standardised regression coefficient (St-b)=0.50, followed by SHBG (St-b=-0.41), free testosterone (St-b=0.28) and free oestradiol (St-b=0.21). Intra-abdominal fat had the weakest associations with all these biomarkers: the highest was for leptin (St-b=0.31); the lowest (and non-significant) for free oestradiol (St-b=0.07).

Change in total body fat, total abdominal and intra-abdominal fat were associated with change in hsCRP, and, in contrast to the above risk biomarkers, to a similar extent (St-b=0.17 or 0.18 for all three fat measures). Change in total abdominal fat, but not intra-abdominal or total body fat, was significantly associated (St-b=0.17) with change in IL-6. None of the fat measures were significantly associated with change in adiponectin, and effects were small (St-b=-0.10 to -0.15).

In the multivariable analysis, with forward stepwise selection for total body fat, TAAT, and IAAT, the final, best fitting models for free oestradiol, free testosterone, SHBG and adiponectin included only total body fat (Supplemental Table 2). The final model for leptin included both total body fat and total abdominal fat. The final model for hsCRP included total body fat and intra-abdominal fat. The final model for IL-6 included only total abdominal fat. The R^2 of the best fitting models was highest for leptin (R^2=0.53) and free oestradiol (R^2=0.44), intermediate for free testosterone, SHBG, CRP and IL-6 (all R^2 were 0.20 to 0.35) and lowest for adiponectin (R^2=0.06).

DISCUSSION

The purpose of this study was twofold. First, we investigated the effects of a diet and exercise intervention, in an RCT design that induced comparable weight loss of 6-7%, on abdominal fat. Total abdominal fat, subcutaneous and intra-abdominal fat all declined

significantly in both the diet and exercise group compared to control. Compared to diet, the decline in total and subcutaneous fat, but not intra-abdominal fat, was significantly larger in the exercise group.

Second, we investigated whether changes in abdominal fat were associated with changes in breast cancer risk biomarkers to a different extent than changes in total body fat. Change in total body fat appeared to play a dominant role in determining sex hormone levels. Whereas abdominal fat contributed to the effects on change in hsCRP and leptin in addition to body fat, and independently to change in IL-6. total fat.

Weight loss trials, evaluating diet interventions with or without exercise in postmenopausal women mainly found that abdominal fat, including subcutaneous and intra-abdominal fat, declined compared with baseline, as we observed[30-32]. In contrast to our findings, these trials found no additional effect of exercise-induced weight loss on abdominal fat compared with diet-induced weight loss.

Nicklas et al randomised 112 postmenopausal women to diet and diet with either moderate or vigorous intensity exercise during 20 weeks[31]. From baseline, abdominal subcutaneous fat decreased by -18% and intra-abdominal fat by -26%; both were statistically significant and comparable across the three study groups. The weight loss was larger than in our trial (-12 kg vs. -5.5 kg), explaining the larger percentages of subcutaneous and intra-abdominal fat losses.

In a randomised trial by Ryan et al. in 77 obese postmenopausal women, six months of diet with or without aerobic exercise induced approximately 7 kg of weight loss[32]. Again, compared with baseline, subcutaneous and intra-abdominal fat declined significantly (both by -15%) and to a similar extent in both weight loss groups. Similar results were found in a smaller trial in 34 obese postmenopausal women wherein the effects of weight loss and resistance training were investigated over 16 weeks[30]. Diet (-5.2 kg body weight) and diet with resistance training (-7.4 kg) led to comparable changes in abdominal subcutaneous fat (both -18%) and intra-abdominal fat (both -22%)[30].

Possible explanations for the fact that we observed greater losses of subcutaneous abdominal fat in the exercise group (-16%) compared with diet (-12.5%) could be that women in our trials were prescribed four hours of exercise per week, instead of three hours as in the above trials. Furthermore, women in the exercise group in our trial lost 0.6 kg more body weight than the diet group, which also might have affected the results slightly.

We did not observe a larger reduction in intra-abdominal fat of exercise- compared to diet-induced weight loss, similar to the above trials. This is in contrast with the findings of two meta-analyses suggesting that exercise may cause visceral fat reductions, irrespective of weight loss[33,34]. These meta-analyses included studies (RCT's and non-randomised intervention studies) in adult men and women of all ages, so results may not be representative for our specific population. The preferable lifestyle-related method for optimal intra-abdominal fat loss is still debated[14].

Body fat produces several markers of inflammation and adipokines[35,36]. Metabolic risk profiles may vary between individuals with equal amounts of body fat. Metabolically unhealthy patients appear to have more visceral fat tissue than healthy counterparts[37]. Abdominal fat, especially intra-abdominal, is thought to be more metabolically active and harmful as it drains directly to the portal circulation leading to more negative health effects[38,39].

Several cross-sectional studies have found associations between CT- or MRI-assessed abdominal fat (both subcutaneous and intra-abdominal) and elevated markers of inflammation and adipokines, also independent of total body fat[32,40-46]. In the Framingham Heart Study, cross-sectional associations were found for both CT-measured subcutaneous and intra-abdominal fat and several markers of inflammation including CRP, IL-6[43] and adiponectin[40]. Another cross-sectional study in 1200 obese subjects found that subcutaneous and intra-abdominal fat, measured by MRI, were associated with adiponectin, CRP and leptin. In general, there was no evidence for a particular abdominal fat depot being of more importance for markers of inflammation[41]. In our study, changes in abdominal fat were also associated with changes in hsCRP, leptin and IL-6.

Sex hormones are associated with an increased breast cancer risk[47,48]. After menopause, the main source of sex hormones is fat tissue[49]. The role of body fat distribution in influencing sex hormone levels is however still unclear. Excess abdominal fat could independently increase bioavailable sex hormone levels as it is associated with hyperinsulinaemia which inhibits SHBG production[50,51].

Results from cross-sectional studies investigating the associations between sex hormones and abdominal fat (mostly measured by DEXA or waist circumference) in postmenopausal women are mixed[52-55]. However, it is known that menopausal-related change in hormone status may also influence fat distribution. Therefore, longitudinal research with interventions that induce changes in abdominal fat, as the SHAPE-2 trial, are needed to unravel the causal mechanism.

To our knowledge, only two other longitudinal intervention studies were performed in healthy postmenopausal women that explored the role of abdominal fat in the relation between exercise-induced weight loss and sex hormones[5,16,17]. Although results are difficult to compare, their findings also suggest that total body fat plays a more important role in determining sex hormone levels than specifically abdominal fat.

Strengths of our study include the relatively large sample size and the randomised design with two different intervention arms aiming for equivalent weight loss. Furthermore, the longitudinal data on intended weight loss enabled the investigation of a causal association between changes in different fat depots and changes in breast cancer risk biomarkers.

Another strength is the use of precise and valid standard methods to measure changes in body composition and abdominal fat, i.e., by DEXA and MRI[56] and changes in biomarkers, i.e., the LC-MS method for sex hormones[57]. Furthermore, subcutaneous and intra-abdominal fat quantifications were obtained from three MRI slices in different levels of the abdomen, instead of single slice analysis which is often used. This more precise method is important when measuring specific changes in abdominal fat.

A limitation to our study is that despite the aim for equivalent weight loss, the exercise group experienced a 0.6 kg greater weight loss than the diet group. This may have slightly affected the results for the first study aim. Furthermore, a longer intervention or follow-up duration might be necessary to show potential effects of exercise-induced weight loss above weight loss by diet only.

In conclusion, we found that a 6-7% weight loss in healthy and overweight-to-obese postmenopausal women led to decreases in abdominal fat, both subcutaneous and intra-abdominal. Weight loss mainly induced by exercise produced greater changes in total abdominal fat, but not intra-abdominal fat, when compared to diet only. Furthermore, we found that a change in total body fat, more than a change in abdominal fat, affected sex hormone levels. Whereas change in abdominal fat seemed of more importance in influencing markers of inflammation and adipokines.

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Supplemental table 1A Pearson correlation coefficients between change in biomarkers and change in fat measures

	Δ Total body fat (kg)	Δ TAAT	Δ SAAT	Δ IAAT
Δ Free oestradiol	0.18‡	0.11	0.11	0.07
Δ Free testosterone	0.27†	0.27†	0.26†	0.18†
Δ SHBG	-0.39†	-0.31†	-0.27†	-0.30†
Δ hsCRP	0.13§	0.16§	0.12	0.19‡
Δ IL-6	0.06	0.16§	0.13	0.16§
Δ Leptin	0.49†	0.37†	0.34†	0.29†
Δ Adiponectin	-0.14§	-0.15§	-0.13§	-0.11

Supplemental table 1B Pearson correlation coefficients between change in different fat measures

	Δ Total body fat (kg)	Δ TAAT	Δ SAAT
Δ Total body fat (kg)	-	-	-
Δ TAAT	0.76†	-	-
Δ SAAT	0.72†	0.92†	-
Δ IAAT	0.54†	0.74†	0.43†

† p<0.001. ‡ p<0.01 *§ p<0.05

Δ: Change, defined as follow-up minus baseline measurement.

