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A Palaeolithic-type diet causes strong tissue-specific effects on ectopic fat deposition in obese postmenopausal women

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Abstract. Ryberg M, Sandberg S, Mellberg C, Stegle O, Lindahl B, Larsson C, Hauksson J, Olsson T (Umeå University, Umeå, Sweden; Max Planck Institute for Developmental Biology & Max Planck Institute for Intelligent Systems, Tuebingen, Germany; MRC Epidemiology Unit, Cambridge, UK; Umeå University, Umeå, Sweden; University of Gothenburg, Gothenburg, Sweden; Umeå University, Umeå, Sweden; Faculty of Medicine, University of Iceland, Reykjavik, Iceland). A Palaeolithic-type diet causes strong tissue-specific effects on ectopic fat deposition in obese postmenopausal women. *J Intern Med* 2013; **274**: 67–76.

Objectives. Ectopic fat accumulation in liver and skeletal muscle may be an essential link between abdominal obesity, insulin resistance and increased risk of cardiovascular disease after menopause. We hypothesized that a diet containing a relatively high content of protein and unsaturated fat [mainly monounsaturated fatty acids (MUFAs)] but limited carbohydrates and saturated fat would reduce lipid content in liver and muscle and increase insulin sensitivity in postmenopausal women.

Subjects. Ten healthy, nonsmoking postmenopausal women with a body mass index (BMI) >27 (28–35) kg m^{-2} were included in the study.

Interventions. Participants were instructed to consume an *ad libitum* Palaeolithic-type diet intended to provide approximately 30 energy percentage (E%) protein, 40 E% fat (mainly MUFAs) and 30 E % carbohydrate. Intramyocellular lipid (IMCL) levels in calf muscles and liver triglyceride levels were quantified using proton magnetic resonance spectroscopy (^{1}H-MRS) before and 5 weeks after dietary intervention. Insulin sensitivity was estimated by homoeostasis model assessment (HOMA) indices and the euglycaemic hyperinsulinaemic clamp technique.

Results. Mean energy intake decreased by 25% with a weight loss of 4.5 kg. BMI, waist and hip circumference, waist/hip ratio and abdominal sagittal diameter also decreased significantly, as did diastolic blood pressure (mean −7 mmHg), levels of fasting serum glucose, cholesterol, triglycerides, LDL/HDL cholesterol, apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1), urinary C-peptide and HOMA indices. Whole-body insulin sensitivity did not change. Liver triglyceride levels decreased by 49%, whereas IMCL levels in skeletal muscle were not significantly altered.

Conclusions. A modified Palaeolithic-type diet has strong and tissue-specific effects on ectopic lipid deposition in postmenopausal women.

Keywords: adipose tissue, diet, fatty liver, insulin resistance, postmenopausal, weight.

Introduction

After menopause, fat is redistributed from peripheral to central depots. This shift is associated with an increased incidence of diabetes and cardiovascular disease in postmenopausal women [1]. An important link between fat redistribution and increased cardiovascular disease risk may be ectopic lipid deposition, that is lipid accumulation outside adipose tissue, notably in liver and skeletal muscle. Indeed, it has been suggested that the incidence of nonalcoholic fatty liver disease (NAFLD) increases after menopause, possibly because of the combination of decreased oestrogen

levels and central fat accumulation [2]. Of note, NAFLD *per se* is an independent predictor of both type 2 diabetes and cardiovascular disease [3].

Increased fat content in the liver is clearly affected by dietary factors. Dietary fatty acids enter the liver through uptake of intestinally derived chylomicron remnants or as free fatty acids from chylomicrons hydrolysed in excess of tissue uptake capacity [4]. A high dietary carbohydrate intake, possibly including fructose, may also regulate *de novo* lipogenesis via activation of specific transcription factors/enzymes, including ChREBP and SREBP-1c [4, 5].

