Strong and persistent effect on liver fat with a Paleolithic diet during a two-year intervention

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ABSTRACT

BACKGROUND/OBJECTIVES: Our objective was to investigate changes in liver fat and insulin sensitivity during a 2-year diet intervention. An ad libitum Paleolithic diet was compared to a conventional, low-fat diet.

SUBJECTS/METHODS: Seventy healthy, obese, postmenopausal women were randomized to either a Paleolithic diet or a conventional, low-fat diet. Diet intakes were *ad libitum*. Liver fat was measured with proton magnetic resonance spectroscopy. Insulin sensitivity was evaluated with oral glucose tolerance tests and calculated as HOMA-IR/Liver IR index for hepatic insulin sensitivity and OGIS/Matsuda for peripheral insulin sensitivity. All measurements were performed at 0, 6, and 24 months. 41 women completed the examinations for liver fat and were included.

RESULTS: Liver fat decreased after 6 months by 64% (95% CI: 54-74%) in the Paleolithic diet group and by 43% (27-59%) in the low-fat diet group ($P < 0.01$ for difference between groups). After 24 months liver fat decreased 50% (25-75%) in the Paleolithic diet group and 49 % (27-71%) in the low-fat diet group. Weight reduction between baseline and 6 months was correlated to liver fat improvement in the low-fat diet group ($r_s = 0.66$, $P < 0.01$) but not in the Paleolithic diet group ($r_s = 0.07$, $P = 0.75$). Hepatic insulin sensitivity improved during the first 6 months in the Paleolithic diet group ($P < 0.001$ for Liver IR index and HOMA-IR), but deteriorated between 6 and 24 months without association to liver fat changes.

CONCLUSIONS: A Paleolithic diet with *ad libitum* intake had a significant and persistent effect on liver fat and differed significantly from a conventional low-fat diet at six months. This difference may be due to food quality, e.g. a higher content of mono- and polyunsaturated fatty acids in the Paleolithic diet. Changes in liver fat did not associate to alterations in insulin sensitivity.

INTRODUCTION

The prevalence of type 2 diabetes is steadily increasing worldwide. Obesity, mainly abdominal obesity, is associated with decreased insulin sensitivity and an increased risk for type 2 diabetes and cardiovascular disease (1).

A link between abdominal adipose tissue accumulation and metabolic–cardiovascular risk may be non-alcoholic fatty liver disease (NAFLD), defined as a liver fat content greater than 5.6% (2). NAFLD affects 30% of the general population and 60–80% of individuals with diabetes and obesity and has been suggested as a key marker for a metabolically unhealthy phenotype in obesity (3, 4). Notably, liver fat levels below the diagnostic threshold for NAFLD may also be associated with impaired insulin sensitivity (5). Moreover, the relationship between liver fat and insulin sensitivity exists independent of weight and the amount of visceral adipose tissue (VAT) (6).

Diet interventions that reduce body weight may ameliorate insulin resistance in patients diagnosed with obesity, but the mechanism by which weight loss improves metabolic balance remains unknown. A reduction in liver fat may be critical. Currently, the main strategy for reducing liver fat is to modify lifestyle by changing diet and increasing exercise (7). The combination of caloric and carbohydrate restriction decreases liver fat substantially within a couple of days (7), but long-term studies on liver fat reduction with diet interventions are lacking. Furthermore, there is a need for studies that control for physical activity and macronutrient intake (7).

Recently, Haufe et al. showed that 6 months of a hypocaloric diet low in either carbohydrate or fat had similar effects in reducing liver fat (8). This beneficial effect was associated with changes in insulin sensitivity. Interestingly, a later follow-up (17–36 months from study start) showed that improvements in liver fat and insulin sensitivity persisted, despite weight regain during the observational period (8).

A Paleolithic diet (PD) emphasizes a high intake of vegetables, fruit, nuts, eggs, fish and lean meat and excludes refined sugar, salt, dairy products and grains. By adhering to these recommendations the intake of mono-and polyunsaturated fatty acids increases compared to a conventional low-fat diet (9, 10). Other diets high in mono- or polyunsaturated fatty acids have shown greater liver fat reduction when compared to conventional low-fat diets (11, 12). A PD improves glucose tolerance and other cardiovascular risk factors, independent of change in waist circumference during short-term studies (9, 13). Furthermore, a PD lowered HbA1c levels more than a consensus diet in a crossover study in patients with type 2 diabetes (9).

Centrally located body fat is linked to a higher prevalence of NAFLD after menopause (14). We have recently reported a significant reduction in liver fat content after a 5-week *ad libitum* PD in 10 healthy, obese, postmenopausal women. Concomitantly, hepatic insulin sensitivity (Homeostasis model assessment-insulin resistance, HOMA-IR) improved (15). Therefore, we were interested in the long-term effects of a PD on liver fat and insulin sensitivity. Our hypothesis was that a PD would improve liver fat and insulin sensitivity more than a conventional, low-fat diet (LFD).