TAAT= total abdominal adipose tissue. SAAT= subcutaneous abdominal adipose tissue. IAAT=intra-abdominal adipose tissue. SHBG= sex hormone binding globulin. hsCRP=high sensitive C-reactive protein. IL=6=interleukine 6

Supplemental table 2 Best fitting final model explaining the different breast cancer risk biomarkers

	St-b	95% CI	R^2
Δ Free oestradiol			
Δ Body fat (kg)	0.21	0.11;0.31	0.44
Δ TAAT (cm2)			
Δ IAAT (cm2)			
Δ Free testosterone			
Δ Body fat (kg)	0.28	0.17;0.38	0.34
Δ TAAT (cm2)			
Δ IAAT (cm2)			
Δ SHBG			
Δ Body fat (kg)	-0.41	-0.53;0.29	0.20
Δ TAAT (cm2)			
Δ IAAT (cm2)			
Δ CRP			
Δ Body fat (kg)	0.11	-0.01;0.24	0.35
Δ TAAT (cm2)			
Δ IAAT (cm2)	0.12	-0.001;0.25	
Δ IL-6			
Δ Body fat (kg)			
Δ TAAT (cm2)	0.18	0.06;0.30	0.20
Δ IAAT (cm2)			
Δ Leptin†			
Δ Body fat (kg)	0.41	0.28;0.55	0.53
Δ TAAT (cm2)	0.12	-0.02;0.25	
Δ IAAT (cm2)			
Δ Adiponectin			
Δ Body fat (kg)	-0.15	-0.28;-0.03	0.06
Δ TAAT (cm2)			
Δ IAAT (cm2)			

Linear regression with forward stepwise model selection, p-in=0.10. Variables that were allowed to enter the model: total body fat (by DEXA scan) (kg), total abdominal adipose tissue (TAAT, cm^2) and intra-abdominal adipose tissue (IAAT, cm^2). Models are adjusted for the baseline biomarker level.

Δ: Change, defined as follow-up minus baseline measurement. Change in fat measure is the independent variable, change in biomarker level is the dependent variable.

St-b: Standardised regression coefficient with a 95% confidence interval.

R^2: percentage of variance in the dependent variable that is explained by the independent variable(s).

† The separate regression coefficients should be considered with caution since the high correlation between body fat and TAAT (*r*=0.76) can cause multicollinearity.

SHBG=sex hormone binding globulin. hsCRP=high sensitive C-reactive protein. IL-6=interleukine 6.

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8

QUALITY OF LIFE AFTER DIET OR EXERCISE-INDUCED WEIGHT LOSS IN OVERWEIGHT TO OBESE POSTMENOPAUSAL WOMEN: THE SHAPE-2 TRIAL

Submitted

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ABSTRACT

Introduction

This study investigates the effect of a modest weight loss either by a calorie restricted diet or mainly by increased physical exercise on health-related quality of life (HRQoL) in overweight-to-obese and inactive postmenopausal women. We hypothesise that HRQoL improves with weight loss, and that exercise-induced weight loss is more effective for this than diet-induced weight loss.

Methods

The SHAPE-2 trial was primarily designed to evaluate any additional effect of weight loss by exercise compared with a comparable amount of weight loss by diet on biomarkers relevant for breast cancer risk. In the present analysis we focus on HRQoL. We randomly assigned 243 eligible women to a diet (n=97), exercise (n=98), or control group (n=48). Both interventions aimed for 5 to 6 kg weight loss. HRQoL was measured at baseline and after 16 weeks by the SF-36 questionnaire.

Results

Data of 214 women were available for analysis. Weight loss was 4.9 kg and 5.5 kg with diet and exercise, respectively. Scores of the SF-36 domain '*health change*' increased significantly by 8.8 points (95% CI: 1.6 to 16.1) with diet, and by 20.5 points (95% CI: 13.2 to 27.7) with exercise when compared with control. Direct comparison of diet and exercise showed a statistically significantly stronger improvement with exercise. Both intervention groups showed a tendency towards improvements in most other domains, which were more pronounced in the exercise group, but not statistically different from control or each other.

Conclusions

In overweight-to-obese and inactive postmenopausal women a reduction of body weight showed improvements in physical and mental HRQoL domains, but results were not statistically significant in either the diet or exercise group. However, a modest weight loss does lead to a positive change in self-perceived health status. This effect was significantly larger with exercise-induced weight loss than with comparable diet-induced weight loss.

INTRODUCTION

Obesity is a growing global public health problem[1]. According to the World Health Organisation, the worldwide prevalence of obesity has doubled since 1980[2]. Obese individuals are at an increased risk of chronic diseases such as diabetes, cardiovascular diseases and cancer[3]. Furthermore, obesity has been associated with a lower health-related quality of life (HRQoL)[4]. Negative effects on quality of life are more pronounced in women than men[5]. And since postmenopausal breast cancer is related to obesity, and breast cancer worldwide is the most frequent cancer type, this age group is a relevant population to study.

A review evaluating nine studies in postmenopausal women found that obese women report a lower HRQoL compared with their lean counterparts[6]. Lifestyle interventions inducing weight loss may therefore provide a self-evident option to improve HRQoL in an overweight to obese population. However, even though some trials observed improvements in HRQoL after reduction in body weight, others did not[7].

Little is known about whether positive effects on quality of life are due to the weight loss or due to the related change in lifestyle[8]. Exercise seems to have a positive influence on HRQoL, also independent of related weight loss[9-12].

The Sex Hormones and Physical Exercise (SHAPE)-2 study was designed to investigate the effect of weight loss, with or without exercise, on health outcomes related to breast cancer risk in postmenopausal women. In this paper, we address the effects on HRQoL. We hypothesise that modest weight loss results in improvements in HRQoL and that effects are larger when weight loss is mainly achieved by exercise compared with an equivalent weight loss induced by diet only.

METHODS

The SHAPE-2 study is a three-armed randomised controlled trial that was conducted in eight municipalities surrounding two research centres in the Netherlands from February 2012 to May 2013. The study protocol was approved by the Medical Ethics Committee of University Medical Centre Utrecht. All participants provided signed informed consent. Detailed methods have been described elsewhere (chapter 4 of this thesis)[13].

In short, women were recruited from the general population by mass mailings and media attention. Inclusion criteria were: aged 50-69 years; postmenopausal (>12 months after last menses); BMI 25-35 kg/m^2; an inactive lifestyle (<2 h/wk of at least moderate intensity activities, ≥4 metabolic equivalent (MET)). Main exclusion criteria were: use of sex hormones; diabetes; smoking; ever diagnosed with breast cancer or other cancer types in the past five years.

All women started with a four to six-week run-in period, wherein a standardised diet was prescribed aiming to stabilise body weight and achieve similarity in diet composition among the study participants. In the intervention period, women were randomised to a diet group (n=97); an exercise group (n=98) or a waiting list control group (n=48). The aim for women in both intervention groups was to lose 5-6 kg of weight. After the weight loss target was reached, or after a maximum of 14 weeks, women entered a maintenance period wherein stable weight was aimed for.

Interventions

The diet group intervention consisted of an energy restricted diet (-500 kcal/day). Dietary composition was comparable to the run-in diet. The diet intervention was provided by a dietitian and consisted of two individual 30 minutes sessions, five one-hour interactive group sessions and eight follow-up telephone calls. The sessions consisted of nutrition education, behaviour change techniques and self-management training[14,15]. Women were requested to maintain their habitual physical activity level.

The exercise group followed a 16-week combined aerobic and strength exercise programme. The programme consisted of four hours/week moderate-to-vigorous intensity exercise, resulting in an estimated average energy expenditure of 350 kcal/day. Per week, two one-hour fitness group sessions at a physiotherapist centre were scheduled (60%-95% of the heart rate reserve (HRR)) and two additional hours of Nordic walking (60%-65% HRR). Participants in the exercise group also received an energy intake restriction of -250 kcal/day during the weight loss period. During the maintenance period, women continued exercising and their diet was adjusted to meet their energy needs in order to keep their weight stable.

Women in the control group were requested to retain their body weight by continuing the run-in diet and to maintain their habitual exercise level. Controls were offered an alternative weight loss programme after completion of the study.

Outcomes and measurements

Study participants visited the research centre for measurements at baseline (i.e., randomisation) and after 16 weeks of intervention. Information on sociodemographic variables and general health were assessed by questionnaires. Anthropometric measures were taken according to standard procedures. A total body dual-energy X-ray absorptiometry (DEXA) scan (Lunar, Prodigy™) was performed to measure body composition (fat and lean mass). Cardio respiratory fitness, expressed as VO_{2peak}, was measured by performing a maximal cycle exercise test. VO_{2peak} is described as the highest 15-second average of VO_2 uptake at the end of the test period.

HRQoL was assessed by the 36-item Short-Form Health Survey (SF-36)[16]. The SF-36 is a generic questionnaire to measure health status and consists of eight dimensions: physical functioning (10 items); role limitations by physical problems (4 items); bodily pain

(2 items); general health perceptions (5 items); vitality (4 items); social role functioning (2 items); role limitations by emotional problems (3 items) and mental health (5 items). For each dimension, a weighted total score is calculated, ranging from 0 to 100. A higher score on the scale indicates a better health status[16]. Based on the 8 domains, two component summary scores are calculated, i.e., the Physical Component Score (PCS, first 5 domains) and a Mental Component Score (MCS, last 5 domains)[17]. The domain 'vitality' and 'general health' are both included in the PCS and MCS. The scores are sex-standardised and represent health scores of, in our case, the Dutch population with a mean of 50, and a standard deviation of 10. For example, a PCS score of 60 indicates that physical health is improved with one standard deviation in our study population compared to the general female population.

An additional question in the SF-36 is on '*health change*' (1 item) and is not included in the summary scores. It asks participants to report on their self-perceived health by comparing it to one year ago[16]. The range is from 0 to 100, and a score of 50 means no change in perceived health. A score below 50 denotes deterioration and scores over 50 represent improvement.

Domains of the SF-36 have a high internal consistency: Cronbach's α range from 0.71 to 0.93 in a Dutch population[18] and were over 0.8 in an elderly population[19]. The construct validity ranges from 0.31 to 0.71[18].

Statistical analyses

Descriptive statistics are presented as mean and standard deviation or median and interquartile range. The main analyses are performed according to the intention-to-treat principle. Complete cases, i.e., women who filled in a questionnaire both at baseline and end-of-study, are included and presented. Linear regression analysis was used to investigate differences in SF-36 scores per domain between the three study groups, and regression coefficients (β) with 95% confidence intervals (95% CI) are presented. Estimates of effect sizes by Cohen's *d* were calculated for the regression coefficients. Cohen's *d* represents the standardised mean difference between two group means. An effect size of 0.5 therefore indicates that the group mean was 0.5 standard deviations higher than the reference group, i.e., the control group, or the diet group in the comparison exercise versus diet[20]. Values below 0.2 are considered small, around 0.5 are considered medium, and large when over 0.8.