To date, strategies to reduce weight by restriction of total and saturated fat (SFA) intake, combined with an increase in physical exercise have been recommended as the basis for reduction of liver fat content [6, 7]. It has been suggested that moderate weight reduction (without a concomitant increase in physical activity) can reduce liver fat content without an accompanying effect on peripheral muscle lipids [4]. Indeed, weight loss of 6–8 kg in obese individuals, with or without type 2 diabetes, has been shown to decrease the amount of liver fat [8–10]. Of importance, few studies have investigated the role of different diets on liver fat content [11]. Recently, a high-protein intake was shown to attenuate the increase in intrahepatic fat induced by eating a hypercaloric high-fat diet [12]. This suggests that investigations of the role of macronutrient composition and food choice in this situation would be of interest.

Partial replacement of carbohydrates and SFAs with protein and monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) according to a Palaeolithic-type diet can be a favourable treatment option for NAFLD. The Palaeolithic-type diet may provide moderate weight loss through a spontaneous reduction in energy intake, even with an *ad libitum* approach. Furthermore, results from animal experiments as well as from small-scale studies in humans suggest that Palaeolithic-type diets may be more efficient than other recommended diets, including a Mediterranean-like diet, with regard to glucose tolerance/levels [13, 14]. However, to our knowledge, whether this type of diet may increase hepatic as well as peripheral insulin sensitivity, in conjunction with reductions in hepatic and/or intramyocellular skeletal muscle fat, has not been evaluated. Recently, Bjermo *et al.* showed that intake of ω-6 PUFAs can reduce liver fat content and improve metabolic status without weight loss in obese subjects, compared with an isocaloric diet rich in SFAs [15].

The aim of this study was to evaluate whether a Palaeolithic-type diet with *ad libitum* intake of food could reduce target organ fat content and increase insulin sensitivity in postmenopausal women.

Methods

Women were recruited from Umeå, Sweden to participate in the study through advertisements in local newspapers. Only postmenopausal (i.e. ≥ 1 year after the end of menstruation) nonsmokers with a body mass index (BMI) >27 kg m^{-2} were enrolled. At inclusion, all participants were healthy with no evidence of heart disease, kidney disease, hyper- or hypothyroidism, osteoporosis or diabetes. Fasting plasma glucose levels were normal, and blood pressure did not exceed 150/90 mmHg. Participants were not taking any prescribed drugs, including oestrogens. Women who were already consuming a carbohydrate-restricted or vegetarian diet or those allergic to key foods in the experimental diet were excluded. After providing written informed consent, each participant visited our clinical research centre for a series of tests. In total, 10 healthy, nonsmoking postmenopausal women who were overweight or obese (BMI 28–35 kg m^{-2}; Table 1) were included in the study. All subjects acted as their own controls; that is, all variables were measured before and 5 weeks after dietary intervention. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Regional Ethical Review Board at Umeå University.

Dietary intervention and assessment

Participants were given prepared meal portions that were intended to provide an average intake of about 30 energy percentage (E%) protein, 40 E% fat (mostly unsaturated) and 30 E% carbohydrates for breakfast, lunch and dinner, together with 40 g nuts (walnuts and sweet almonds) on a daily basis for 5 weeks. All meals were prepared by the food service at Umeå University Hospital and were weighed and frozen after preparation. The diet included lean meat, fish, fruit, vegetables (including root vegetables), eggs and nuts. Dairy products, cereals, beans, refined fats and sugar, added salt, bakery products and soft drinks were excluded.

Table 1 *Anthropometric variables in overweight postmenopausal women before and after 5 weeks of dietary intervention (n = 10)*

Variable	Baseline		5 weeks		*P*
Body weight (kg)	86.4	(81.3, 89.9)	81.8	(78.0, 85.8)	**
BMI (kg m^{-2})	31.3	(29.2, 34.0)	30.2	(27.7, 32.5)	**
Waist circumference (cm)	106	(100, 109)	98.0	(91.6, 107)	**
Hip circumference (cm)	113	(111, 115)	110	(106, 112)	**
WHR	0.92	(0.91, 0.96)	0.90	(0.86, 0.93)	*
Abd sag diameter (cm)	26.3	(25.1, 29.6)	24.4	(23.7, 26.3)	**
SBP (mmHg)	125	(119, 140)	115	(105, 128)	0.057
DBP (mmHg)	82.0	(75.0, 88.5)	75.0	(70.0, 78.5)	*
Heart rate (beats/min)	74.0	(70.0, 76.0)	64.0	(52.5, 68.0)	**
PAEE (kJ day^{-1} kg^{-1})	30.2	(21.6, 50.1)	32.7	(26.9, 53.2)	NS

