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Long-term effects of a Palaeolithic-type diet in obese postmenopausal women: a two-year randomized trial

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Abstract

Background/Objectives—Short-term studies have suggested beneficial effects of a Palaeolithic-type diet (PD) on body weight and metabolic balance. We now report long-term effects in obese postmenopausal women of a PD on anthropometric measurements and metabolic balance, in comparison with a diet according to the Nordic Nutrition Recommendations (NNR).

Subjects/Methods—Seventy obese postmenopausal women (mean age 60 years, body mass index 33 kg/m^2) were assigned to an *ad libitum* PD or NNR diet in a 2-year randomized controlled trial. The primary outcome was change in fat mass as measured by dual energy X-ray absorptiometry.

Results—Both groups significantly decreased total fat mass at 6 months (−6.5 and −2.6 kg) and 24 months (−4.6 and −2.9 kg), with a more pronounced fat loss in the PD group at 6 months (P<0.001), but not at 24 months (P=0.095). Waist circumference and sagittal diameter also decreased in both groups, with a more pronounced decrease in the PD group at 6 months (−11.1 vs. −5.8 cm, P=0.001 and −3.7 vs. −2.0 cm, P<0.001, respectively). Triglyceride levels decreased

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significantly more at 6 and 24 months in the PD group versus the NNR group ($P<0.001$ and $P=0.004$). Nitrogen excretion did not differ between groups.

Conclusions—A PD has greater beneficial effects versus an NNR diet regarding fat mass, abdominal obesity and triglyceride levels in obese postmenopausal women; effects not fully sustained for anthropometric measurements at 24 months. Adherence to protein intake was poor in the PD group. The long-term consequences of these changes remain to be studied.

Keywords

adipose tissue; diet; insulin resistance; postmenopausal; weight

INTRODUCTION

The incidence of obesity has increased substantially since the early 1980s, resulting in major public health challenges.[1, 2] Related to this, dietary risk factors and physical inactivity collectively accounted for 10% of global deaths and disability-adjusted life years (DALYs) in a recent comparative risk assessment of the burden of disease and injury.[2] Central fat accumulation, i.e., the accumulation of visceral adipose tissue (VAT), is clearly associated with an increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).[3] In menopause, fat is redistributed from peripheral to central depots, which is associated with an increased incidence of diabetes and CVD among postmenopausal women.[4]

Diets moderately high in protein (25-30% of energy intake) have been suggested to be beneficial for weight loss when used *ad libitum*, at least up to 12 months.[5] This includes a more pronounced reduction in body fat and blood pressure and improved lipid profile when compared to high-carbohydrate diets.[5] However, it is not clear if an increased protein intake is sustainable during long-term diet interventions. An increased intake of monounsaturated fats (MUFA), and omega-3 fatty acids, and moderately decreased intake of carbohydrates (40% of energy intake) may also be beneficial regarding weight loss as well as have protective effects on CVD.[6, 7]

Such dietary properties are possible with a Palaeolithic-type diet (PD), making it seem worthy of randomized controlled trials (RCTs). Short-term studies with this type of diet have suggested beneficial effects on energy intake, weight, waist circumference, body mass index (BMI), and metabolic balance, including insulin sensitivity, as well as cardiovascular risk markers, even when administered *ad libitum* versus other types of diets.[8-11] Our hypothesis was that a PD would be more efficient than a conventional low-fat/high-fibre diet[12] at reducing fat mass during a 2-year randomized dietary intervention trial in obese postmenopausal women. Furthermore, we wanted to analyse if a PD would have beneficial effects on cardiovascular risk markers.

SUBJECTS AND METHODS

Subjects

The subjects were recruited in 2007 through advertisements in local newspapers. In total, 210 women expressed an interest in participating in the study (Figure 1) out of which 70 postmenopausal non-smoking women with a BMI ≥ 27 kg/m^2 fulfilled the inclusion criteria. Exclusion criteria include consumption of a restricted or vegetarian diet, allergy to key components in the intervention diets, history of heart disease, kidney disease, hyper- or hypothyreosis, osteoporosis, or diabetes. Other exclusion criteria were abnormal fasting plasma glucose levels (≥7 mmol/L), blood pressure exceeding 150/90 mmHg, hormone replacement therapy, statins, beta-blockers, or any medication for psychiatric disorders. Three women on monotherapy with an ACE inhibitor for mild hypertension were included in the study. Each woman met a physician for clinical assessment, followed by a series of baseline tests at the Clinical Research Center at Umeå University Hospital.

Diet intervention

After baseline measurements, the women were randomized to a PD or a Nordic Nutrition Recommendations (NNR) diet for 24 months. All study personnel (except the dieticians) were blinded to the dietary allocation of the participants. Both diets were consumed ad libitum. The PD provided 30% of energy intake (E%) from protein, 40 E% fat, and 30 E% carbohydrates and included a recommendation for a high intake of MUFA and polyunsaturated fatty acids (PUFA). The diet was based on lean meat, fish, eggs, vegetables, fruits, berries, and nuts. Dairy products, cereals, added salt, and refined fats and sugar were excluded. The NNR diet[12] was aiming at a daily intake of 15 E% protein, 25-30 E% fat, and 55-60 E% carbohydrates, with emphasis on low fat dairy products and high fibre products. Each group took part in a total of 12 group sessions held by a trained study dietician (one dietician per diet) throughout the 24-month study period. The group sessions consisted of information on and cooking of the intervention diets, dietary effects on health, behavioral changes, and group discussions. The subjects were given recipes and written instructions to facilitate the preparation of meals at home. Eight group sessions (four cooking classes and four follow-up sessions) were held during the first 6 months of the intervention. Additional group meetings were held at 9, 12, 18, and 24 months. The protocol was in accordance with the Helsinki declaration and approved by the Regional Ethical Review Board at Umeå University, Sweden. This trial was registered at clinicaltrials.gov as NCT00692536.