Additional analyses were done to study effects of weight loss and change in fitness (as independent variables), regardless of the group assignment. Bi-variable and multivariable regression analyses were used. Multivariable models were adjusted for intervention group, age, education, baseline weight and baseline SF-36 score (model 2). Additionally, the model was adjusted for change in physical fitness (VO_{2peak}) or change in weight (depending on the determinant of interest, i.e., change in weight or physical fitness, respectively) (model 3).

All analyses were performed using SPSS software (version 21.0). An alpha level of 0.05 for two-sided tests was chosen as significant.

RESULTS

In total, 243 women were randomised (Figure 1) of which complete case HRQoL data of 214 (88.1%) women was available and used for the current analysis. Participants were on average 60 years old, had a body weight of 80 kg and had a BMI of 29.2 kg/m^2 (Table 1). There were no differences in baseline characteristics between the study groups.

The results of the SHAPE-2 trial on body composition are reported extensively in chapter 5 of this thesis. In short, the diet and the exercise group both lost the aimed body weight (-4.9, 95% CI: -5.4 to -4.4, and -5.5 kg, 95% CI: -5.9 to -5.1, respectively), whereas the control group remained weight stable (+0.06 kg, 95% CI: -0.34 to -0.46). Body fat percentage changed by -4.1% (95% CI: -4.6 to -3.7) in the exercise group, by -2.5% (95% CI: -3.0 to -2.1) in the diet group and by +0.2% (95% CI: -0.2 to 0.6) in controls. Fitness (VO_{2peak}), increased significantly in the exercise group (+119 ml/min, 95% CI: 80.5 to 157.0), and showed non-significant decreases with diet (-44.9 ml/min, 95% CI: -82.5 to -7.4) and control (-78.6 ml/min, 95% CI: -133.3 to -23.8). The median number of group sessions attended by women in the diet group was four (out of five offered). In the exercise group, the median attendance of all exercise sessions was 84%.

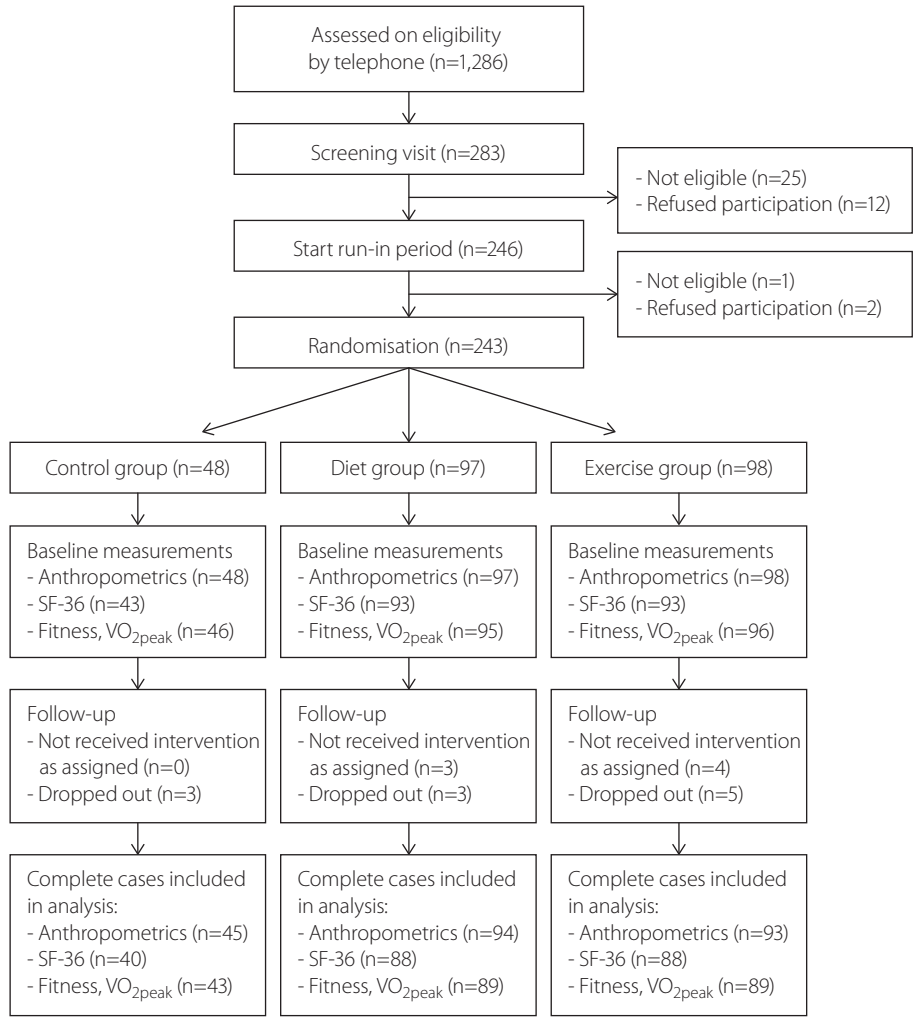
Intervention effects on HRQoL domains

Baseline domain scores of the SF-36 were comparable in the three study groups (Table 1). At baseline scores for physical functioning, role physical and role emotional were slightly higher in the control group compared with both intervention groups.

Except for *health change*, no significant differences in change in HRQoL were observed when comparing the intervention groups to control (Table 2). Only self-perceived health (domain *health change)* increased significantly in both intervention groups versus control (regression coefficient (β)=8.8, 95% CI: 1.5 to 16.1, for diet and β=20.5, 95% CI: 13.2 to 27.7, for exercise). The improvement was larger in the exercise group when directly compared to diet (β=11.7, 95% CI: 5.9 to 17.4). Also for all other domains, the exercise group showed larger improvements when compared with the diet group, except for general health. However, none of these differences were statistically significant.

The effect sizes of differences in HRQoL between the study groups were small to medium for all SF-36 domains, except for *health change* which had medium to large effect sizes for all comparisons, with the largest effect size in the exercise group versus control of 1.18.

Figure 1 Flow-chart of the inclusion, random assignment, and follow-up of the SHAPE-2 study participants



Associations between change in weight or physical fitness and change in HRQoL in the total study population

Both weight loss and an increase in fitness level were significantly associated with an increase in *health change* score (β=3.3, 95% CI: 2.3 to 4.3, and β=0.3, 95% CI: 0.2 to 0.5, respectively, in the unadjusted models (model 1)). After adjustment for covariates, these associations slightly attenuated but remained significant.

Table 1 Baseline characteristics of the SHAPE-2 study participants

	Control group (n=48)	Diet group (n=97)	Exercise group (n=98)
	Mean (standard deviation)		
Age (years)	60.0 (4.9)	60.5 (4.6)	59.5 (4.9)
Time since last menses (years)	11.4 (7.8)	10.7 (6.1)	10.9 (7.7)
Education†, number (%)			
Low	15 (31.3%)	27 (27.8%)	33 (33.6%)
Middle	15 (31.3%)	27 (27.8%)	20 (20.4%)
High	18 (37.5%)	42 (43.3%)	44 (44.9%)
Married or living with partner, number (%)	37 (77.1%)	77 (81.1%)	73 (75.3%)
Weight (kg)	80.9 (10.0)	80.0 (8.6)	80.4 (9.0)
BMI (kg/m^2)	29.5 (2.6)	29.3 (2.5)	29.0 (2.9)
Body fat percentage (%)	43.6 (5.0)	44.1 (3.8)	43.8 (4.0)
Total body fat (kg)	34.2 (7.4)	33.9 (5.7)	33.9 (6.2)
Lean mass (kg)	43.4 (3.9)	42.7 (4.0)	43.1 (4.1)
$VO_{2peak,}$ relative (ml/kg/min)	22.1 (4.7)	21.9 (4.0)	21.8 (3.7)
VO_{2peak} (ml/min)	1751 (363)	1742 (310)	1749 (293)
Physical activity, by activity monitor ‡ (min/day)	*Median (interquartile range)*		
Sedentary	652 (600 - 691)	637 (606 - 685)	630 (593 - 678)
Light	179 (164 - 226)	194 (175 - 214)	197 (157 - 229)
Moderate	35 (25 - 39)	35 (22 - 46)	33 (27 - 46)
Vigorous	0.3 (0.2 - 0.6)	0.4 (0.2 - 0.5)	0.3 (0.1 - 0.5)
SQUASH moderate and vigorous activity § (min/wk)	270 (120 - 495)	184 (115 - 420)	248 (90 - 465)
SF-36 domains	*Mean (standard deviation)*		
1-Physical functioning	89.0 (10.3)	85.1 (15.2)	86.9 (10.5)
2-Role-physical	89.5 (24.5)	84.7 (31.7)	87.8 (27.4)
3-Bodily pain	84.1 (20.3)	83.3 (19.3)	82.9 (18.4)
4-General health	72.9 (14.4)	71.3 (14.4)	72.0 (14.4)
5-Vitality	69.3 (12.5)	68.9 (15.8)	69.8 (14.9)
6-Social functioning	88.4 (16.5)	87.5 (17.1)	87.4 (16.5)
7-Role-emotional	95.4 (18.7)	87.8 (30.2)	90.6 (25.8)
8-Mental health	78.2 (15.6)	79.6 (12.2)	77.2 (12.9)
PCS	53.5 (5.2)	52.3 (7.3)	53.1 (6.1)
MCS	52.8 (6.8)	52.7 (8.1)	52.2 (8.0)
9-Health change	52.3 (17.9)	51.1 (16.9)	50.3 (13.0)

Table 1 Continued

Data available for: VO_{2peak} n=237 (97.5%); alcohol intake, n=226 (93.0%); SQUASH physical activity questionnaire, n=236 (97.1%); ActiGraph accelerometer, n=161 (out of 215 (74.9%)). For SF-36: domains social functioning, health change, PCS and MCS, n=229 (94.2%, control group, n=43; diet group, n=93; exercise group, n=93); physical functioning, role-physical and role-emotional, n=228 (93.8%); bodily pain, n=227 (93.4%).