Data are presented as median (25th, 75th percentiles). *P*-values refer to change from baseline to week 5 using the Wilcoxon matched-pair signed-rank test: *$P < 0.05$, **$P < 0.01$. abd sag, abdominal sagittal; BMI, body mass index; DBP, diastolic blood pressure; NS, nonsignificant; PAEE, physical activity energy expenditure; SBP, systolic blood pressure; WHR, waist/hip ratio.

Participants were instructed to complement the provided food with other included food items from the list, *ad libitum*. To enable preparation of additional complete meals at home, the women received 14-day menus together with recipes and instructions regarding portion sizes. They were also advised to use only rapeseed or olive oil in food preparation. The recommended alcohol intake was less than two glasses of red wine per week. Once a week, participants had one-to-one meetings with the study dietician to check their weight, collect prepared meals for the next 7 days, and review and complete their food records.

Food intake was assessed by individual diet history interviews at baseline (i.e. before the start of the study) and at the end of the study. During the interviews, food intake during the previous month including meal pattern, meal content, beverage intake, between-meal snacks, frequency of intake of individual food types and portion sizes were assessed. In addition, participants kept daily food records during the intervention of intake of provided food as well as food and beverages that they chose. Women were instructed to record the weight of any leftovers from the provided meals using a household scale. To estimate the amounts of food eaten, participants were instructed to use household utensils (e.g. cup, spoon) and a book of photographs of food portions [16] and standard weights of food items [17]. The reported food intake was converted to energy and nutrients using personal computer nutrient software package MATS version 4.06 (Rudans Lättdata, Västerås, Sweden). For nutrient calculations, we used the Swedish Food Database PC version 02_1 (National Food Agency, Uppsala, Sweden).

Physical activity

Free-living physical activity energy expenditure (PAEE) was estimated from data collected using a combined accelerometer and heart rate monitor (Actiheart Minimitter®, Respironics-Philips, Bend, Oregon, USA), as described in detail previously [18]. The sensor was attached to the left side of the chest using standard ECG pads. An 8-min step test on a 20-cm high step was used for individual calibration of heart rate to PAEE [19]. Acceleration and heart rate were recorded at a resolution of 15 × 15 s over a 7-day period, with participants carrying the heart rate monitor for 24 h day^{-1} (except when showering or swimming). Data collected during the 7-day period were downloaded to a computer, and each heart rate trace was processed using a two-stage inference scheme involving robust Gaussian process regression to handle potential measurement noise, as previously described [20]. Because of a lack of availability of auxiliary variables, the first stage of processing (noise classification) was adapted as follows: observations outside the range of 30–200 beats per min were classified as 'very noisy', and the remaining observations were classified as 'seminoisy' if the

fraction of very noisy observations exceeded 0.3 in the surrounding region (±40 observations); otherwise it was classified as 'clean'.

Instantaneous PAEE (J min^{-1} kg^{-1}) was estimated from the combination of individually calibrated heart rate and movement data [20] using a branched equation framework [21]. Periods of non-wear were inferred from the combination of non-physiological heart rate and prolonged periods of inactivity, which were taken into account to minimize diurnal information bias when summarizing the intensity time series into average daily PAEE (kJ kg^{-1} day^{-1}). Estimates of PAEE using the branched equation model compare favourably with those using the double-labelled water method [18].

Anthropometric data and blood pressure

Participants were weighed in light indoor clothing without shoes to the nearest 0.1 kg on a calibrated electronic digital scale (Tanita BWB-800 MA, Umedico AB, Rosersberg, Sweden). BMI was calculated as body weight divided by the square of height (kg m^{-2}). Using a tape measure, waist circumference was measured midway between the lower rib margin and the iliac crest during gentle exhalation and hip circumference was measured as the maximum circumference over the buttocks (both to the nearest 0.5 cm). Systolic and diastolic blood pressure measurements were recorded twice, with a 2-min interval, using an automatic blood pressure monitor (Boso-Medicus, Bosch, Germany).