MATERIALS AND METHODS

Study design

Overweight postmenopausal women were randomized to either a Paleolithic diet (PD) or a conventional low-fat diet (LFD). Examinations were performed at 0, 6 and 24 months.

Subjects and randomization

Study participants were recruited through advertisements in local newspapers and posters within the Umeå University Hospital area. We included women after menopause with a BMI

above 27 kg/m^2. Exclusion criteria were smoking, hypertension, heart disease, diabetes, kidney disease, osteoporosis, thyroid disease, and medication with statins or beta-blockers, allergy to a key component of the intervention diets or consumption of a restricted diet. 210 women were interested in participating. 70 fulfilled the inclusion criteria and were randomized (Fig. 1). The randomization was carried out by a statistician blinded to the study with a block size of four and an allocation ratio of 1:1. There were no significant differences at baseline between the 41 included and the 29 excluded participants (data not shown). Nurses and technicians that carried out the examinations were blinded to group affiliation. Also the researchers conducting the statistical analyses were blinded. The study protocol was approved by the Regional Ethics Review Board at Umeå University, Umeå, Sweden and was in accordance with the Helsinki declaration. All participants gave written informed consent before inclusion.

Diet intervention

The Paleolithic diet (PD) was based on fish, seafood, lean meat, eggs, nuts, fruits and vegetables. Cereals, dairy products, legumes, added salt, and sugar were excluded. The PD aimed to provide 40 E% as fat with a high intake of mono- and polyunsaturated fatty acids. 30 E% were planned to come from carbohydrates and 30 E% from protein.

The conventional low-fat diet (LFD) was based on the Nordic Nutrition Recommendations (16). The women were advised to increase their intake of fruit, vegetables, whole grain, and fish. Meat and dairy products were to be low-fat. The LFD aimed to provide 25-30 E% as fat, 55-60 E% as carbohydrates, and 15 E% as protein.

Energy intake in both diet groups was *ad libitum*. Each study group met with a separate dietician on a regular basis but most frequently during the first 6 months. For details regarding diet validation see Mellberg et al (10).

Measurements of body composition

Weight was measured on a digital scale. Height was measured to the nearest 0.5 cm. Waist circumference was measured with a tape midway between the iliac crest and the lowest rib during gentle exhalation. Abdominal height was recorded at the umbilical level as the height of the abdomen measured when lying down on the examination couch with the legs straight. All measurements were performed after a four-hour fast.

Lean mass (kg), fat mass (kg) and body fat (%) were measured with dual energy X-ray absorptiometry (GE Medical Systems, Lunar Prodigy X-ray Tube Housing Assembly, Brand BX-1L, Model 8743, Madison, WI, USA) after a four-hour fast.

Measurement of physical activity energy expenditure

Physical activity energy expenditure was estimated using a heart rate monitor combined with an accelerometer (Actiheart, CamNtech Ltd, Cambridge, UK) as described previously (17-19).

Measurement of liver fat and visceral/subcutaneous adipose tissue

Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured with magnetic resonance imaging. Liver fat was determined by proton resonance spectroscopy. All magnetic resonance investigations were performed with a 1.5T ACS NT MR scanner (Philips, Best, The Netherlands). The details of the setup and measurement protocols have been described previously (15, 20, 21).

Measurement of insulin sensitivity and glucose metabolism

Baseline blood samples (glucose and insulin) were drawn after overnight fasting. The participants were given a solution containing 75 g glucose to drink within 5 min. Blood samples for plasma glucose and plasma insulin were then drawn every 30 min for a total of 2 h. Hepatic insulin sensitivity was calculated as the liver IR index [–0.091 + (log area under the insulin curve from 0–120 min × 0.4) + (log % fat mass × 0.346) – (log HDL-cholesterol × 0.408) + (log BMI × 0.435)] (22) and the HOMA-IR index [(fasting glucose × fasting insulin)/22.5] (23). Based on our recent meta-analysis, the oral glucose insulin sensitivity (OGIS) and Matsuda indices were chosen as surrogate measures for peripheral insulin sensitivity (24-26).

Measurement of blood lipids and blood pressure

Total cholesterol, triglycerides and HDL were analyzed at the Department for Clinical Chemistry, Umeå University Hospital, Umeå, Sweden. LDL was calculated as (cholesterol – HDL – triglycerides)/2.2. Blood pressure was measured in the sitting position with and automated blood pressure meter (Boso Medicus, Bosch, Jungingen, Germany).