Anthropometry and metabolic parameters

Anthropometric measurements were made at baseline and after 6, 12, 18, and 24 months, as described earlier.[13] The sagittal abdominal diameter was recorded at the umbilical level as the height of the abdomen measured when lying down on the examination couch with the legs straight. Body composition, including an estimation of the total percent of body fat, was measured by dual energy X-ray absorptiometry (DXA) (GE Medical Systems, Lunar Prodigy X-ray Tube Housing Assembly, Brand BX-1L, Model 8743, Madison, WI, USA). Systolic and diastolic blood pressure was measured twice at 2-minute intervals in the sitting position after five minutes rest, using an automatic blood pressure meter (Boso-medicus, Bosch, Germany). Fasting blood samples were drawn from the antecubital vein after a

resting period. Aliquots were frozen immediately at −20°C until analysed for plasma glucose, plasma insulin, serum cholesterol, triglycerides, and HDL cholesterol, tissue plasminogen activator (tPA) activity, plasminogen activator inhibitor type 1 (PAI-1) activity and high sensitivity C-reactive protein (hsCRP). Serum LDL was calculated as (serum cholesterol - serum HDL - serum triglycerides)/2.2.

Dietary assessment

Dietary intake was assessed using 4-day estimated self-reported food records conducted at baseline (2 × 4 days) and monthly until 6 months, thereafter at 9, 12, 18 and 24 months. Subjects were instructed to keep a record of all food items consumed over four consecutive days (three weekdays and one weekend day) and to describe and estimate the amount of food eaten by using coloured food-portion photographs representing known weights and household measuring utensils (e.g., cup, spoon, and standard weight of food items). The reported food intake was converted to estimates of energy and nutrient intake using the nutritional analysis package Dietist XP (version 3.0) based on the food composition database of the Swedish National Food Administration (2008-03-06).

Adherence to protein intake

Nitrogen excretion in urine (NU) was used as a biomarker for protein intake, with three 24-h urine samples collected at baseline and after 6 and 24 months. The para-aminobenzoic acid (PABA) method[14] was used to verify the completeness of the urine collections. NU was determined using the Kjeldahl technique with a Kjeltec analyser (model NMKL nr 6, Eurofins Food & Agro AB, Lidköping, Sweden). Duplicates for 10 percent of the samples were included for quality control. The analytical precision was ±10%.

Measurement of energy expenditure

Resting energy expenditure (REE) was measured at baseline and after 6 and 24 months using indirect calorimetry (Datex-Ohmeda Deltatrac II) with breath-by-breath sampling. Free-living physical activity energy expenditure (PAEE) was estimated at the same time-points using data collected over a 7-day period using a combined accelerometer and heart rate monitor (Actiheart®, CamNtech Ltd, Cambridge, UK) as described previously.[15-17] Diet-induced thermogenesis, i.e. the production of heat after eating, was fixed at 10% of the total energy expenditure (TEE) for all individuals in the study. TEE was calculated for each participant as the sum of the PAEE and REE, divided by 0.9 and expressed as kcal or MJ per 24-h day.

Statistical methods and randomization

The primary outcome of the study was the change in fat mass over a period of 2 years, which was also used to calculate power. Thirty-five subjects were estimated to be needed in each diet arm to achieve a significant outcome ($p<0.05$) with 80% power. Block randomization, with a block size of four and an allocation ratio of 1:1, was done by a statistician, blinded to the study. Most variables were normally distributed, but serum insulin, serum TG, PAI-1, hsCRP, total lean mass and PUFA (g) required logarithmic transformation. Differences between groups at baseline were tested by independent sample

t-tests. We used generalized estimating equations (GEEs) to evaluate repeated measurements over time. A two-sided *P*-value <0.05 was considered significant. Statistical analyses were performed using SPSS for Windows (version 20.0). The primary analysis in this trial was an intention-to-treat analysis.

RESULTS

Subject characteristics

No differences were found in the baseline characteristics between the diet groups, except for higher HDL cholesterol in the PD group (Tables 1 and 3). A total of 30% (n=21) of the participants were lost to follow-up (Figure 1). A higher proportion of participants completed the PD arm than the NNR arm (27 PD, 22 NNR). Medication use did not change during the study period.

Energy expenditure and dietary intake

REE, PAEE, and TEE did not change or differ between groups during the study period (Table 2) with the exception of a decrease in REE at 6 months in the study population as a whole. The change in reported nutrient intake between baseline, 6 months, and 24 months and the difference between groups at each time point is presented in Table 2. No difference was found between diet groups regarding nutrient intake at baseline. Reported daily energy intake decreased over time, without significant differences between groups (Table 2). The PD group had a 19% and 20% lower reported energy intake and the NNR group 18% and 12% lower reported energy intake at 6 and 24 months, respectively.