Blood and urinary samples

All participants underwent routine laboratory screening including haematology and liver, kidney and thyroid function analyses. Fasting blood samples were drawn from the antecubital vein after at least 15 min resting period. Aliquots were frozen immediately and stored at −20 °C until required for analyses. Serum levels of glucose, cholesterol, triglycerides, HDL, LDL, apolipoprotein (ApoA1), apolipoprotein B (ApoB), C-reactive protein (CRP), Na, K, Cl, CO_2, creatinine, phosphate, albumin and Ca were analysed with Vitros Slides technology (Ortho Clinical Diagnostics, Johnson & Johnson, Sollentuna, Sweden). Serum levels of insulin, C-peptide, cortisol, thyroxine and thyroid-stimulating hormone were analysed with Elecsys kits (Roche Diagnostic Scandinavia AB, Bromma, Sweden). High-sensitivity CRP was analysed using an immunoassay method (IMMULITE, Diagnostic Products Corporation, Los Angeles, CA, USA). Levels of C-peptide were determined in aliquots from 24-h urine samples by enzyme-linked immunosorbent assay (ELISA) after a 1:10 dilution of the samples, using a kit from Mercodia (Uppsala, Sweden). Leptin and adiponectin were analysed using ELISAs.

Magnetic resonance imaging and spectroscopy

Magnetic resonance (MR) imaging and spectroscopy were performed with a 1.5 T ACS NT MR scanner (Philips, Best, the Netherlands), using the SENSE 5 element cardiac coil or an extremity coil (Philips) as the receiver coil. Participants lay in the supine position head first in the bore of the magnet.

Liver fat determination by ^1H MR spectroscopy

High-resolution T_1-weighted MR images were used for placement of the volume for spectroscopic investigations. The spectroscopic volumes were placed in the right lobe of the liver, carefully avoiding vascular structures and subcutaneous fat tissue. MR spectra were recorded using the point-resolved spectroscopy sequence with a repetition time (TR) of 3000 ms and an echo time (TE) of 25 ms, acquiring 64 signal averages from a volume element of 20 × 20 × 20 mm^3. In total, 1024 data points were acquired over a 1000-Hz spectral width. The spectra were acquired without presaturation of the water resonance. Respiratory triggering of spectroscopic data acquisition was accomplished using a software patch written by GyrTools Ltd, Zürich, Switzerland.

Spectra were analysed using LCModel version 6.2, written by Dr. Stephen Provencher (GyrTools Ltd, Zürich, Switzerland.). The areas of the combined lipid peak from the LCModel analysis (L16 + L09 + L13) and the area of the water peak were used to determine the hepatic liver content expressed as the ratio of lipid protons to water protons after correction for T_2 relaxation effects. A T_2 time for lipids of 78 ms and a T_2 time for water of 40 ms were used for correction of the T_2 relaxation effect [22].

Analysis of muscular lipid content

Transverse T_1-weighted turbo spin echo images were acquired for clear identification of the individual muscle groups, using a TR of 500 ms, TE of 12 ms, matrix size 256 × 256 and slice thickness of 10 mm. MR spectroscopy volumes (20 × 20 × 20 mm^3) were placed in both the soleus and the tibialis anterior muscles on the transverse

T_1-weighted turbo spin echo images that were acquired as described above. MR spectra were recorded using the same parameters as for the liver spectra. Two spectra were acquired, one with and one without water presaturation.

The muscle spectra were analysed using LCModel. The most important signal is the CH_2 resonance of intramyocellular lipids (IMCLs) at 1.3 p.p.m (IMCL13) [23]. The areas of the MR peaks were corrected for T_2 relaxation effects. A T_2 relaxation time for water of 40 ms and a T_2 relaxation time of 86 ms for IMCL were used [22]. The IMCL/water ratio was calculated [23].