Statistical analyses

Generalized estimation equations were used to test differences between diet groups during the entire period of intervention. When the overall model effect was significant ($P < 0.05$), analyses were conducted to determine whether the diet groups showed significant differences over the time periods of 0–6 months and/or 0–24 months. In addition, generalized estimation equations were used to analyze the change over time within each intervention group. Again, when the overall model effect was significant for the whole study period, we performed separate analyses for the 0–6 month and 0–24 month periods. Also the difference between baseline values was assessed with generalized estimation equations. Before conducting the

analyses, we logarithmically transformed the values for waist circumference, abdominal height, lean mass, fat mass, body fat, liver fat, fasting glucose, glucose 120 min after OGTT, fasting insulin, HOMA-IR, systolic blood pressure, diastolic blood pressure, triglycerides and HDL.

For correlation analyses, we used Spearman (r_s) because not all variables were normally distributed. Data are also reported after Bonferroni corrections for the number of analyses. All statistical analyses were performed with IBM SPSS Statistics for Mac, Version 21.0 (Armonk, NY, IBM Corp). Data are presented as means (SD) if not otherwise stated.

RESULTS

Subject characteristics

The results from the diet intervention have been published separately (10). The study participants were 61 (2) years respectively 62 (6) years old (PD, LFD). They had a BMI of 32.6 (3.9) kg/m^2 and 32.0 (2.7) kg/m^2 respectively (PD, LFD). There was no difference in baseline characteristics between diet groups (Table 1). Physical activity energy expenditure did not change during the study (10).

Body composition

Both diet groups lost weight and decreased their BMI, waist circumference, fat mass, visceral adipose tissue and subcutaneous adipose tissue throughout the study (Table 1). At 6 months the PD group showed a greater reduction in BMI, body fat (%), fat mass (kg) and subcutaneous adipose tissue than the LFD group. However, the LFD group lost less lean mass compared to the PD group. At 24 months there were no significant differences in body composition between diet groups except the better preservation of lean mass in the LFD group.

202

203 *Liver fat*

204 Both intervention groups reduced their liver fat significantly during the 24 months study
205 period (Fig.2, Table 1). After the first 6 months, the PD group had a more pronounced liver
206 fat reduction compared to the LFD group (Fig. 2). In the LFD group weight reduction was
207 strongly associated with liver fat reduction (Fig. 3). The strong correlation persisted ($r_s = 0.58$,
208 $P < 0.05$) even if calculated without a subject that reduced her weight by 17 kg and her liver
209 fat by 14%. However, in the PD group weight reduction was not associated with liver fat
210 reduction (Fig. 3). After six months all individuals in the PD group had reduced their liver fat
211 below 5% and 13 of the 25 participants below 1%. In the PD group liver fat changes during
212 the first six months of the study were strongly associated with baseline liver fat content ($r_s =$
213 0.92, $P < 0.001$). These analyses used only data from individuals that had completed all liver
214 fat examinations. Inclusion of all available liver fat data did not alter these results (data not
215 shown).

216

217 *Insulin sensitivity*

218 Hepatic insulin sensitivity measured with the Liver IR index and HOMA-IR improved
219 significantly after 6 months in the PD group but not in the LFD group (Table 1). Between 6
220 and 24 months, hepatic insulin sensitivity deteriorated significantly in the PD group ($P <$
221 0.001 for Liver IR index and HOMA-IR respectively), with a similar trend in the LFD group.
222 Peripheral insulin sensitivity improved non-significantly in both groups after the first 6
223 months (Table 1). The diet groups did not differ significantly regarding hepatic or peripheral
224 insulin sensitivity after the intervention (Fig.2, Table 1). These analyses used only data from
225 individuals that had completed all liver fat examinations. Inclusion of all available insulin

sensitivity data did not influence the interpretation of the results to any major extent (data not shown).

Association between liver fat, visceral adipose tissue and insulin sensitivity

VAT and liver fat correlated with insulin sensitivity measures at baseline (Table 2). These results did not change after adjustment for BMI (data not shown). In contrast, SAT was not correlated with liver fat or insulin sensitivity measures at baseline.

Changes in VAT and liver fat did not correlate significantly with changes in insulin sensitivity between baseline and 6 months (data not shown) and between baseline and 24 months (Table 3).

When we compared participants with liver fat >10% at baseline with those with <5.6%, we found that women with more liver fat had a greater increase in hepatic and peripheral insulin sensitivity than those with low amounts of liver fat, but this difference was not significant (data not shown).

Blood lipids and blood pressure

Triglycerides, total cholesterol and LDL improved significantly more in the PD group during the first 6 months of the study (Table 1). At 24 months both study groups showed an improvement of HDL (Table 1). Systolic blood pressure improved in both study groups at 6 months. However, diastolic blood pressure improved only in the PD group.