† Education, low: primary school and technical/professional school. Middle: college degree. High: university degree

‡ GT3X+ ActiGraph activity monitor. Minutes/day of activity spent in each activity category. Activity categories are based on Freedson 1998 cut-off points.

§ Activities performed ≥4 METs.

HRQoL=Health-related quality of life. BMI=Body mass index. PCS=Physical Component Score (SF-36 domains 1-5). MCS=Mental Component Score (SF-36 domains 4-8).

Although the coefficients of both change in weight and change in fitness were pointing in the hypothesised direction for the physical and mental component summary scores (i.e., weight loss and increased physical activity increase summary scores), the effects were small and not statistically significant.

DISCUSSION

We investigated the effects of comparable exercise- and diet-induced weight loss (6-7%) on HRQoL, among overweight and inactive postmenopausal women. Only self-perceived health status improved in comparison to one year ago (referring to the period before study enrolment). The effect is medium to large, according to Cohen's *d*, and statistically significant in both intervention groups versus control. Moreover, the improvement was significantly larger in the exercise group when compared to diet. Although most domains of HRQoL improved with diet and exercise, and improvements were on average larger in the exercise group, none of these improvements were statistically significant and effects were small to medium according to Cohen's *d* effect size.

A meta-analysis by Warkentin et al. evaluated 53 RCTs wherein weight loss was induced by several modalities in different types of populations[7]. They concluded that weight loss may increase physical, but not mental health. However, the authors concluded that compelling evidence for the association between weight loss and HRQoL is still lacking as many studies, in line with our results, do not find significant effects of weight reduction[7].

The only effect we observed that was large and significant was on self-perceived health (domain *health change)*. However, other domains of the SF-36 showed small to moderate and non-significant results. This largely null result could be explained by a ceiling effect. We included a rather healthy group of women who scored relatively high at baseline on all SF-36 domains, meaning there was not much room for improvement.

Table 2 Baseline and 16-week differences in HRQoL and intervention effects between study groups

	Baseline mean (SD)	16 weeks mean (SD)	Change 16 weeks (%)	Treatment effect (95% CI): Intervention vs Control	P-value	Cohen's *d* effect size	Treatment effect (95% CI): Exercise vs Diet	P-value	Cohen's *d* effect size
1-Physical functioning (n=211)									
Control	90.0 (9.5)	90.0 (11.5)	0.0 (0.0)						
Diet	85.6 (15.1)	87.9 (13.8)	2.3 (2.7)	0.77 (-2.74;4.28)	0.67	0.08			
Exercise	87.0 (10.5)	91.0 (11.1)	4.0 (4.6)	2.97 (-0.51;6.46)	0.09	0.33	2.20 (-0.55;4.95)	0.12	0.24
2-Role-physical (n=211)									
Control	91.0 (21.1)	89.1 (27.4)	-1.9 (2.1)						
Diet	85.9 (30.2)	87.1 (29.0)	1.2 (1.4)	-0.91 (-11.0;9.17)	0.86	0.03			
Exercise	88.2 (26.4)	91.4 (24.7)	3.2 (3.6)	2.89 (-7.14;12.93)	0.57	0.11	3.81 (-4.13;11.8)	0.35	0.15
3-Bodily pain (n=208)									
Control	86.7 (18.7)	89.7 (14.7)	3.0 (3.4)						
Diet	85.0 (17.8)	83.5 (21.7)	-1.5 (1.8)	-5.41 (-11.8;1.00)	0.10	0.33			
Exercise	83.4 (17.9)	86.4 (16.6)	3.0 (3.6)	-1.88 (-8.27;4.51)	0.56	0.11	3.53 (-1.55;8.62)	0.17	0.21
4-General health (n=209)									
Control	73.8 (13.8)	73.4 (14.3)	-0.4 (0.5)						
Diet	71.8 (14.4)	74.8 (14.7)	3.0 (4.2)	2.77 (-1.48;7.01)	0.20	0.26			
Exercise	72.0 (14.7)	74.7 (14.8)	2.7 (3.8)	2.52 (-1.69;6.74)	0.24	0.23	-0.24 (-3.52;3.04)	0.88	0.02
5-Vitality (n=210)									
Control	71.2 (11.0)	70.7 (14.7)	-0.5 (0.7)						
Diet	69.4 (15.6)	70.5 (17.1)	1.1 (1.6)	0.86 (-4.22;5.95)	0.74	0.07			
Exercise	69.8 (15.1)	72.3 (15.0)	2.5 (3.6)	2.46 (-2.60;7.51)	0.34	0.19	1.59 (-2.33;5.52)	0.42	0.12
6-Social functioning (n=214)									
Control	90.1 (16.0)	90.4 (16.1)	0.3 (0.3)						
Diet	88.1 (16.7)	86.2 (20.5)	-1.9 (2.2)	-3.37 (-9.49;2.75)	0.28	0.21			
Exercise	87.2 (16.8)	89.1 (14.4)	1.9 (2.2)	-0.16 (-6.27;5.96)	0.96	0.01	3.21 (-1.59;8.00)	0.19	0.20
7-Role-emotional (n=210)									
Control	97.4 (11.8)	95.7 (13.6)	-1.7 (1.7)						
Diet	87.7 (30.1)	89.3 (29.8)	1.6 (1.8)	-3.65 (-12.4;5.05)	0.41	0.16			
Exercise	90.0 (26.5)	93.1 (20.4)	3.1 (3.4)	-0.50 (-9.13;8.12)	0.91	0.02	3.15 (-3.67;9.96)	0.36	0.14
8-Mental health (n=210)									
Control	80.3 (14.0)	79.9 (12.4)	-0.4 (0.5)						
Diet	79.8 (12.1)	80.4 (13.7)	0.6 (0.8)	0.75 (-3.42;4.93)	0.72	0.07			
Exercise	77.2 (13.0)	79.8 (12.4)	2.6 (3.4)	1.62 (-2.55;5.79)	0.44	0.15	0.87 (-2.37;4.11)	0.60	0.08
PCS (n=214)									
Control	54.0 (5.1)	54.5 (6.8)	0.5 (0.9)						
Diet	52.6 (7.2)	53.3 (6.5)	0.7 (1.3)	-0.39 (-2.34;1.56)	0.70	0.08			

Table 2 Continued

	Baseline mean (SD)	16 weeks mean (SD)	Change 16 weeks (%)	Treatment effect (95% CI): Intervention vs Control	P-value	Cohen's *d* effect size	Treatment effect (95% CI): Exercise vs Diet	P-value	Cohen's *d* effect size
MCS (n=214)									
Control	53.7 (6.2)	53.2 (6.6)	-0.5 (0.9)						
Diet	52.7 (8.0)	52.6 (8.2)	-0.1 (0.2)	-0.11 (-2.46;2.23)	0.92	0.02			
Exercise	52.1 (8.2)	52.9 (6.8)	0.8 (1.5)	0.49 (-1.86;2.84)	0.68	0.08	0.60 (-1.24;2.45)	0.52	0.10
9-Health change (n=214)									
Control	53.8 (17.7)	52.6 (17.0)	-1.2 (2.2)						
Diet	51.4 (17.2)	61.8 (20.5)	10.4 (20)	8.81 (1.55;16.1)	0.02	0.53			
Exercise	50.3 (12.9)	73.6 (19.1)	23.3 (46)	20.5 (13.2;27.7)	<0.01	1.18	11.7 (5.91;17.4)	<0.01	0.65

Complete cases are presented, i.e., women who filled in a questionnaire both at baseline and follow-up, Therefore, baseline scores may differ from the baseline scores as presented in table 1.
*n=214 (88.1%) (control group, n=39; diet group, n=87; exercise group, n=88). Linear regression with adjustment for the baseline SF-36 domain score.

HRQoL=Health-related quality of life. PCS=Physical Component Score (SF-36 domains 1-5). MCS=Mental Component Score (SF-36 domains 4-8).

Intervention studies in older women with physical impairments show generally lower baseline HRQoL scores and weight loss results in better improvements[21,22]. Furthermore, despite its validation, the SF-36 is a generic HRQoL questionnaire which might not be specific enough to detect subtle changes in our study population.

The strengths of this study are the randomised controlled design and the relatively large study population. Furthermore, there was high adherence in both intervention groups to the different weight loss programmes. Moreover, the design enabled us to study effects of different intervention methods, because the amount of weight loss was comparable in both groups.

Our weight loss, though, was rather modest, but some trials found small but significant effects on HRQoL after modest weight loss in a population of healthy postmenopausal women[23-27]. Two large RCTs, the NEW trial[23] and a trial by Villareal et al.[26], investigated the individual and combined effects of a hypocaloric diet and/or exercise interventions on HRQoL during one year in healthy older women. In both trials improvements in HRQoL were observed in the intervention groups that concordantly lost body weight, that is, the diet-only and combined diet and exercise intervention group.

In the NEW trial, the combined diet and exercise intervention group showed improvements in five SF-36 domain scores, whereas the diet-only group only improved in two domains including vitality and mental health[23]. In contrast to our current study the NEW trial did not aim for a specific and equivalent weight loss across the intervention groups. This combined intervention group lost approximately -9 kg (-10.8%) body weight whereas the diet-only lost -7 kg (-8.5%). In the exercise only intervention group of the NEW trial (-2 kg, -2.4% weight loss) no effects were observed on HRQoL.

Villareal et al. reported on the effects of the interventions on the Physical and Mental Component Summary scores only. Both the diet and combined diet plus exercise groups achieved a weight loss of 9-10% and showed the same significant improvement in the PCS of the SF-36[26]. No effect of either of the study interventions was seen on MCS.

In the SHAPE-2 study we had a slightly smaller weight loss (6-7%) and our intervention duration was much shorter (16 weeks compared to one year) which could also explain the different results.