Estimation of insulin sensitivity

Insulin sensitivity was estimated using homoeostasis model assessment (HOMA) indices and hyperinsulinaemic euglycaemic clamps. HOMA was estimated using the formula $(G_0 \times I_0)/22.5$, where G_0 is fasting plasma glucose and I_0 is fasting plasma insulin [24]. In the clamp study, participants fasted overnight and then received an insulin infusion at a supraphysiological level of 56 mU m^{-2} min^{-1} for 120 min. In parallel, subjects received a glucose infusion (200 mg mL^{-1}) the rate of which was adjusted to maintain euglycaemia, the infused amount of glucose to maintain euglycemia was used to estimate insulin sensitivity [25]. The M-value was calculated by dividing the glucose infusion rate during the last 60 min of the clamp by body weight [25].

Statistical analysis

Wilcoxon matched-pair signed-rank test was used to evaluate differences between baseline and 5 weeks. Statistical analyses were performed using SPSS (IBM SPSS, v19, Chicago, IL, USA).

Results

The characteristics including anthropometric data of the study group are shown in Table 1 and the daily dietary intake at baseline and after 5 weeks as reported in the diet history interviews and daily food records are shown in Table 2. There was a significant decrease in energy intake (−22%) and absolute intake (g day^{-1}) of carbohydrates (−58%), sucrose (−60%) and SFA (−57%), with a concomitant significant increase in intake of protein (+27%), total fat expressed as E% (+32%), MUFAs (+37%), PUFAs (+122%) and cholesterol (+90%). Thus, the ratio between energy intake from the macronutrients protein, total fat and carbohydrates expressed as E% changed significantly from 16:33:50 at baseline to 28:44:25 after 5 weeks, whereas the ratio between the fatty acids SFA, MUFA and PUFA changed from 13:12:5 to 8:21:12. No significant differences in reported absolute intake (g day^{-1}) of total fat, dietary fibre or alcohol were observed.

Median body weight decreased by 4.6 kg (−5%; Table 1). BMI, waist and hip circumference, waist/hip ratio and abdominal sagittal diameter also decreased significantly (−4%, −7%, −3%, −2% and −7%, respectively) as did diastolic blood pressure (−9%) and resting heart rate (−14%). Fasting serum levels of glucose, leptin, cholesterol, triglycerides, HDL, LDL, ApoB and apolipoprotein A1 (ApoA1) and percentage HDL also decreased significantly (Table 3).

There was a nonsignificant increase of about 8% in physical activity (kJ day^{-1} kg^{-1}) during the study (Table 1). Objective estimates of physical activity indicate that the participants in this study had a sedentary lifestyle.

Hepatic lipid content, as measured by ^{1}H-MRS, decreased significantly (mean −49%; Fig. 1). There were no significant changes in lipid levels in the different muscle compartments (Table 3). Urinary C-peptide excretion and HOMA indices decreased significantly, whereas whole-body insulin sensitivity, measured using the hyperinsulinaemic euglycaemic clamp technique, was not significantly changed (Table 3).

Discussion

We found that a short-term intervention of *ad libitum* intake of a Palaeolithic-type diet with a relatively high content of protein and unsaturated fat (mainly MUFAs) resulted in striking metabolic effects in obese postmenopausal women. A considerable decrease in liver triglyceride content was associated with lower fasting insulin and glucose levels. This change was accompanied by a profound decrease in C-peptide secretion, indicating increased hepatic insulin sensitivity in combination with decreased insulin secretion. Leptin levels also decreased significantly, possibly due to an effect of reduced adipose tissue and a catabolic state [26]. Furthermore, blood lipids demonstrated highly beneficial changes (except for a slight

Table 2 *Average daily dietary intake at baseline and after 5 weeks of dietary intervention (*n = *10)*