DISCUSSION

Liver fat decreased more after 6 months of a Paleolithic diet (PD) compared to a conventional, low-fat diet (LFD) in obese postmenopausal women. Hepatic insulin sensitivity

improved after 6 months of PD. After 24 months of PD liver fat was still low, but hepatic insulin sensitivity had deteriorated between 6 and 24 months.

One may argue that greater liver fat reduction in the PD group compared to the LFD group depends on the difference in weight reduction between both groups. In fact there is a strong correlation between weight reduction and liver fat improvement in the LFD group, but there was no such association in the PD group. After six months all participants in the PD group had reduced their liver fat below 5%, but this was not the case for the LFD group. Other factors than weight loss and calorie restriction may therefore play an important role for this effect, i.e. macronutrient composition and food quality. Westerbacka et al showed that two weeks of an isocaloric low-fat diet decreased liver fat content while an isocaloric diet with high fat content increased liver fat (27). In contrast, an isocaloric diet high in mono-unsaturated fatty acids was reported to reduce liver fat content in patients with diabetes (11). Moreover, a 6-week Mediterranean diet intervention improved insulin sensitivity and liver fat without weight reduction in individuals without diabetes (12). Preliminary analyses of serum fatty acid composition suggest a significant increase in polyunsaturated fatty acids between baseline and 6 months in the PD group compared to the LFD group (Blomquist et al, unpublished data). Thus, the benefits we observed with the PD may be associated with its high content of polyunsaturated fatty acids. Notably, a recent meta-analysis confirmed that n-3 polyunsaturated fatty acids have a positive effect on liver fat content (28). Furthermore, Rosqvist et al. showed that overfeeding with either polyunsaturated or saturated fat resulted in weight gain, but only study participants overfed with saturated fat had increased liver fat (29). Another possible explanation for the difference in liver fat at 6 months is the lower amount of carbohydrates in the Paleolithic diet which may cause decreased de novo lipogenesis (30).

We found a clear divergence between changes in liver fat and changes in insulin sensitivity after diet intervention. Both liver fat and visceral fat had improved at the end of the

study period. In contrast, hepatic insulin sensitivity improved initially but deteriorated between 6 and 24 months. Consistent with this finding, changes in liver fat content did not correlate with changes in insulin sensitivity.

A relationship between liver fat and hepatic insulin sensitivity was reported previously in individuals with type 2 diabetes (31), but this association may not be as clear-cut in individuals without diabetes (5, 32). In our cohort of overweight, postmenopausal women without diabetes, we found that liver fat content at baseline was moderately associated with hepatic insulin sensitivity. In line with this, both normal and impaired suppression of endogenous glucose production has been reported in subjects with NAFLD (6, 32).

Whether accumulation of liver fat is the cause or consequence of hepatic insulin resistance remains unclear (33). We found an improvement in hepatic insulin sensitivity in the PD group after the first 6 months, which deteriorated between 6 and 24 months. Concomitantly, liver fat content decreased between baseline and 6 months but remained unaltered during the remaining study period. This finding is in line with earlier studies showing short-term effects on hepatic insulin sensitivity after gastric bypass surgery and a very low-calorie diet (34, 35). Thus, a profound change in energy balance may rapidly improve hepatic insulin sensitivity. We therefore analyzed the ketone body beta-hydroxybutyrate as a marker of negative energy balance but found only a slight (non-significant) increase in beta-hydroxybutyrate in the PD group after 6 months (data not shown). This argues against a profound alteration in energy balance as a main factor underlying the metabolic improvement.

In the PD group, hepatic insulin sensitivity returned to baseline values from 6 to 24 months. Comparable results have been reported from studies on gastric bypass and a very low-calorie diet, in which a decrease in hepatic insulin sensitivity followed an initial

299 improvement (34, 35). In contrast, liver fat continued to decrease on the very low-calorie diet
300 until the end of the 8-week study (35).
301 Haufe et al. found that insulin sensitivity improved with a concomitant decrease in liver
302 fat after a hypocaloric, 6-month intervention with a diet reduced in either carbohydrates or fat
303 (36). After 2 years, liver fat and hepatic insulin sensitivity remained improved compared to
304 baseline levels. Notably, our participants were older than those in the Haufe et al. study,
305 which may have influenced insulin sensitivity (37). This influence may include effects of
306 menopause *per se* because estrogen may have a protective effect against the development of
307 liver steatosis (37). Furthermore, possible changes in fatty acid patterns during the diet
308 intervention are of interest for further studies as this can reflect alterations in compliance to
309 especially the PD.
310 Taken together, the results indicate that hepatic insulin sensitivity and liver fat can
311 change quickly after alterations in energy balance. However, after an initial rapid
312 improvement, hepatic insulin sensitivity seems to deteriorate gradually, despite reduced
313 intrahepatic fat levels.
314 At baseline, we found that VAT, liver fat, and insulin sensitivity were closely related, as
315 described previously (5, 8, 31, 32, 38-40). Therefore, the triad of VAT, liver fat, and insulin
316 sensitivity seems to be an important determinant of metabolic health, but this association was
317 not consistent after our diet intervention. It may therefore be advisable in future studies to
318 stratify by, for example, VAT (or waist circumference as a substitute) to eliminate potential
319 differences between study groups at baseline.
320 Major strengths of our study include the combination of a long-term trial with reliable
321 measurements of liver fat and visceral fat, with concomitant control of putatively confounding
322 factors, such as changes in physical activity and different adherence to diets (10). Notably,
323 protein intake did not differ between groups, despite different target levels, as estimated by