The PD group reported a significantly lower intake (E% and g/d) of carbohydrates, higher intake (E% and g/d) of protein, MUFA, PUFA, cholesterol, and higher total fat (E%), MUFA:SFA and PUFA:SFA ratios compared to the NNR group (Table 2). The PD group reported a more pronounced change in the ratio (E%) protein:carbohydrates:total fat from baseline to 6 and 24 months (17:46:33, 23:29:44, 22:34:40; respectively) compared to the NNR group (17:45:35, 19:48:32, 17:43:34; respectively). Target intakes were not fully achieved; the PD did not reach the target amounts of percent energy of protein (30 E%) at 6 and 24 months, and the NNR group did not reach the target amounts of carbohydrates (55-60%).

Adherence to protein intake

A total of 406 urine collections from 65 subjects (34 PD, 31 NNR) at baseline, 51 subjects (30 PD, 21 NNR) at 6 months, and 39 subjects (21 PD, 18 NNR) at 24 months were available for comparison of reported protein intake and NU. Mean NU were 13 g/d in both groups at baseline (Table 2). There was no difference in NU within- or between groups at 6 or 24 months follow-up, indicating poor adherence to the target protein intake (30 E%) in the PD group.

Anthropometry and cardiometabolic risk markers

Both diet groups decreased their total fat mass (TFM) −6.5 and −2.6 kg, at 6 months, and −4.6 and −2.9 kg at 24 months for the PD and NNR group respectively; with a significant

difference between groups at 6 months but not at 24 months (P<0.001 and P=0.095 respectively; Figure 2). The PD group also lost more total lean mass (−1.3 kg vs. −0.4 kg; P=0.005) during the first 6 months. Both diet groups had a significant weight loss during the whole study period, with significantly greater weight loss in the PD group at all follow-up time points except 24 months (Figure 2). The largest weight loss was measured at the 12-month follow-up; −8.7 kg in the PD group and −4.4 kg in the NNR group. BMI (mean (±SEM)) decreased 3.0 (±0.30), 3.3 (±0.36), and 2.4 (±0.41) kg/m^2 in the PD group and 1.2 (±0.27), 1.7 (±0.38), and 1.4 (±0.34) kg/m^2 in the NNR group at 6, 12, and 24 months, respectively. The loss in BMI was more pronounced in the PD group at 6 (P<0.001) and 12 months (P=0.002), but not 24 months (P=0.059). In both diet groups, waist circumference decreased significantly during the whole study period, with a significantly more pronounced decrease in the PD group at 6 months (−11.1 vs. −5.8 cm; P=0.001) (Figure 2). In addition, the sagittal diameter decreased significantly over time in a similar manner in both groups, with a larger decline in the PD group at 6 months (−3.7 vs. −2.0 cm; P<0.001; Figure 2). Concomitantly, hip circumference decreased over time with a significant difference between groups at 6 months (−6.8 with PD vs. −2.7 cm with NNR at 6 months; P<0.001).

Triglyceride levels decreased significantly in the PD group over time with a 0.26 mmol/L and 0.22 mmol/L difference between the diet groups at 6 and 24 months (P<0.001 and P=0.004), respectively (Table 3). Other beneficial cardiometabolic changes occurred in the study population as a whole over time (Table 3). At both 6 and 24 months diastolic blood pressure, heart rate, CRP, LDL cholesterol, and PAI-1 activity decreased, as well as systolic blood pressure and total cholesterol at 6 months, and HDL cholesterol increased at 24 months. No differences were measured over time or between groups in regards to fasting glucose, fasting insulin concentrations, and tPA activity.

DISCUSSION

We found that a diet with reduced carbohydrate and saturated fatty acid (SFA) intake and a relative increase in the intake of protein, MUFA, and PUFA has strong and long-lasting effects on fat mass, body weight and abdominal obesity in postmenopausal women, but there were no significant differences in anthropometric measurements at 24 months between groups. Notably, triglyceride levels decreased significantly more in the PD group versus the control group based on the NNR diet. However, adherence to the target intake of protein was poor in the PD group.

Increased visceral fat mass, associated with liver fat (i.e., ectopic fat accumulation), may contribute to increased risk for the metabolic and cardiovascular complications after the menopause.[18] Triglyceride elevations are an essential part of the metabolic dysfunction seen with ectopic fat accumulation.[13] Of interest is that we recently showed a 5-week PD to be associated with a 49% decrease in liver fat and increased insulin sensitivity linked to a 41% decrease in serum triglycerides. A diet with a similar macronutrient composition as the present study was also shown previously to reduce visceral fat more than total fat on a long-term basis, albeit with a high dropout from the study after two years.[19] Further studies on putative effect by a PD on visceral fat and ectopic fat seem therefore warranted.