Variable	Baseline		5 weeks		*P*
Energy intake (kcal)	2408	(2210, 2860)	1888	(1795, 1969)	*
Energy intake (MJ)	10.0	(9.2, 11.9)	7.9	(7.5, 8.2)	*
Protein (E%)	16.0	(14.8, 18.0)	28.0	(27.8, 29.2)	**
Protein (g)	105	(91.3, 110)	133	(127, 142)	**
Carbohydrate (E%)	49.5	(45.8, 52.0)	25.0	(22.8, 29.0)	**
Carbohydrate (g)	281	(253, 362)	118	(102, 149)	**
Total fat (E%)	33.0	(29.5, 36.0)	43.5	(41.8, 46.2)	**
Total fat (g)	92.1	(76.4, 104)	94.8	(89.8, 98.2)	NS
SFA (E%)	13.0	(12.0, 16.0)	8.00	(7.75, 8.00)	**
SFA (g)	39.2	(33.4, 43.6)	16.7	(15.5, 17.8)	**
MUFA (E%)	12.0	(10.0, 13.0)	20.5	(18.8, 22.2)	**
MUFA (g)	32.8	(26.7, 37.0)	44.9	(41.0, 46.4)	**
PUFA (E%)	5.00	(3.00, 6.00)	12.0	(11.8, 12.2)	**
PUFA (g)	11.6	(9.8, 17.0)	25.8	(25.4, 27.1)	**
Cholesterol (g)	0.30	(0.24, 0.34)	0.57	(0.53, 0.61)	**
Fibre (g)	25.0	(20.7, 27.0)	26.9	(24.4, 30.1)	NS
Alcohol (E%)	1.50	(0.75, 2.50)	1.50	(1.00, 2.25)	NS
Alcohol (g)	4.35	(2.40, 9.48)	3.85	(2.12, 6.08)	NS
Sucrose (E%)	11.0	(8.5, 14.0)	6.00	(5.00, 6.50)	**
Sucrose (g)	69.5	(48.0, 85.2)	27.5	(24.8, 31.6)	**

Data are presented as median (25th, 75th percentiles) obtained from dietary history interviews at baseline and the combination of dietary history interviews and a 14-day daily food record at endpoint. *P*-values refer to change from baseline to week 5 using the Wilcoxon matched-pair signed-rank test: $*P < 0.05$, $**P < 0.01$. E%, energy percentage; MUFA, monounsaturated fat; NS, nonsignificant; PUFA, polyunsaturated fat; SFA, saturated fat.

decrease in HDL cholesterol levels and a reduction in ApoA1). Concomitantly, lipid content in skeletal muscles, as determined by ^{1}H-MRS, was unaltered, as was peripheral insulin sensitivity as quantified by the hyperinsulinaemic euglycaemic clamp technique.

To date, a few small short-term studies including the present study have shown beneficial effects of a Palaeolithic-type diet on weight, metabolic functions (including glucose levels/glucose tolerance) and cardiovascular disease risk markers in healthy people, as well as in patients with type 2 diabetes and/or ischaemic heart disease [13, 14, 27, 28]. Our findings suggest that a reduction in liver fat content may be essential for these beneficial effects.

The dietary regimen resulted in a significantly reduced energy intake (520 kcal day^{-1} reduction) despite the *ad libitum* approach. This may be one of the factors contributing to the striking decrease in liver fat content. A series of studies have indeed shown that hypocaloric diets reduce the amount of liver fat [8, 29]. Amongst eight obese individuals with type 2 diabetes (men and women), Petersen *et al.* found a significant reduction in liver steatosis with unaltered peripheral insulin sensitivity [8], as confirmed in the present study. However, the diet in the current study resulted in only a moderately decreased caloric intake (corresponding to an energy intake of approximately 1900 kcal day^{-1}vs.1200 kcal day^{-1} in the study by Petersen *et al.* [8]). This suggests that macronutrient composition is important, although the possibility cannot be excluded that the same result would be obtained with different food choices of identical macronutrient compositions.