nitrogen excretion in the urine (10). A limitation is the lack of quantification of the intake of different carbohydrates with a method that is more accurate than food records. Two separate dieticians introduced the study participants to the different diets. This may have introduced a bias. However, both dieticians had earlier experience of educating individuals in the respective diet. The aim was therefore to decrease the risk that the participants in the LFD group felt as participants in a control group. Furthermore, future detailed studies with clamp techniques for more precise estimations of hepatic and peripheral insulin sensitivity are of interest.

In conclusion, a Paleolithic diet had a significant and persistent effect on liver fat and differed significantly from a conventional low-fat diet at six months. This difference may not be due to greater body weight reduction but to a difference in food quality, e.g. a higher content of mono- and polyunsaturated fatty acids in the Paleolithic diet.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

J.O. performed the statistical analysis, interpreted the data, drafted the figure and tables, and wrote the manuscript. C.M. recruited participants, collected the data, performed the statistical analysis, and wrote the manuscript. M.R. recruited participants, collected the data, and edited the manuscript. S.S. recruited participants and collected the data. J.K. analyzed the VAT and SAT data. C.L. and B.L. designed the study and interpreted the data. J.H. analyzed the liver spectroscopy data. T.O. designed the study, recruited participants, collected the data, interpreted the data, and wrote the manuscript. All authors actively participated in revising the paper and gave approval of the final version. J.O. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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FIGURE LEGENDS

Figure 1. CONSORT flow diagram

* The main analysis in the low-fat diet group was conducted with 16 subjects. Two additional subjects underwent liver spectroscopy at 24 months but did not have data from baseline/6 months. These individuals were included in a separate analysis including all available liver fat data.

Figure 2. Relative changes of liver fat and hepatic insulin sensitivity after a Paleolithic diet (PD) compared to a conventional, low-fat diet (LFD) in a 2-year intervention with postmenopausal obese women. Data represent mean relative changes in percent ± SEM. ** $P < 0.01$. Hepatic insulin sensitivity (b) (c) was estimated by the liver insulin resistance index (liver IR index) and homeostasis model assessment of insulin resistance (HOMA-IR).

Figure 3. Association between changes observed in weight and liver fat during 6 months of a conventional, low- fat diet and a Paleolithic diet

Table 1. Body composition, glucose metabolism, blood lipids and blood pressure

	Paleolithic diet			Low-fat diet		
	Baseline (n = 25)	6 months (n = 25)	24 months (n = 25)	Baseline (n = 16)	6 months (n = 16)	24 months (n= 16)
Age	61 (6)			62 (6)		
Body composition						
BMI (kg/m^2)	32.6 (3.9)	29.2 (3.8)**§§§	29.5 (3.9)§§§	32.0 (2.7)	30.4 (3.2)**§§§	30.2 (3.4)§§§
Weight (kg)	85.9(10.9)	76.8 (10.7)***§§§	77.9 (11.7)§§§	84.0 (7.7)	79.7 (9.2)***§§§	79.0 (9.7)§§§
Waist circumference (cm)	106 (11)	93 (10)§§§	94 (12)§§§	106 (7)	97 (7)§§§	94 (8)§§§
Abdominal height (cm)	21.4 (2.1)	17.7 (1.7)**§§§	17.9 (2.2)§§§	21.4 (1.9)	19.1 (1.6)**§§§	18.6 (2.1)§§§
Lean mass (kg)	41.6 (5.0)	40.0 (4.9)**§§§	40.0 (4.5)*§§§	41.1 (3.9)	40.9 (3.5)**	40.5 (3.9)*
Fat mass (kg)	39.6 (7.6)	32.4 (7.8)**§§§	34.4 (7.7)§§§	38.6 (5.3)	34.9 (6.7)**§§§	34.7 (7.1)§§
Body fat (%)	48.5 (4.4)	44.3 (6.0)*§§§	45.8 (5.7)§§§	48.3 (3.3)	45.8 (4.0)*§§§	45.7 (5.0)§
Visceral adipose tissue (L)	2.2 (0.7)	1.7 (0.6)§§§	1.8 (0.8)§§§	2.4 (0.8)	2.1 (0.6)§§	2.1 (0.8)§§
Subcutaneous adipose tissue (L)	6.5 (1.5)	5.2 (1.3)***§§§	5.7 (1.4)§§§	6.2 (1.0)	5.7 (1.3)***§§	5.5 (1.3)§§§