Even though some studies showed that exercise even without concurrent weight loss may increase HRQoL[9-12], the above trials did not find effects in the exercise only group or additional effects of exercise when weight loss was reached[23,26].

In a non-randomised study among 298 overweight women, aged 50-75 years, it was tried to investigate the individual contribution of weight loss and change in physical fitness to change in HRQoL[24]. After a 6-month lifestyle treatment for obesity, approximately 9 kg body weight (9.5%) was lost and physical fitness (measured by a 6-minute walk test) increased by 5.5%. Weight loss appeared to contribute significantly to improvements in seven out of nine HRQoL domains, whereas physical fitness did not further improve HRQoL, not even in the domain *health change*.

To conclude, our study showed that modest weight loss resulted in a positive change in self-perceived health status in a population of healthy overweight and obese, inactive, postmenopausal women. This change was significantly larger when weight loss was achieved mainly by exercise compared with equivalent weight loss by diet alone, indicating an effect of exercise beyond weight loss. Furthermore, there was a tendency towards improvements in HRQoL in physical and mental SF-36 domains in both intervention groups, which were most pronounced in the exercise group.

Table 3A Association between change in weight and change in HRQoL, regardless of study group

	Crude Model 1†			Adjusted model 2‡			Adjusted model 3§		
	β¥	95 % CI	P-value	β	95 % CI	P-value	β	95 % CI	P-value
PCS	0.16	-0.11;0.42	0.24	0.28	-0.07;0.63	0.12	0.20	-0.15;0.55	0.27
MCS	0.17	-0.16;0.50	0.32	0.33	-0.09;0.74	0.12	0.23	-0.17;0.62	0.25
Health change	3.32	2.33;4.31	<0.001	2.32	1.05;3.59	<0.001	2.23	0.99;3.46	<0.001

¥ The β (with 95% confidence interval, 95% CI) is the regression coefficient from linear regression models with one unit of change (Δ baseline and end-of-study) in weight (independent variable) and change in HRQoL (dependent variable). E.g., a β of 0.16 means that if body weight decreases by 1 kg, the mean SF-36 domain score increases by 0.16 points.

† Crude model 1= Weight loss (i.e., Weight at baseline minus Weight at 16 weeks)=independent, (SF-36 Score at 16 weeks minus SF-36 Score at baseline)= dependent.

‡ Adjusted model 2= model 1 adjusted for intervention group, age, education, baseline SF-36 score and baseline weight; § Adjusted model 3= model 1 adjusted for intervention group, age, education, baseline SF-36 score, baseline weight and change in VO_{2peak} (ml/min)

HRQoL=Health-related quality of life. PCS=Physical Component Score (SF-36 domains 1-5). MCS=Mental Component Score (SF-36 domains 4-8).

Table 3B Association between change in fitness (VO_{2peak}) and change in HRQoL, regardless of study group

	Crude Model 1†			Adjusted model 2‡			Adjusted model 3§		
	β¥	95 % CI	P-value	β	95 % CI	P-value	β	95 % CI	P-value
PCS	0.001	-0.04;0.04	0.98	0.01	-0.03;0.06	0.54	0.01	-0.03;0.06	0.54
MCS	0.02	-0.03;0.07	0.44	0.04	-0.01;0.09	0.11	0.04	-0.01;0.09	0.11
Health change	0.31	0.15;0.47	<0.001	0.21	0.05;0.04	0.01	0.20	0.05;0.04	0.01

* The β (with 95% confidence interval, 95% CI) is the regression coefficient from linear regression models with one measurements of change (Δ baseline and end-of-study) of both change in fitness (VO_{2peak}, per 10 ml/min, independent variable) and change in HRQoL (dependent variable). e.g., a β of 0.001 means that if fitness level increases by 1 unit (10 ml/min), the mean SF-36 domain score increases by 0.001 points.

† Crude model 1= (Fitness at 16 weeks minus Fitness at baseline) = independent, (SF-36 Score at 16 weeks minus SF-36 Score at baseline) = dependent. ‡ Adjusted model 2= model 1 adjusted for intervention group, age, education, baseline SF-36 score and baseline VO_{2peak} (per 10 ml/min); § Adjusted model 3= model 1 adjusted for intervention group, age, education, baseline SF-36 score, baseline VO_{2peak} (per 10 ml/min) and change in weight.

HRQoL=Health-related quality of life. PCS=Physical Component Score (SF-36 domains 1-5). MCS=Mental Component Score (SF-36 domains 4-8).

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9

GENERAL DISCUSSION

Postmenopausal breast cancer is the most prevalent cancer among women in the Western world[1]. There are many known risk factors for breast cancer, few of these are lifestyle-related. Since lifestyle is modifiable this can lead to options for primary prevention of breast cancer.

Overweight or obesity, and physical inactivity are recognised lifestyle-related risk factors for postmenopausal breast cancer, based on many observational studies[2]. Unfortunately, these risk factors are highly prevalent in the Western world. According to the WHO the prevalence of overweight and obesity has doubled since 1980. Globally, around 35% of women are estimated to be overweight or obese, and reported numbers in developed countries are even higher[3,4]. Furthermore, 34% of the world's female population are estimated to be inactive[5], according to international physical activity guidelines that recommend at least 30 minutes of moderate-to-vigorous physical activity per day, on at least five days/week[6]. In this thesis, we estimated that around 9% of all postmenopausal breast cancer cases in the Netherlands is attributable to overweight or obesity and 5.5% to physical inactivity (**Chapter 2**).

In the next paragraphs, I will first focus on the effects of diet or exercise-induced weight loss on breast cancer risk that we investigated in the SHAPE-2 trial and the role of abdominal fat in breast cancer risk. Hereafter, I will elaborate on the clinical impact of our findings and if intervention effects are likely to sustain.

Exercise or diet-induced weight loss and breast cancer risk

The mechanisms whereby overweight and physical inactivity influence breast cancer risk are not fully understood. Several pathways have been proposed, such as via serum sex hormones, markers of inflammation, insulin and insulin-like growth factors and adipokines[7]. Few randomised controlled trials were conducted to investigate the effect of exercise on breast cancer risk. The primary outcome measures in these trials were serum sex hormones as these are most consistently associated with postmenopausal breast cancer risk. In the SHAPE(-1) trial (2005)[8] and two other comparable sized exercise trials among healthy postmenopausal women[9-11], beneficial effects of exercise were observed only in women who lost concordant body weight or fat.

These results led to the question: *are the observed beneficial effects on sex hormones - as biomarkers for breast cancer risk - fully attributable to weight loss, or is there an additional effect when weight loss is reached by exercise?* In order to investigate this, we designed the Sex Hormones And Physical Exercise (SHAPE)-2 trial wherein 243 Dutch healthy postmenopausal women were randomly allocated to a diet-induced weight loss group, an exercise-induced weight loss group or a stable weight control group (**Chapter 4**). Both interventions aimed for an equivalent weight loss in 16 weeks' time.

The diet group was prescribed a diet with a caloric deficit of -500 kcal/day. The exercise group participated in an exercise programme of four hours endurance and strength training per week. Furthermore, the exercise group was prescribed a small caloric restriction (-250 kcal/day).

We added a diet component to the exercise intervention to ensure sufficient weight loss. Meta-analyses and reviews concluded that exercise-only interventions in RCTs result in modest weight loss, mainly ranging from 0.1 to 3 kg[12-14]. Moreover, in the SHAPE(-1) study among comparable Dutch postmenopausal women as included in the SHAPE-2 trial, no differences in body weight were observed between the exercise and control group[15]. Behavioural and metabolic compensatory mechanisms - including a reduced resting metabolic rate, an increased energy intake, or a decrease in non-exercise activities - prevent modest exercise from inducing substantial weight loss[16,17]. Trials with high dosages of exercise of 29 to 70 h/wk have showed that exercise-induced weight loss, up to 12.5 kg, is possible[14]. However, adherence becomes a major challenge, as for most individuals these exercise durations are practically unfeasible. Despite the addition of a diet component to the exercise programme, the emphasis in the SHAPE-2 exercise intervention group was on increased physical activity and women were encouraged to increase their activity pattern also outside the study programme.

In the SHAPE-2 study, we observed that women in the diet group on average lost 4.9 kg (-6.1%) of body weight and women in the exercise group lost a comparable 5.5 kg (-6.9%), body composition improved more by exercise-induced weight loss when compared to diet-induced comparable weight loss. More body fat was lost and lean mass was preserved when weight loss was induced mainly by exercise. Physical fitness increased with exercise, but not with diet only (**Chapter 5**). Furthermore, exercise-induced weight loss led to a larger improvement of self-perceived health than diet-induced weight loss (**Chapter 8**).

Regarding serum sex hormones, the primary outcome of the SHAPE-2 study, we found that both weight loss programs led to a decrease in oestrogens and androgens, and a (beneficial) increase in sex hormone binding globulin (SHBG). The effects on almost all measured sex hormones were most pronounced in the exercise group, which seemed to be largely mediated by fat loss (**Chapter 5**).

Our results show that modest weight loss, either induced by diet or mainly by exercise, leads to reductions in sex hormones and markers of inflammation, as breast cancer risk biomarkers. Our results furthermore show that exercise-effects on serum sex hormones and markers of inflammation are largely caused by the accompanying larger reduction in body fat when compared to diet-induced weight loss. We conclude that weight loss that is induced mainly by exercise is preferred over weight loss by diet only to reduce breast cancer risk.

A recently completed Canadian trial (not yet published) aimed to get more insight in the most optimal dosage and type of exercise for affecting breast cancer risk biomarkers by randomising postmenopausal women to different exercise regimes. Based on our findings we speculate that the exercise programme that produces the greatest body fat loss, most likely a combined aerobic and strength training programme, will influence serum sex hormones most beneficially.