Of interest, a high-protein diet was recently shown to blunt an increase in intrahepatic fat when added to a hypercaloric high-fat diet [12]. This was associated with an increase in bile acid concentration

Table 3 *Metabolic variables in overweight postmenopausal women at baseline and after 5 weeks of dietary intervention (n = 10)*

Variable	Baseline		5 weeks		*P*
Glucose (mmol L^{-1})	5.35	(4.95, 5.80)	5.00	(4.85, 5.28)	*
Insulin (mIU L^{-1})	8.35	(6.03, 12.5)	6.75	(5.18, 9.15)	*
C-peptide (nmol L^{-1})	0.84	(0.77, 1.15)	0.83	(0.71, 0.90	NS
Urinary C-peptide (nmol L^{-1})	9.67	(7.43, 24.7)	6.15	(4.42, 10.2)	**
Cortisol (nmol L^{-1})	272	(236, 332)	288	(221.0, 339)	NS
Cholesterol (mmol L^{-1})	4.45	(3.98, 5.10)	3.60	(3.28, 3.88)	**
Triglycerides (mmol L^{-1})	1.06	(0.96, 1.30)	0.67	(0.58, 0.75)	**
HDL (mmol L^{-1})	1.35	(1.23, 1.46)	1.17	(1.06, 1.37)	*
LDL (mmol L^{-1})	2.85	(1.95, 3.28)	2.20	(1.68, 2.45)	**
LDL/HDL ratio	2.06	(1.50, 2.49)	1.98	(1.44, 2.26)	NS
HDL%	29.5	(25.0, 33.5)	31.5	(28.3, 36.5)	*
ApoB (mg L^{-1})	902	(723.3, 1000)	773	(637, 810)	**
ApoA1 (mg L^{-1})	1358	(1231, 1421)	1210	(1074, 1270)	**
ApoB/ApoA1 ratio	0.71	(0.54, 0.76)	0.62	(0.53, 0.77)	NS
hsCRP (μg $m^{-1}L$)	3.61	(2.10, 4.35)	2.58	(1.17, 3.98)	NS
Adiponectin (ng mL^{-1})	3.45	(2.06, 8.04)	5.21	(2.93, 7.25)	NS
Leptin (ng mL^{-1})	34.0	(31.2, 47.6)	19.3	(14.2, 25.8)	**
M-value (mg kg^{-1} min^{-1})	5.97	(4.15, 6.83)	5.66	(4.67, 6.53)	NS
HOMA	2.24	(1.33, 3.19)	1.50	(1.16, 2.03)	**
Tibialis ant (IMCL/w)	93	(51, 166)	114	(100, 173)	NS
Soleus (IMCL/w)	343	(187, 573)	560	(243, 795)	NS

Data are presented as median (25th, 75th percentiles). *P*-values refer to change from baseline to week 5 using the Wilcoxon matched-pair signed-rank test: $*P < 0.05$, $**P < 0.01$.
ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; HDL, high-density lipoprotein; HOMA, homoeostatic model assessment [24]; hsCRP, high-sensitivity C-reactive protein; IMCL/w, intramyocellular fat/water ratio; LDL, low-density lipoprotein; M-value, euglycaemic hyperinsulinaemic clamp [25]; NS, nonsignificant.

during the period of high-protein intake. Increased bile acids may inhibit lipogenesis and favour hepatic lipid oxidation through stimulation of the liver X receptor and farnesoid receptor A [12]. In addition, high-protein diets are associated with increased satiety and thermogenesis, with a relatively more pronounced loss of fat versus lean mass and improved blood lipid profile compared with low-protein diets [12, 30–32]. The increased protein intake may thus play an important role, possibly overriding the effects of decreased energy intake in reducing liver fat content.

It is noteworthy that similar proportions of fat intake as in our study, but through mainly saturated fat, may induce obesity-independent NAFLD [11]. Westerbacka and co-workers suggested that the relative amount of fat may be important, demonstrating that isocaloric diets with low (16%) or high (56%) fat content resulted in a 20% decrease and a 35% increase, respectively, in liver fat content [10]. Concomitantly, the amounts of SFAs, MUFAs and PUFAs were reduced in the low-fat group. The role of fat composition thus requires further study. Of importance, a higher proportion of MUFAs in the diet may provide cardiovascular benefits because diets rich in MUFAs but deficient in SFAs and carbohydrates may improve insulin sensitivity, blood lipid levels and blood pressure in healthy individuals [33, 34]. It is also worth noting that at least part of the beneficial effects of a Mediterranean-like dietary pattern on reducing body weight, BMI, waist circumference, glucose and insulin resistance and improving several other