Liver fat (%)	4.6 (5.2)	1.2 (1.2)**§§§	1.6 (1.8)§§§	8.6 (8.7)	5.2 (7.9)**§§§	4.3 (6.0)§§§
Hepatic insulin sensitivity						
Liver IR index	4.78 (0.24)	4.60 (0.23)§§§	4.70 (0.24)§	4.75 (0.26)	4.70 (0.28)	4.81 (0.23)
HOMA-IR	1.97 (1.06)	1.31 (0.50)§§§	1.79 (0.91)	2.15 (1.08)	2.10 (1.07)	2.56 (1.48)
Peripheral insulin sensitivity						
OGIS	400 (75)	412 (67)	422 (50)	379 (83)	396 (68)	373 (68)
Matsuda	93 (50)	111 (44)	105 (62)	78 (47)	86 (50)	75 (45)
Glucose metabolism						
Fasting glucose (mmol/L)	5.0 (0.8)	4.9 (0.5)	4.9 (0.5)	5.4 (1.2)	5.4 (1.1)	5.4 (0.7)
Glucose 120 min after OGTT (mmol/L)	7.2 (2.4)	6.6 (1.5)	7.0 (1.8)	6.9 (2.2)	7.2 (1.8)	7.4 (2.0)
Fasting insulin (mIU/L)	8.6 (4.0)	6.0 (2.1)§§§	8.0 (3.6)	9.0 (4.3)	8.5 (3.6)	10.3 (4.7)

Blood lipids						
Total cholesterol (mmol/L)	6.0 (0.8)	5.2 (0.9)*§§§	5.7 (0.7)§	5.5 (0.9)	5.2 (1.0)*	5.6 (0.9)
Triglycerides (mmol/L)	1.2 (0.4)	0.8 (0.4)**§§§	0.9 (0.4)*§§§	1.2 (0.5)	1.1 (0.3)**	1.1 (0.3)*
HDL (mmol/L)	1.5 (0.3)	1.4 (0.3)	1.7 (0.3)§§§	1.3 (0.2)	1.3 (0.3)	1.6 (0.3)§§§
LDL (mmol/L)	4.0 (0.7)	3.4 (0.8)*§§§	3.6 (0.7)§§§	3.6 (0.8)	3.4 (0.8)*	3.5 (0.9)
Blood pressure						
Systolic (mm Hg)	136 (11)	127 (17)§§§	137 (24)	134 (14)	128 (15)§	140 (20)
Diastolic (mm Hg)	82 (8)	75 (9)§§§	79 (10)§	79 (6)	76 (7)	82 (7)

All data are presented as mean (SD). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for the difference between diet groups.

§ $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ for the change over time vs. baseline and within diet group.

Table 2. Correlations between body composition, liver fat, and insulin sensitivity at baseline.

Baseline measure	BMI (n = 41)	Subcutaneous adipose tissue (n = 41)	Visceral adipose tissue (n = 41)	Liver fat (n = 41)
Liver fat (n = 41)	0.27	0.03	0.49**	
Hepatic insulin sensitivity				
Liver IR index (n = 41)	0.48**	0.32*	0.56***	0.23
HOMA-IR (n = 40)	0.57***	0.23	0.68***	0.42**
Peripheral insulin sensitivity				
OGIS (n = 39)	−0.33*	−0.09	−0.68***	−0.46**
Matsuda (n = 39)	−0.50**	−0.17	−0.72***	−0.43**

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$ (with Bonferroni correction, $P < 0.001$ was considered significant). IR, insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; OGIS: oral glucose insulin sensitivity.

Table 3. Correlations between changes observed in body composition, liver fat, and insulin sensitivity. Changes were evaluated from baseline to 24 months after either a Paleolithic diet (PD) or a conventional, low-fat diet (LFD).

	Paleolithic diet			Low-fat diet		
Change 0 - 24 months (n for PD, and LFD)	BMI (n = 25)	Visceral adipose tissue (n = 25)	Liver fat (n = 25)	BMI (n = 15)	Visceral adipose tissue (n = 15)	Liver fat (n = 16)
Liver fat (n = 25, 16)	0.09	0.06		0.54*	0.52*	
Hepatic insulin sensitivity						
Liver IR index (n = 22, 15)	0.41	0.51*	0.22	0.53*	0.75**	0.43
HOMA-IR (n = 24, 16)	0.44*	0.45*	−0.16	0.50	0.40	0.45
Peripheral insulin sensitivity						
OGIS (n = 24, 13)	−0.22	−0.23	−0.03	−0.50	−0.55	−0.40
Matsuda (n = 24, 14)	−0.32	−0.29	0.05	−0.53	−0.43	−0.35

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$ (with Bonferroni correction, $P < 0.001$ was considered significant)

IR, insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; OGIS: oral glucose insulin sensitivity.