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The lack of a significant decrease in fasting glucose and insulin levels in our study may be explained in part by the fact that the subjects had normal glucose tolerance at baseline (i.e., “obese but healthy”), thereby reducing the possibility of improving the metabolic status and these cardiovascular risk markers. In contrast we found significant effects over time, but not between groups, of PAI-1 and CRP levels, with a mean decrease in the PD group that was approximately twice that of the NNR group. Analyses of the putative effects of a PD in subjects with a more pronounced metabolic dysfunction are encouraged by short-term studies indicating an improvement in the glucose tolerance and cardiovascular risk markers of patients with T2DM and CVD.[9, 10, 20]

The profound decrease in energy intake (20 % versus 12 % for the PD and NNR groups, at 24 months) is in line with earlier short-term studies from us and others.[9-11, 13, 20] This may be due to effects on satiety by increased intake of protein and low energy-dense foods, as well as PUFAs.[21-28] Notably, no significant differences were found in urinary nitrogen excretion between the diet groups at any time point, which may have limited the differences between groups. Adherence to the target protein intake was thus poor in the PD group, in line with earlier studies with the aim of increasing protein intake.[29, 30] The magnitude of effects on body composition and triglyceride levels do however suggest that larger randomized trials are done regarding putative differences between a PD and other types of diet with different macronutrient compositions. The reductions in fat mass, weight and abdominal obesity were thus profound, but less different between groups than expected from our power analysis.

Despite the stratification for BMI the PD group had a more beneficial metabolic profile at baseline, including higher HDL cholesterol levels. The NNR study group also had higher variability in some of the study variables, which may have influenced our ability to detect differences between the groups. Therefore, we may have underestimated the effects of the intervention on various outcome variables. In addition, the dropout rate from the study was larger than expected (37% and 23%, respectively for the NNR and PD group); the latter in line with recent data from Jönsson et al.[22]

We did not include an observational study group. Therefore, we cannot conclude whether the changes observed in these intervention groups differ from the natural course regarding the study parameters in this population. However, our aim was to investigate the possible effect of a diet regimen in comparison with the low-fat, high-fibre diet in Nordic countries generally recommended when the study started.

In conclusion, a PD during two years with *ad libitum* intake of macronutrients including an increased intake of PUFAs and MUFAs has sustained effects on fat mass and abdominal obesity with significantly better long-term effect on triglyceride levels versus an NNR diet. Adherence to the prescribed protein intake was poor in the PD group, suggesting that other components of the PD diet are of greater importance. The putative long-term beneficial effects of different components of the PD on obesity-related diseases, notably T2DM, need to be explored.

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Abbreviations

PD	Palaeolithic-type diet
NNR	Nordic nutrition recommendations
VAT	visceral adipose tissue
CVD	cardiovascular disease
RCT	randomized clinical trial
DXA	dual energy X-ray absorption
NI	nitrogen intake
REE	resting energy expenditure
TEE	total energy expenditure
PAEE	physical activity energy expenditure
GEE	generalized estimating equations
TFM	total fat mass

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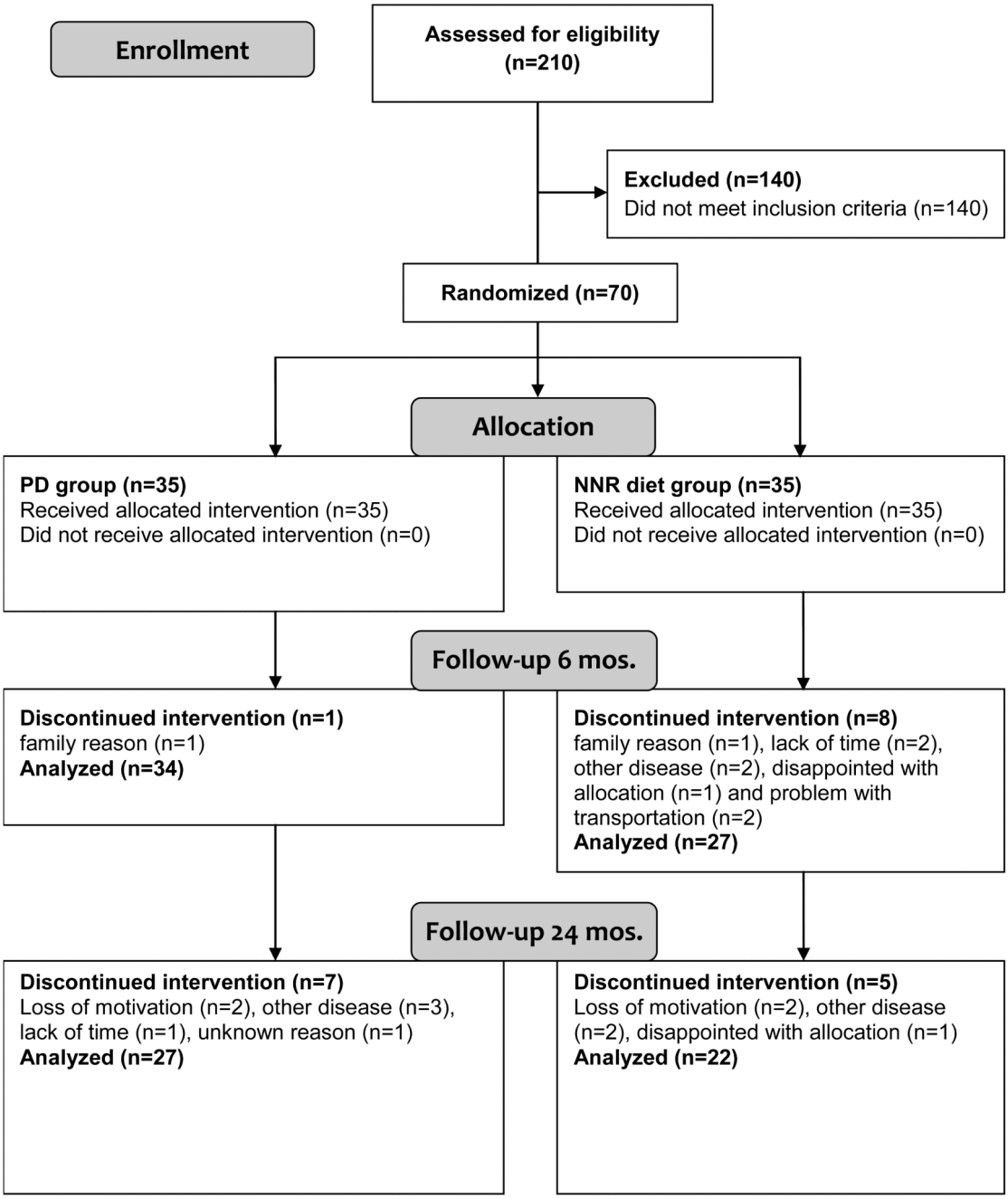