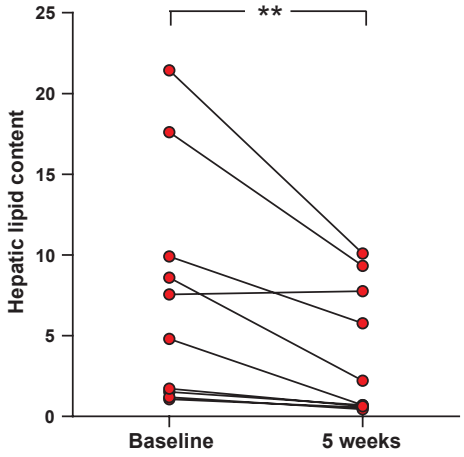

Fig 1 *Hepatic lipid content at baseline and after 5 weeks of dietary intervention (n = 10). Hepatic liver content expressed as the ratio of lipid protons to water protons. P-value refers to change from baseline to week 5 using the Wilcoxon matched-pair signed-rank test:* $^{**}P < 0.01$.

cardiovascular disease risk markers may be related to fatty acid balance with increased MUFAs [35, 36]. An increased intake of PUFAs *per se*, especially ω-3 fatty acids from fish oil such as Eicosapentaenoic acid and Docosahexaenoic acid, has also been shown to be beneficial. For example, a 6-month dietary intervention study with a PUFA-enriched diet showed beneficial effects on liver function and insulin sensitivity [37].

A relative decrease in carbohydrate intake may also contribute to the reduced liver fat content. A high carbohydrate intake, in association with insulin resistance, can thus lead to a change in ingested carbohydrate disposition from skeletal muscle glycogen synthesis towards hepatic *de novo* lipogenesis [38]. Importantly, *de novo* lipogenesis produces only SFAs, and NAFLD is associated with a relative increase in SFAs with a deficiency of PUFAs [32]. A reduced intake of carbohydrates, including fructose, may therefore provide multiple benefits in individuals with liver steatosis.

There may also be other sources of lipids in the liver including dietary chylomicron remnants, adipose tissue lipolysis and postprandial lipolysis of chylomicrons that can be produced in excess of tissue uptake (i.e. spill over) [11]. Of note, a reduced ability to increase adipose tissue blood flow in obese postmenopausal women may contribute to the high incidence of liver steatosis in this group [39].

Randomized controlled trials should be conducted including larger cohorts and different macronutrient dietary compositions. These studies should also include premenopausal women as well as men. A major strength of the present study is the careful evaluation of whole-body insulin sensitivity in combination with estimation of insulin production, through measurements of urinary C-peptide levels. In addition, using validated methods, we were able to show that energy expenditure was unaltered [18], verifying the beneficial effects of a reduction in liver steatosis on hepatic insulin sensitivity even though exercise regimens must be included to demonstrate effects on muscle/whole-body insulin sensitivity. The actual weight loss demonstrated in this study corresponds to a net energy deficit of 1000 kcal day^{-1}, but reported energy intake (1900 kcal day^{-1}), estimated resting metabolic rate (1200–1400 kcal day^{-1}, using FAO/WHO/UNU equations) [40] and estimated mean PAEE (600 kcal day^{-1}) rather suggest a steady-state of energy balance in our subjects. The reason for the pronounced weight loss with this dietary regimen is therefore not clear. Possible explanations include overreporting of energy intake, increased thermogenic effects of protein (versus other macronutrients) [41] and loss of glycogen, which may contribute to loss of body water during the study period. Of note, increased urinary volumes were commonly reported amongst participants during this intervention. Nonetheless, measurement uncertainties for all energy components on either side of the energy balance equation may be part of the apparent contradiction of achieving weight loss during energy balance.

In conclusion, a Palaeolithic-type diet led to a 49% reduction in liver triglyceride levels during a 5-week intervention. This was associated with improved hepatic insulin sensitivity. By contrast, whole-body insulin sensitivity and muscle fat content were unaltered. Whether a similar diet can be beneficial in the long-term in patients with NAFLD with associated metabolic dysfunction remains to be determined in randomized controlled trials.

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Conflict of interest statement

None of the authors has any conflicts of interest to declare.

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