Fig. 1

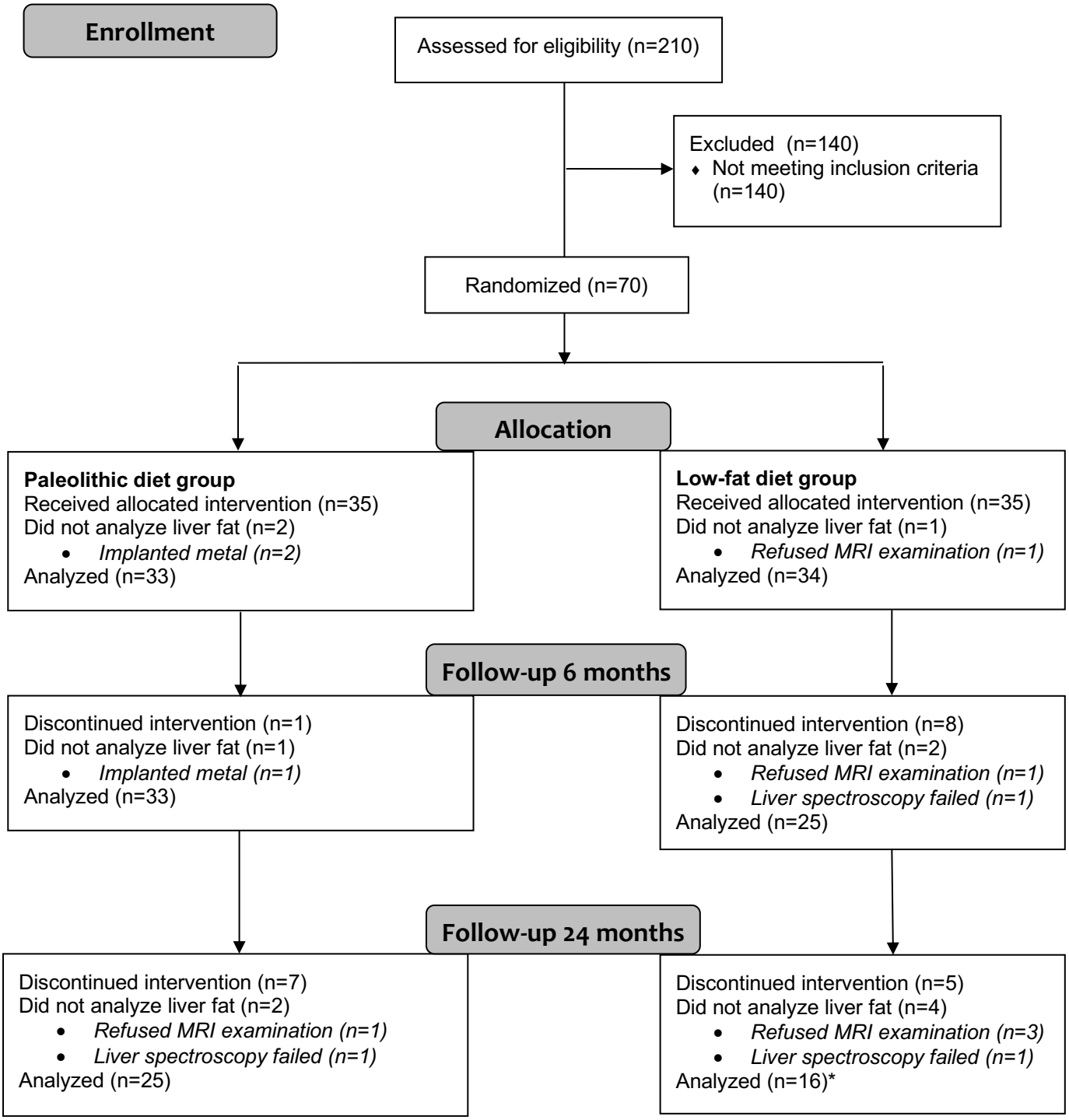

Enrollment
Assessed for eligibility (n=210)
Excluded (n=140)
♦ Not meeting inclusion criteria (n=140)
Randomized (n=70)
Allocation
Paleolithic diet group
Received allocated intervention (n=35)
Did not analyze liver fat (n=2)
• Implanted metal (n=2)
Analyzed (n=33)
Low-fat diet group
Received allocated intervention (n=35)
Did not analyze liver fat (n=1)
• Refused MRI examination (n=1)
Analyzed (n=34)
Follow-up 6 months
Discontinued intervention (n=1)
Did not analyze liver fat (n=1)