Figure 1. Flow diagram of subjects participation in the trial.

Participants were prescribed to eat either a Palaeolithic type diet (PD) or a diet according to the Nordic Nutrition Recommendations (NNR).

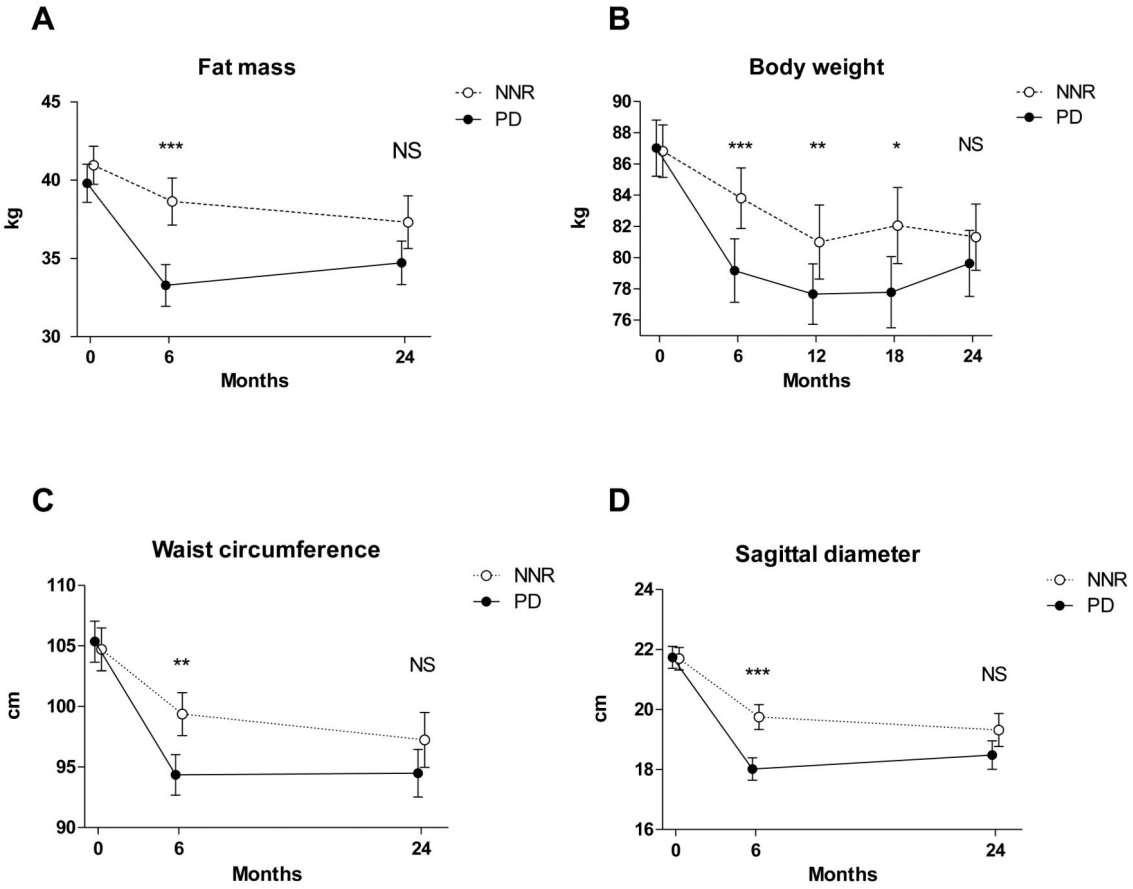

Figure 2. The effect of diet intervention on different anthropometric measurements.

Generalized estimated equations were used, with the Palaeolithic diet group as a reference group. *P*-values for the diet×time interaction for fat mass <0.001, weight <0.001, waist = 0.001, and sagittal diameter < 0.001. Data are presented as mean±SE. * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ for differences between diet groups at respective time points based on estimated marginal means. PD - Palaeolithic type diet; NNR - a diet according to Nordic Nutrition Recommendations.