The role of body fat in breast cancer risk: total versus abdominal fat

Our findings in **Chapter 5 and 6** suggest that reduction of fat mass is important for influencing breast cancer risk by exercise. In recent literature, fat tissue is often described as an endocrine organ since it secretes numerous hormones, markers of inflammation and adipokines[18]. These fat secreted markers have been associated with an increased risk of several chronic diseases as cardiovascular disease, diabetes and different types of cancer[18,19].

After menopause, the most important source of sex hormones is from adipose tissue[20]. Enzymes, such as aromatase and 17b hydroxyl-dehydrogenase, which are active in body fat converse precursor hormones to sex hormones[21].

The risks associated with excess fat mass seems to depend on specific fat storage sites[18,22,23]. Roughly, fat distribution can be classified in either storage predominantly around the waist, storage on the hips or overall coverage, i.e., general adiposity[24]. For cardiovascular diseases and diabetes, there is strong evidence that abdominal fat, consisting of subcutaneous and intra-abdominal fat, is an independent risk factor[22,25]. On the contrary, it seems that subcutaneous thigh fat has an independent protective effect on cardiovascular diseases[26]. For postmenopausal breast cancer risk, the role of body fat distribution is less clear. Few cohort studies suggest that abdominal fat increases breast cancer risk, but results are inconsistent[27]. A recent follow-up study found that general fat was more important than intra-abdominal fat in determining cancer risk in women[28]. Moreover, two intervention studies did not find evidence for an independent effect of abdominal fat on serum sex hormones associated with breast cancer risk[10,29,30], but in fact showed an effect of total body fat.

There are several hypothesised mechanisms that may explain the harmful effect of abdominal fat. Abdominal fat, especially intra-abdominal fat, is associated with a metabolic unhealthy profile[22] and is thought to be more metabolically active than other fat depots[25,31]. Furthermore, its unfavourable anatomical location may be of influence. Abdominal fat tissue drains cytokines and free fatty acids directly to the liver via the portal vein[24]. This may lead to accumulation of free fatty acids in the liver which causes insulin resistance and increased dyslipidaemia[24], i.e., the metabolic syndrome. Hyperinsulinaemia (resulting from insulin resistance) inhibits SHBG production and can, thereby, increase sex hormone availability[32,33]. This is a suggested biological mechanism through which abdominal fat might influence breast cancer risk[32]. We did not measure insulin in the SHAPE-2 study yet, but this might be performed in the remaining blood samples.

It is proposed that the beneficial effect of thigh fat on cardiovascular diseases could be due to a different lipolytic activity. Thigh fat has a lower lipolysis rate resulting in a lower release of free fatty acids in the circulation. Furthermore, thigh fat seems to be able to absorb free fatty acids from the circulation for long-term storage. In addition, it is hypothesised that thigh fat produces less pro-inflammatory adipokines and more beneficial anti-inflammatory adipokines that corresponds to a more healthy metabolic profile.

The exact mechanisms and magnitude by which different fat depots might influence chronic diseases as cardiovascular diseases and especially cancer, however, still need to be unravelled. In this thesis, we found that change in abdominal fat seems to play a role in markers of inflammation (CRP, IL-6 and leptin). Serum sex hormone levels were most strongly associated with changes in total body fat, and not abdominal fat (**Chapter 7**). Unfortunately, we were not able to investigate the role of thigh fat on breast cancer risk biomarkers, which would be interesting for future research.

We speculate that abdominal fat may play an additional role in breast cancer risk, but it is likely that the indirect effect on sex hormones by abdominal fat is overruled by the direct production of sex hormones from general adipose tissue in case of overweight or obesity.

Clinical impact of biomarker studies: what are the effects on breast cancer risk?

The few intervention studies investigating the effect of exercise on breast cancer risk, including the SHAPE-2 trial, use sex hormones as a surrogate intermediate outcome. Both SHAPE-2 weight loss interventions resulted in reductions of sex hormones, which indicate a reduction in breast cancer risk. However, to estimate the magnitude of the clinical impact of the observed effects is still a challenge since there are no absolute cut-off values defined that correspond to a certain change in future breast cancer risk.

In **Chapter 5**, we estimated that our observed 13% reduction in oestradiol corresponds to a 5-10% reduction in breast cancer risk. These speculations are based on observed oestradiol lowering effects of aromatase inhibitors in breast cancer patients, and estimates of breast cancer risk reduction among high risk women using these drugs. The NEW trial estimated that their reductions of 30% in oestradiol could correspond to a maximum of 50% reduction in breast cancer risk[34]. However, all these speculations are based on many assumptions.

Until now, it is assumed that the distributions and rankings of sex hormone levels, rather than the absolute values, correspond to breast cancer risk. Observational studies that linked sex hormone levels to breast cancer risk mainly show that women whose hormone levels are in the highest quintiles of the distribution have an up to twofold increased risks when compared to women with levels in the lowest quintiles[35,36]. The absolute values corresponding to these quintiles vary largely between studies. For example, a study by the Endogenous Hormones and Breast Cancer Collaborative Group evaluated nine prospective studies that measured sex hormones in postmenopausal breast cancer cases and samples of healthy postmenopausal controls[36]. Median hormone levels varied substantially, e.g., oestradiol levels differed up to five-fold between the studies, ranging from 22 pmol/l to 101 pmol/l in control women. Besides population heterogeneity (in ages, BMI and other determinants of hormone levels such as reproductive factors and nutritional habits), the large variation in absolute values is probably mainly caused by differences in laboratory assays.

In large scale epidemiologic studies, two widely available assay methods are generally used: direct and indirect immunoassays. These methods are rather quick, widely available and relatively cheap, but they suffer from cross-reactivity making results less reliable[37]. More sophisticated methods include gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) (used in SHAPE-2). LC-MS is the current reference standard for measuring sex hormones in postmenopausal women with low levels, cross-reactivity is much lower and sensitivity and specificity higher[37,38].

Another reason why it is important to have knowledge about absolute cut-off points of sex hormones and breast cancer risk, is to be able to use sex hormone measurements in individual risk prediction. High risk women could possibly be identified and targeted for primary prevention interventions, such as lifestyle programmes or chemoprevention, or secondary prevention in the form of a more intensive screening. A consensus meeting by the Breast Cancer Prevention Collaborative Group in 2007 concluded that sex hormone measurements in women would possibly add to existing risk prediction scores as for example the Gail model[39].

A recent study assessed the additional prognostic capacity of hormone levels to current risk scores for predicting future breast cancer risk[40]. The authors found that the predictive capacity of the Gail[39] and Rodner-Colditz[41] models improved maximum by 5.9% and 3.4%, respectively, to a total predictive capacity of approximately 60%. However, the authors face the same limitation as described above. They note that different methods were used to measure the hormones in their study population and, therefore, were not able to report on breast cancer risk associated with absolute hormone values. So the risk score cannot be implemented in clinic to predict an individuals' breast cancer risk. The Breast Cancer Prevention Collaborative Group underlined the importance of proper and uniform ways to measure hormones[42].

A standardised, valid and precise method (such as the LC-MS method), of measuring sex hormones is mandatory for the interpretation of absolute sex hormone levels and linked breast cancer risk. By this, the effects on breast cancer risk from clinical weight loss and exercise trials can be estimated. Furthermore, it could possibly lead to the incorporation of sex hormones in clinical risk models so that high risk women can be identified and be targeted for primary or secondary prevention.

Do behavioural and physical effects sustain after weight loss and exercise interventions?

For health outcomes and policy making, it is important that weight loss and exercise intervention effects sustain over a long period of time. However, in the first year after weight loss treatment, generally around one third of the lost body weight is regained, and half of the patients reach their baseline weight again within five years after treatment[43]. This can be explained by several physiological and behavioural compensatory responses that occur after a period of weight loss.

Physiological responses include changes in hormone levels, such as insulin, leptin and ghrelin, that play an important role in energy homeostasis as regulated by the central nervous system[44]. In reaction, energy expenditure decreases (by a lower resting metabolic rate) and appetite and hunger increase[43-46]. A weight loss trial in 50 obese individuals empirically proved this concept. Diet-induced weight loss (-13 kg) resulted in an altered hormone profile known to encourage weight gain. One year after weight loss, the hormone levels had still not normalised completely[47]. Another possible physiological mechanism that might explain the difficulty in sustaining weight loss includes a decreased gastrointestinal motility[48].

In addition to the above physiological reactions, behaviour plays an important role in the prevention of weight gain. After weight loss, an individuals' lifestyle, i.e., caloric intake and physical activity pattern, should be chronically adapted to the current body's caloric needs. Internal motivation, self-efficacy, autonomy and realistic goal setting are important factors for behavioural change and increase the chance of persistent weight loss[49,50].

Guidelines on the treatment of obesity advise a period of weight maintenance after approximately 10% initial weight loss to reduce the risk of relapse[23,51]. Exercise helps for weight maintenance[14,52] and is, therefore, recommended in addition to diet. A meta-analysis concluded that interventions including both diet and exercise are moderately effective in slowing down weight regain after initial loss, even up to 24 months[52].

Follow-up studies of exercise interventions indicate that effects on physical activity level – at least partly – sustain on the long-term[53-56]. The previous SHAPE trial (2005) showed that the exercise intervention group was still more active than the control group one year after trial completion. compared to during the intervention period, activity levels had decreased.

Follow-up data of the current SHAPE-2 trial were also gathered one year after the trial completion. Questionnaires and an activity monitor were used to assess physical activity, and women were asked to report their current body weight. Preliminary results reveal that the exercise group remained more active than the diet or control group. Also, the present physical activity level was higher compared to baseline, but somewhat lower than during the study. Participants in both intervention groups experienced a slight weight regain, but body weight was still significantly lower compared to baseline. This indicates that behavioural effects of the SHAPE-2 exercise and diet weight loss programmes largely sustained on the long-term.