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Table 1

Baseline characteristics of the study participants[1]

	Palaeolithic diet (n=35)	Nordic Nutrition Recommendations diet (n=35)	*P*-value
Age (years)	59.5 ± 5.5	60.3 ± 5.9	NS
Body weight (kg)	87.0 ± 10.6	86.8 ± 10.0	NS
Body mass index (kg/m^2)	32.7 ± 3.6	32.6 ± 3.3	NS
Waist circumference (cm)	105.4 ± 10.0	104.7 ± 10.4	NS
Sagittal diameter (cm)	21.7 ± 2.2	21.7 ± 2.2	NS
Total fat mass (kg)	39.8 ± 7.2	40.9 ± 8.6	NS
Total lean mass (kg)[2]	42.6 ± 5.0	41.7 ± 4.0	NS

NS - Not significant (*P*>0.05).

[1]Data are reported as mean ± SD. Differences between groups at baseline were tested by independent sample t-tests.

[2]Hypothesis testing after logarithmic transformation.

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Table 2

Changes in energy expenditure, dietary intake and biomarker of protein intake during 2 years of intervention among obese postmenopausal women[1]

	Palaeolithic-type diet	Nordic Nutrition Recommendations diet	Model effect diet×time[2]	Difference between groups[3]
REE (kcal/d) [MJ/d]			0.231	
Baseline	1324 ± 21.0 [5.54 ± 0.09]	1292 ± 25.1 [5.41 ± 0.11]		
Change 0 to 6 months	−50.0 ± 22.9 [−0.21 ± 0.10]	−27.8 ± 14.5 [−0.12 ± 0.06]		
Change 0 to 24 months	−29.9 ± 13.4 [−0.13 ± 0.06]	3.1 ± 14.8 [0.01 ± 0.06]		
PAEE (kcal/d) [MJ/d]			0.559	
Baseline	769 ± 33.4 [3.22 ± 0.14]	750 ± 43.0 [3.14 ± 0.18]		
Change 0 to 6 months	−16.7 ± 33.4 [−0.07 ± 0.14]	1.9 ± 33.4 [0.008 ± 0.14]		
Change 0 to 24 months	33.4 ± 43.0 [0.14 ± 0.18]	−7.2 ± 35.8 [−0.03 ± 0.15]		
TEE (kcal/d) [MJ/d]			0.956	
Baseline	2331 ± 47.8 [9.76 ± 0.20]	2288 ± 64.5 [9.58 ± 0.27]		
Change 0 to 6 months	−52.6 ± 45.4 [−0.22 ± 0.19]	−38.2 ± 43.0 [−0.16 ± 0.18]		
Change 0 to 24 months	4.78 ± 54.9 [0.02 ± 0.23]	0.72 ± 50.2 [0.003 ± 0.21]		
Energy intake (kcal/d) [MJ/d]			0.304	
Baseline	2000 ± 59.0 [8.37 ± 0.25]	2019 ± 59.1 [8.45 ± 0.25]		
Change 0 to 6 months	−375 ± 61.0 [−1.57 ± 0.26]	−359 ± 61.2 [−1.50 ± 0.26]		
Change 0 to 24 months	−401 ± 89.5 [−1.68 ± 0.38]	−251 ± 62.2 [−1.05 ± 0.26]		
Protein (E%)			<0.001	
Baseline	17.1 ± 0.32	17.2 ± 0.41		
Change 0 to 6 months	6.30 ± 0.54¶	1.59 ± 0.45¶		<0.001
Change 0 to 24 months	4.79 ± 0.72¶	0.16 ± 0.40		<0.001
Protein (g/d)			<0.001	
Baseline	84.4 ± 2.60	85.2 ± 2.44		
Change 0 to 6 months	9.34 ± 3.34§	−8.74 ± 2.48¶		<0.001
Change 0 to 24 months	0.39 ± 3.28	−8.84 ± 2.99§		0.037
Carbohydrate (E%)			<0.001	
Baseline	46.2 ± 0.67	45.3 ± 0.86		

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	Palaeolithic-type diet	Nordic Nutrition Recommendations diet	Model effect diet×time[2]	Difference between groups[3]
Change 0 to 6 months	−16.9 ± 1.09¶	−1.05 ± 0.89		<0.001
Change 0 to 24 months	−12.7 ± 1.68¶	−2.02 ± 1.84		<0.001
Carbohydrate (g/d)			<0.001	
Baseline	224 ± 7.83	222 ± 8.15		
Change 0 to 6 months	−104 ± 7.38¶	−40.8 ± 7.88¶		<0.001
Change 0 to 24 months	−87.2 ± 11.9¶	−32.5 ± 9.56§		<0.001
Total fat (E%)			<0.001	
Baseline	33.4 ± 0.60	34.6 ± 0.73		
Change 0 to 6 months	10.1 ± 1.13¶	−2.34 ± 0.87§		<0.001
Change 0 to 24 months	7.02 ± 1.40¶	0.26 ± 1.76		0.003
Total fat (g/d)			<0.001	
Baseline	74.6 ± 2.45	78.6 ± 3.00		
Change 0 to 6 months	3.43 ± 3.56	−18.0 ± 3.32¶		<0.001
Change 0 to 24 months	−3.77 ± 4.39	−9.98 ± 4.65‡		0.331
SFA (E%)			0.09	
Baseline	12.7 ± 0.34	13.5 ± 0.35		
Change 0 to 6 months	−2.87 ± 0.53	−1.71 ± 0.43		
Change 0 to 24 months	−2.16 ± 0.54	0.17 ± 1.02		
SFA (g/d)			0.227	
Baseline	28.2 ± 1.07	30.5 ± 1.32		
Change 0 to 6 months	−10.6 ± 1.24	−8.41 ± 1.57		
Change 0 to 24 months	−8.91 ± 1.83	−3.68 ± 2.43		
MUFA (E%)			<0.001	
Baseline	13.0 ± 0.31	13.1 ± 0.38		
Change 0 to 6 months	7.69 ± 0.69¶	−1.07 ± 0.39§		<0.001
Change 0 to 24 months	5.08 ± 0.88¶	0.24 ± 0.65		<0.001
MUFA (g/d)			<0.001	
Baseline	28.8 ± 1.07	29.6 ± 1.24		

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	Palaeolithic-type diet	Nordic Nutrition Recommendations diet	Model effect diet×time[2]	Difference between groups[3]
Change 0 to 6 months	8.20 ± 1.84¶	−7.06 ± 1.35¶		<0.001
Change 0 to 24 months	2.41 ± 2.02	−3.37 ± 1.60‡		0.025
PUFA (E%)			<0.001	
Baseline	5.39 ± 0.21	5.54 ± 0.22		
Change 0 to 6 months	4.32 ± 0.52¶	0.07 ± 0.30		<0.001
Change 0 to 24 months	3.19 ± 0.58¶	0.31 ± 0.50		<0.001
PUFA (g/d)			<0.001	
Baseline	12.0 ± 0.59	12.4 ± 0.59		
Change 0 to 6 months	5.75 ± 1.28¶	−1.84 ± 0.59§		<0.001
Change 0 to 24 months	2.82 ± 1.21‡	−1.04 ± 0.93		0.012
MUFA:SFA (ratio)			<0.001	
Baseline	1.05 ± 0.04	0.99 ± 0.03		
Change 0 to 6 months	1.14 ± 0.10¶	0.06 ± 0.03		<0.001
Change 0 to 24 months	0.75 ± 0.11¶	0.05 ± 0.05		<0.001
PUFA:SFA (ratio)			<0.001	
Baseline	0.44 ± 0.03	0.42 ± 0.02		
Change 0 to 6 months	0.60 ± 0.07¶	0.07 ± 0.03‡		<0.001
Change 0 to 24 months	0.44 ± 0.07¶	0.04 ± 0.04		<0.001
Omega-3 fatty acids (g/d)			0.001	
Baseline	2.68 (0.18)	2.62 (0.13)		
Change 0 to 6 months	1.13 (0.29)¶	−0.20 (0.23)		<0.001
Change 0 to 24 months	4.67 (0.52)¶	2.65 (0.44)¶		0.003
Omega-6 fatty acids (g/d)			0.005	
Baseline	9.04±0.45	9.22±0.52		
Change 0 to 6 months	3.56±1.01¶	−0.65±0.81		0.001
Change 0 to 24 months	−4.79±0.54¶	−4.81±0.62¶		0.986
Dietary cholesterol (mg/day)			<0.001	

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	Palaeolithic-type diet	Nordic Nutrition Recommendations diet	Model effect diet×time[2]	Difference between groups[3]
Baseline	305 ± 14.3	342 ± 17.4		
Change 0 to 6 months	276 ± 27.8[¶]	−43.6 ± 20.0[‡]		<0.001
Change 0 to 24 months	146 ± 28.2[¶]	−19.0 ± 20.6		<0.001
Dietary fibre (E%)			0.869	
Baseline	2.45 ± 0.06	2.18 ± 0.08		
Change 0 to 6 months	0.44 ± 0.12	0.50 ± 0.15		
Change 0 to 24 months	0.29 ± 0.10	0.24 ± 0.13		
Dietary fibre (g/d)			0.548	
Baseline	24.6 ± 1.03	21.9 ± 0.97		
Change 0 to 6 months	−0.94 ± 1.17	−0.35 ± 0.98		
Change 0 to 24 months	−2.70 ± 1.36	−0.77 ± 1.15		
Sucrose (g/d)			0.547	
Baseline	35.3 ± 2.11	40.9 ± 3.18		
Change 0 to 6 months	−8.36 ± 1.77	−11.2 ± 3.24		
Change 0 to 24 months	−7.24 ± 2.52	−11.3 ± 2.85		
Nitrogen in urine (g/d)[4]			0.374	
Baseline	13.0 ± 0.44	13.0 ± 0.40		
Change 0 to 6 months	0.04 ± 0.33	−0.68 ± 0.43		
Change 0 to 24 months	−0.47 ± 0.54	−0.98 ± 0.34		

Generalized estimating equations, with the PD group as a reference group, were used to estimate the change from baseline to 6 and 24 months within each treatment group and to test the difference between treatment groups.

Resting energy expenditure (REE), physical activity energy expenditure (PAEE), total energy expenditure (TEE), per cent of energy (E%), saturated fat (SFA), monounsaturated fat (MUFA), polyunsaturated fat (PUFA).