Conclusions and recommendations

The data presented in this thesis show that weight loss, by diet or mainly exercise, produces favourable effects on body composition and breast cancer risk biomarkers in healthy postmenopausal women. Weight loss by exercise, in combination with a small caloric intake restriction, leads to more beneficial health outcomes than weight loss induced by diet only. This includes improvement of physical fitness, body composition and self-perceived health status. Furthermore, the larger reduction in fat mass experienced with exercise, leads to larger decreases in serum sex hormone levels indicating a decrease in breast cancer risk. Unfortunately, current knowledge about the relation between absolute levels of sex hormones and subsequent breast cancer risk is unsufficient to precisely quantify the decrease in breast cancer risk. In addition to a decrease in total body fat, exercise may also result in larger decreases of abdominal fat, which could possibly affect markers of breast cancer risk through other pathways. Exercise helps for weight maintenance and sustainment of beneficial health effects on the long-term. We, therefore, recommend a combination of diet and exercise to induce weight loss, improve general health, self-perceived health, and decrease breast cancer risk in postmenopausal women.

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SUMMARY

Postmenopausal breast cancer is the most prevalent cancer type in Western women. The incidence increases which is not only due to aging of the population but also due to a larger exposure to risk factors. There are several known risk factors for postmenopausal breast cancer of which few are lifestyle related. Since lifestyle is modifiable, these risk factors provide an opportunity for primary prevention of breast cancer.

In **Chapter 2**, we estimated the fraction of postmenopausal breast cancer cases that is attributable to lifestyle and in theory preventable. We included lifestyle-related risk factors for which a large body of evidence exists and which are still present, and modifiable, at middle-age. These are: overweight or obesity, physical inactivity, alcohol consumption, dietary fibre intake and cigarette smoking. The largest body of evidence exists for the first three risk factors. Recent literature provides increasing evidence for the negative effect of (long-term) smoking and insufficient dietary fibre intake on breast cancer risk.

To calculate the population attributable fraction (PAF), we used prevalence rates of risk factor exposure in women aged 40 years and older in the Netherlands, which are derived from large national surveys and registration databases around the year 2000. Relative risks for postmenopausal breast cancer of each risk factor were derived from meta-analyses. We found that overweight/obesity has the highest PAF (8.8%), followed by alcohol consumption (6.6%), physical inactivity (5.5%), smoking (4.6%) and fibre intake (3.2%). We estimated that one out of four postmenopausal breast cancer cases (25.7%) was attributable to these five lifestyle-related risk factors in the Netherlands in 2010, corresponding to 2,665 cases. We conclude that a more healthy lifestyle could substantially reduce breast cancer risk in the Dutch population.

In **Chapter 3**, we used data of the SHAPE(-1) study to investigate the effect of a one-year exercise intervention on insulin sensitivity in healthy normal- to overweight and inactive postmenopausal women. In this study, 189 postmenopausal women were randomised to a one-year exercise intervention of 150 minutes of aerobic and strength exercise per week or control. We observed no effect of exercise on insulin sensitivity. Possible explanations could be that the exercise dosage was too low or that the improvements in insulin sensitivity were too subtle to measure, since in this population of healthy women there is not much room for improvement. Furthermore, it could be that weight loss, which was not induced by the exercise intervention, is important for substantial effects to reveal.

In **Chapter 4**, we described the rationale and design of the Sex Hormone And Physical Exercise (SHAPE)-2 trial. The SHAPE-2 trial aimed to investigate the effects of equivalent weight loss, with or without exercise, on breast cancer risk biomarkers. The study was conducted in the vicinities of Utrecht and Enschede, the Netherlands, from February 2012 to June 2013. In total, 243 postmenopausal women who were overweight to obese and had an inactive lifestyle were included. All women started with a four to

six-week run-in period, aiming for stable body weight and a comparable diet composition among all participants. Hereafter, women were randomised to a diet group, an exercise group or control. Women in both intervention groups had the aim to lose 5 to 6 kg of body weight. Our reasoning was that an equivalent amount of weight loss induced by diet or mainly by exercise allows to study the possible additional effect of exercise on biomarkers for breast cancer risk. Women in the diet group were prescribed a caloric restriction of -500 kcal/day. Women in the exercise group participated in an exercise programme containing four hours of aerobic and strength training. Furthermore, to ensure substantial weight loss within a short time frame, a caloric intake restriction of -250 kcal/day was prescribed.

In **Chapter 5**, we presented the results of the SHAPE-2 weight loss interventions on body composition, physical fitness and serum sex hormone levels. Both the diet (-4.9 kg) and exercise group (-5.5 kg) achieved the target weight loss and the control group remained weight stable (+0.06 kg). Exercise-induced weight loss resulted in a significantly larger loss of fat mass compared to diet whereas lean mass was preserved. Furthermore, fitness increased in the exercise group only. Weight loss, either by diet or exercise, resulted in significantly favourable effects on sex hormones (decreased) and SHBG (increased). Significantly larger effects on free testosterone as well as suggestive effects for androstenedione and SHBG were observed in the exercise group compared to diet. Although the effects on oestrogens were in favour of the exercise group, the differences did not reach significance. Effects of exercise on sex hormones seem to be largely mediated by the larger amounts of fat loss.

In **Chapter 6**, we investigated the effects of the SHAPE-2 weight loss interventions on inflammatory markers (high sensitive C-reactive protein (hsCRP) and interleukine-6 (IL-6)) and adipokines (leptin and adiponectin). HsCRP and leptin reduced by both diet- and exercise-induced weight loss. There was a suggestively larger effect of exercise on hsCRP compared with diet. No effects were seen on IL-6 and adiponectin. In addition to change in body fat, change in physical fitness also contributed to the effects on hsCRP. Also the effect on leptin was mediated by body fat, however, the effect in the diet group remained after adjustment for fat. It could be that diet-induced hunger plays a role, as leptin is a hormone involved in the process of hunger and satiety.

In **Chapter 7**, we investigated the effects of the SHAPE-2 interventions on abdominal fat, including subcutaneous and intra-abdominal fat. Second, we investigated the role of total body fat and abdominal fat in relation to the previously assessed breast cancer risk biomarkers. We found that both subcutaneous and intra-abdominal fat decreased with weight loss and that subcutaneous fat decreased to a larger extent in the exercise group compared to diet. Furthermore, we found that change in total body fat, more than a change in abdominal fat, affected sex hormone levels. Whereas change in abdominal fat seemed of more or equal importance in influencing markers of inflammation and adipokines.

In **Chapter 8**, we investigated whether weight loss, by diet or exercise, improved Health Related Quality of Life (HRQoL) in our SHAPE-2 study-population. HRQoL was measured by the SF-36 questionnaire. We found that weight loss induced improvements in physical and mental HRQoL domains, but the results were not statistically significant in either of the intervention groups. However, weight loss did result in a positive change in self-perceived health status, which was significantly greater in the exercise group compared with diet.

In **Chapter 9**, we discussed some outstanding questions and elaborated on the impact of our findings.

For health outcomes and the implementation of weight loss programmes, long-term effects are more important. Preliminary results of a follow-up study one year after completion of the SHAPE-2 trial reveal that body weight remained below the baseline body weight and that women in the exercise group were still more active than both other groups.

The reductions in sex hormones that were observed by the SHAPE-2 weight loss programmes indicate a decrease in breast cancer risk. The clinical impact of these effects, however, remains unclear. A standardised method for measuring sex hormones in large groups of women is essential in order to link absolute levels of sex hormones to breast cancer risk.

Weight loss, especially fat loss is important to establish favourable effects on sex hormones. Body fat distribution may also play a role. Abdominal fat has been shown to increase the risk of several chronic diseases, however, its role in breast cancer risk is still unknown.

In conclusion, we found that weight loss, by diet or mainly exercise, produces favourable effects on body composition and breast cancer risk biomarkers in healthy postmenopausal women. Weight loss by exercise, in combination with a small caloric intake restriction, leads to more beneficial health outcomes than weight loss induced by diet only. Furthermore, the larger reduction in fat mass experienced with exercise, leads to larger decreases in serum sex hormone levels indicating a decrease in breast cancer risk. In addition to a decrease in total body fat, exercise may also result in larger decreases of abdominal fat, which could possibly affect markers of breast cancer risk through other pathways.

SAMENVATTING

Borstkanker is de meest voorkomende vorm van kanker bij Westerse vrouwen, met name na de menopauze. Het aantal vrouwen met borstkanker neemt de laatste jaren toe. Dit komt niet alleen door vergrijzing, maar ook doordat vrouwen steeds meer blootgesteld zijn aan risicofactoren. Er zijn verschillende risicofactoren voor borstkanker na de overgang, waarvan enkele leefstijl gerelateerd zijn. Aangezien leefstijl kan worden veranderd, zou blootstelling aan deze risicofactoren in de bevolking verminderd kunnen worden. Dit zou kunnen leiden tot een verlaging in het optreden van borstkanker.

In **Hoofdstuk 2** hebben we onderzocht welk deel van het optreden van borstkanker in Nederland toe is te schrijven aan leefstijl gerelateerde risicofactoren: de zogenoemde populatie attributieve fractie. Dit aantal zou in theorie voorkomen kunnen worden. In de analyse hebben we risicofactoren meegenomen waarvoor sterk bewijs is dat zij bijdragen aan het ontstaan van borstkanker en welke aanwezig en te veranderen zijn bij vrouwen vanaf middelbare leeftijd. Deze factoren zijn: overgewicht en/of obesitas, lichamelijke inactiviteit, alcohol consumptie, een laag vezelgehalte van de gebruikelijke voeding en roken.

Om de populatie attributieve fractie te berekenen hebben we cijfers gebruikt van het voorkomen van de risicofactoren (prevalentiecijfers) in de Nederlandse vrouwelijke bevolking van 40 jaar en ouder. Deze cijfers hebben we verkregen uit grootschalige onderzoeken op basis van een vragenlijst en registraties uit het jaar 2000. De mate van invloed van de risicofactoren op het optreden van borstkanker (relatieve risico's) hebben we verkregen uit wetenschappelijke literatuur.

We hebben geschat dat, in het jaar 2010, 8.8% van alle borstkankers bij vrouwen boven de 40 jaar toe te schrijven is aan overgewicht/obesitas, 6.6% aan alcoholconsumptie, 5.5% aan te weinig lichaamsbeweging, 4.6% aan roken en 3.2% aan een te lage vezel inname. In totaal is één-vierde van alle gevallen van borstkanker toe te schrijven aan een van deze vijf leefstijlfactoren, wat absoluut gezien neerkomt op 2655 vrouwen met borstkanker. Dit betekent dat een gezondere leefstijl het voorkomen van borstkanker substantieel kan verminderen in de Nederlandse populatie.

In **Hoofdstuk 3** hebben we het effect van een één-jaar durend beweegprogramma op insulinegevoeligheid bij inactieve postmenopauzale vrouwen met een normaal gewicht of licht overgewicht onderzocht. Hiervoor hebben we gegevens van de SHAPE studie (een wetenschappelijk onderzoek uit 2005) gebruikt. In de SHAPE studie werden 189 vrouwen gerandomiseerd (willekeurig geloot) in een beweeggroep (150 minuten aerobics en krachttraining per week gedurende een jaar), of controlegroep (zonder interventie). We vonden geen effect van het beweegprogramma op insulinegevoeligheid.

In **Hoofdstuk 4** hebben we de aanleiding, het doel en de opzet van de SHAPE-2 studie beschreven. De afkorting SHAPE staat voor *Sex Hormones And Physical Exercise*, ofwel: geslachtshormonen en lichaamsbeweging. Het doel van de SHAPE-2 studie was

om de effecten te onderzoeken van een gelijk aantal kilo's gewichtsverlies bereikt door middel van dieet of door middel van lichaamsbeweging op hormonen die samenhangen met het borstkankerrisico, waaronder geslachtshormonen. Het onderzoek werd uitgevoerd in (de omgeving van) Utrecht en Enschede van februari 2012 tot juni 2013. In totaal deden 243 postmenopauzale vrouwen met overgewicht of obesitas en een inactieve leefstijl mee aan het onderzoek.

Alle vrouwen startten met een vier tot zes weken durende run-in periode waarin een dieet werd voorgeschreven met als doel het lichaamsgewicht stabiel te houden. Hierna werden de deelnemers willekeurig verdeeld in een dieetgroep, een beweeggroep of een controlegroep. Vrouwen in de dieetgroep kregen een calorierestrictie van 500 kcal/dag ten opzichte van het run-in dieet. Vrouwen in de beweeggroep volgden een beweegprogramma met vier uur sport per week: twee uur fitness, een combinatie van duur- en krachttraining, en twee uur Nordic walking. Daarnaast kregen vrouwen in de beweeggroep een calorierestrictie van 250 kcal/dag.

Het doel van de dieet- en beweeggroep was om vijf tot zes kilogram af te vallen, zodat we konden onderzoeken of er een extra gunstig effect was van beweging, ten opzichte van dieet, op hormonen samenhangend met het borstkankerrisico. **Hoofdstuk 5** beschrijft de resultaten van de SHAPE-2 studie op lichaamssamenstelling, fysieke fitheid (conditie) en geslachtshormonen in het bloed. Zowel de dieetgroep (-4.9 kg) als de beweeggroep (-5.5 kg) bereikten het gewenste gemiddelde gewichtsverlies. De controlegroep bleef stabiel in gewicht (+0.06 kg). Vrouwen in de beweeggroep verloren significant meer lichaamsvet vergeleken met vrouwen in de dieetgroep. De spiermassa bleef behouden bij vrouwen in de beweeggroep, terwijl dit afnam in de dieetgroep. De fysieke fitheid nam toe bij vrouwen in de beweeggroep, maar niet in de dieetgroep. Gewichtsverlies, zowel door dieet als beweging, leidde tot een significante verbetering in niveaus van geslachtshormonen (verlaging) en SHBG, een eiwit dat geslachtshormonen bindt (verhoging). Verder waren er grotere positieve effecten zichtbaar in de beweeggroep ten opzichte van de dieetgroep voor testosteron (significant), androsteendion en SHBG (beide zeer suggestief). Hoewel de effecten op de oestrogenen ook positiever waren in de beweeggroep dan in de dieetgroep, waren deze niet significant. De (grotere) effecten van beweging op geslachtshormonen leken voornamelijk veroorzaakt te zijn door het grotere verlies aan vetmassa in de beweeggroep.

Hoofdstuk 6 beschrijft de resultaten van de SHAPE-2 studie op ontstekingsfactoren (C-reactive protein (hsCRP) en interleukine-6 (IL-6)) en adipokines (leptine en adiponectine), die gerelateerd zijn aan het borstkankerrisico. Zowel de dieet- als de beweeggroep lieten een verlaging in HsCRP en leptine niveaus zien. Er was een trend richting een grotere verlaging in hsCRP in de beweeggroep ten opzichte van de dieetgroep. Beide gewichtsverliesprogramma's lieten geen effect zien op IL-6 en adiponectine. De grotere verlaging van lichaamsvet in de beweegroep zorgde met name voor het grotere effect op hsCRP. Daarnaast droeg verandering in fysieke fitheid ook bij aan de effecten op hsCRP.

In **Hoofdstuk 7** hebben we de effecten onderzocht van de SHAPE-2 programma's op buikvet, zowel subcutaan (onder de huid) als visceraal vet (rondom de organen). Daarnaast onderzochten we de rol van veranderingen in totaal lichaamsvet en buikvet in relatie tot geslachtshormonen, ontstekingsfactoren en adipokines (zoals gemeten in **Hoofdstukken 5** en **6**). We vonden dat zowel subcutaan als visceraal vet verminderden door gewichtsverlies en dat in de beweeggroep meer subcutaan vet werd verloren dan in de dieetgroep. Daarnaast vonden we dat afname van totaal lichaamsvet, meer dan afname van buikvet, een verlagend effect had op geslachtshormonen. Terwijl voor de verandering in ontstekingsfactoren en adipokines verlies van buikvet meer van invloed leek dan verlies van totaal lichaamsvet.

In **Hoofdstuk 8** beschrijven we de resultaten van de SHAPE-2 studie op kwaliteit van leven. Kwaliteit van leven werd gemeten met de SF-36 vragenlijst. We vonden dat gewichtsverlies leidde tot subtiele, maar niet significante, verbeteringen in fysieke en mentale gezondheid. Daarnaast resulteerde gewichtsverlies in een significant meer positieve waardering van de gezondheid wanneer gevraagd werd hoe de huidige gezondheid was veranderd ten opzichte van een jaar geleden. Deze verbetering in gezondheid was groter in de beweeggroep dan in de dieetgroep.

In **Hoofdstuk 9** bediscussiëren we enkele overgebleven vragen en weiden we uit over de betekenis van onze bevindingen in de huidige praktijk.

Voor gezondheidsuitkomsten en de implementatie van gewichtsverliesprogramma's zijn de lange termijn effecten van groot belang. Onze vervolgstudie één jaar na beëindiging van de SHAPE-2 studie laat zien dat het lichaamsgewicht nog steeds lager is dan bij aanvang van de studie en dat vrouwen in de beweeggroep actiever waren dan bij aanvang en tevens actiever waren dan vrouwen uit de beide andere groepen.

De veranderingen in geslachtshormonen die zijn waargenomen in de SHAPE-2 studie wijzen op een verlaging van het borstkankerrisico. De exacte verlaging van het borstkankerrisico door deze effecten blijft echter nog onduidelijk. Een gestandaardiseerde methode voor het meten van geslachtshormonen in grote groepen vrouwen is essentieel om de absolute veranderingen in hormonen te kunnen omzetten in een absolute afname van het borstkankerrisico.

Gewichtsverlies, met name vetverlies, is belangrijk voor de positieve effecten op geslachtshormonen. De verdeling van vet over het lichaam, bijvoorbeeld voornamelijk op de heupen of in de buik, zou ook een rol kunnen spelen. Buikvet is een risicofactor gebleken voor verschillende chronische ziekten zoals hart- en vaatziekten. De rol die buikvet speelt in het (borst)kankerrisico is echter nog grotendeels onbekend.

Concluderend hebben we gevonden dat gewichtsverlies, door middel van dieet of voornamelijk beweging, leidt tot gunstige effecten op lichaamssamenstelling en factoren in het bloed samenhangend met het borstkankerrisico bij gezonde postmenopauzale vrouwen. Gewichtsverlies door middel van beweging, in combinatie met een kleine calorierestrictie, leidt tot meer positieve gezondheidsuitkomsten dan gewichtsverlies door middel van

dieet alleen. Beweging zorgt daarnaast voor een groter verlies aan vetmassa, wat leidt tot een grotere verlaging van geslachtshormonen, wijzend op een verlaging van het borstkankerrisico. Ook kan beweging resulteren in een groter verlies van buikvet, wat mogelijk via andere wegen het borstkankerrisico kan verlagen.

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The author of this thesis, Willemijn van Gemert, was born on the 19th of May 1986, in 's-Hertogenbosch and grew up in Nuland, the Netherlands. She attended high school in Oss at Maasland-college and graduated in 2004. She moved to Nijmegen to study medicine at the Radboud University. During her medical education she performed a research internship at the department of Medical Oncology in the Radboud University Medical Center where she investigated the incidence, treatment and follow-up of cancer in pregnancy in the Netherlands. After completing her final clinical internship in tropical medicine (Berecum, Ghana) she obtained her medical degree in 2011. In June of that same year she started her PhD research in Cancer Epidemiology, at the Julius Center for Health Sciences and Primary Care, of the University Medical Center Utrecht. She worked under supervision of Petra H.M. Peeters, MD, PhD, Albertine (Jantine) J. Schuit, PhD, and Evelyn M. Monninkhof, PhD. Parallel to the PhD-project she followed a postgraduate master programme in Clinical Epidemiology at Utrecht University of which she received her degree in May 2014.