[1] All values are mean ± SE obtained from 4 days estimated food records. Change over time vs. baseline and within group;

[‡] $P<0.05$,

[§] $P<0.01$ and

[¶] $P<0.001$ (only presented if overall model effect was significant).

[2] The *P*-value represents the overall model effect of the diet×time interaction during the 24-month study.

[3] The *P*-value represents the effect of the diet×time interaction at 6 and 24 months, and was only calculated if the overall model effect was significant (post-hoc analysis).

[4] Nitrogen in urine, used as a biomarker for protein intake, was determined by analysis of nitrogen excretion in 24 h urine (Kjeldahl technique).

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Table 3

Changes in bioindicators during 2 years of intervention among obese postmenopausal women[1]

	Palaeolithic-type diet	Nordic Nutrition Recommendations diet	Model effect diet×time[2]	Difference between groups[3]
Insulin (mIU/L)			0.500	
Baseline	8.43 ± 0.69	9.02 ± 0.77		
Change 0 to 6 months	−1.29 ± 0.91	−-0.10 ± 0.91		
Change 0 to 24 months	−0.18 ± 0.53	0.87 ± 0.77		
Glucose (mmol/L)			0.465	
Baseline	5.13 ± 0.15	5.17 ± 0.20		
Change 0 to 6 months	−0.21 ± 0.15	0.05 ± 0.24		
Change 0 to 24 months	−0.04 ± 0.13	−0.002 ± 0.22		
Systolic blood pressure (mmHg)			0.293	
Baseline	141 ± 2.2	138 ± 2.2		
Change 0 to 6 months	−12.2 ± 2.2	−8.5 ± 1.9		
Change 0 to 24 months	−3.7 ± 3.5	1.7 ± 2.2		
Diastolic blood pressure (mmHg)			0.349	
Baseline	83.0 ± 1.3	82.9 ± 1.5		
Change 0 to 6 months	−6.6 ± 1.1	−5.0 ± 1.5		
Change 0 to 24 months	−4.8 ± 1.5	−1.5 ± 1.8		
Heart rate (beats/min)			0.401	
Baseline	72 ± 1.6	71 ± 1.7		
Change 0 to 6 months	−2.2 ± 1.4	−3.2 ± 1.1		
Change 0 to 24 months	−3.6 ± 1.8	−1.4 ± 1.7		
High sensitive CRP (mg/L)			0.464	
Baseline	2.14 ± 0.29	2.40 ± 0.31		
Change 0 to 6 months	−0.42 ± 0.20	−0.22 ± 0.17		
Change 0 to 24 months	−0.45 ± 0.23	−0.05 ± 0.29		
Tissue plasminogen activator (IU/mL)			0.544	
Baseline	0.51 ± 0.05	0.46 ± 0.06		
Change 0 to 6 months	0.06 ± 0.05	−0.03 ± 0.06		
Change 0 to 24 months	0.05 ± 0.06	0.05 ± 0.06		

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	Palaeolithic-type diet	Nordic Nutrition Recommendations diet	Model effect diet×time[2]	Difference between groups[3]
PAI-1 (U/mL)			0.431	
Baseline	21.3 ± 2.7	24.3 ± 2.3		
Change 0 to 6 months	−5.6 ± 2.3	−3.3 ± 2.3		
Change 0 to 24 months	−4.5 ± 2.1	−2.3 ± 3.3		
Cholesterol (mmol/L)			0.127	
Baseline	5.91 ± 0.14	5.52 ± 0.23		
Change 0 to 6 months	−0.67 ± 0.13	−0.39 ± 0.15		
Change 0 to 24 months	−0.20 ± 0.10	0.07 ± 0.11		
HDL (mmol/L)			0.896	
Baseline	1.49 ± 0.06 *	1.28 ± 0.05		
Change 0 to 6 months	−0.05 ± 0.05	−0.04 ± 0.04		
Change 0 to 24 months	0.16 ± 0.04	0.18 ± 0.04		
LDL (mmol/L)			0.291	
Baseline	3.87 ± 0.13	3.64 ± 0.21		
Change 0 to 6 months	−0.43 ± 0.10	−0.29 ± 0.11		
Change 0 to 24 months	−0.26 ± 0.08	−0.07 ± 0.09		
Triglycerides (mmol/L)			0.001	
Baseline	1.22 ± 0.09	1.27 ± 0.10		
Change 0 to 6 months	−0.38 ± 0.07 ¶	−0.12 ± 0.07		<0.001
Change 0 to 24 months	−0.23 ± 0.07 ¶	−0.01 ± 0.06		0.004

Generalized estimating equations, with the PD group as a reference group, were used to estimate the change from baseline to 6 and 24 months within each treatment group and to test differences between treatment groups.

[1] All values are mean ± SE. Change over time vs. baseline and within group;

¶ $P<0.001$ (only presented if overall model effect was significant).

* $P=0.01$ for difference between groups at baseline.

[2] The *P*-value represents the overall model effect of the diet×time interaction during the 24-month study.

[3] The *P*-value represents the effect of the diet×time interaction at 6 and 24 months, and was only calculated if the overall model effect was significant (post-hoc analysis).