• Implanted metal (n=1)
Analyzed (n=33)
Discontinued intervention (n=8)
Did not analyze liver fat (n=2)
• Refused MRI examination (n=1)
• Liver spectroscopy failed (n=1)
Analyzed (n=25)
Follow-up 24 months
Discontinued intervention (n=7)
Did not analyze liver fat (n=2)
• Refused MRI examination (n=1)
• Liver spectroscopy failed (n=1)
Analyzed (n=25)
Discontinued intervention (n=5)
Did not analyze liver fat (n=4)
• Refused MRI examination (n=3)
• Liver spectroscopy failed (n=1)
Analyzed (n=16)*

Fig. 2

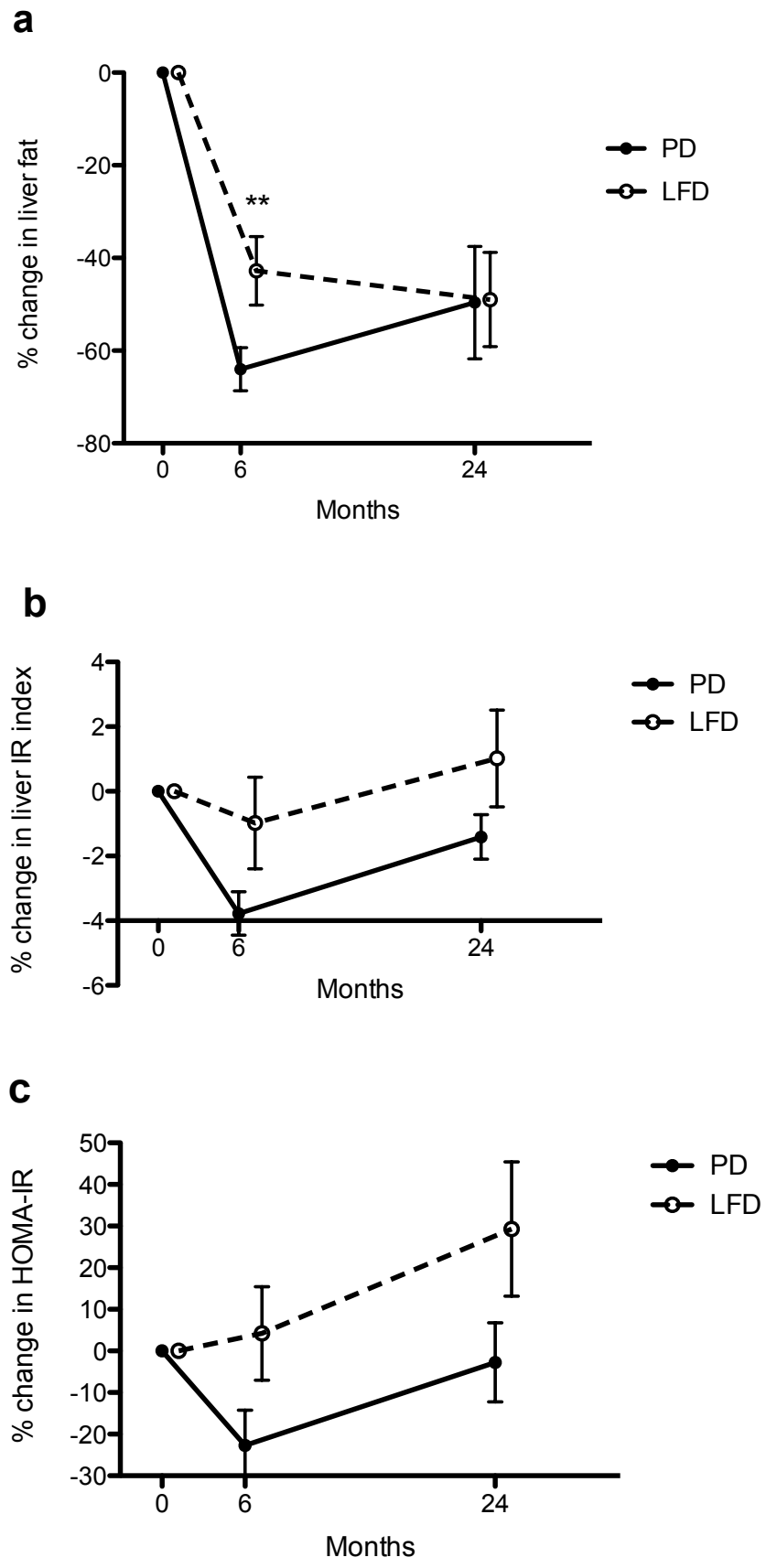
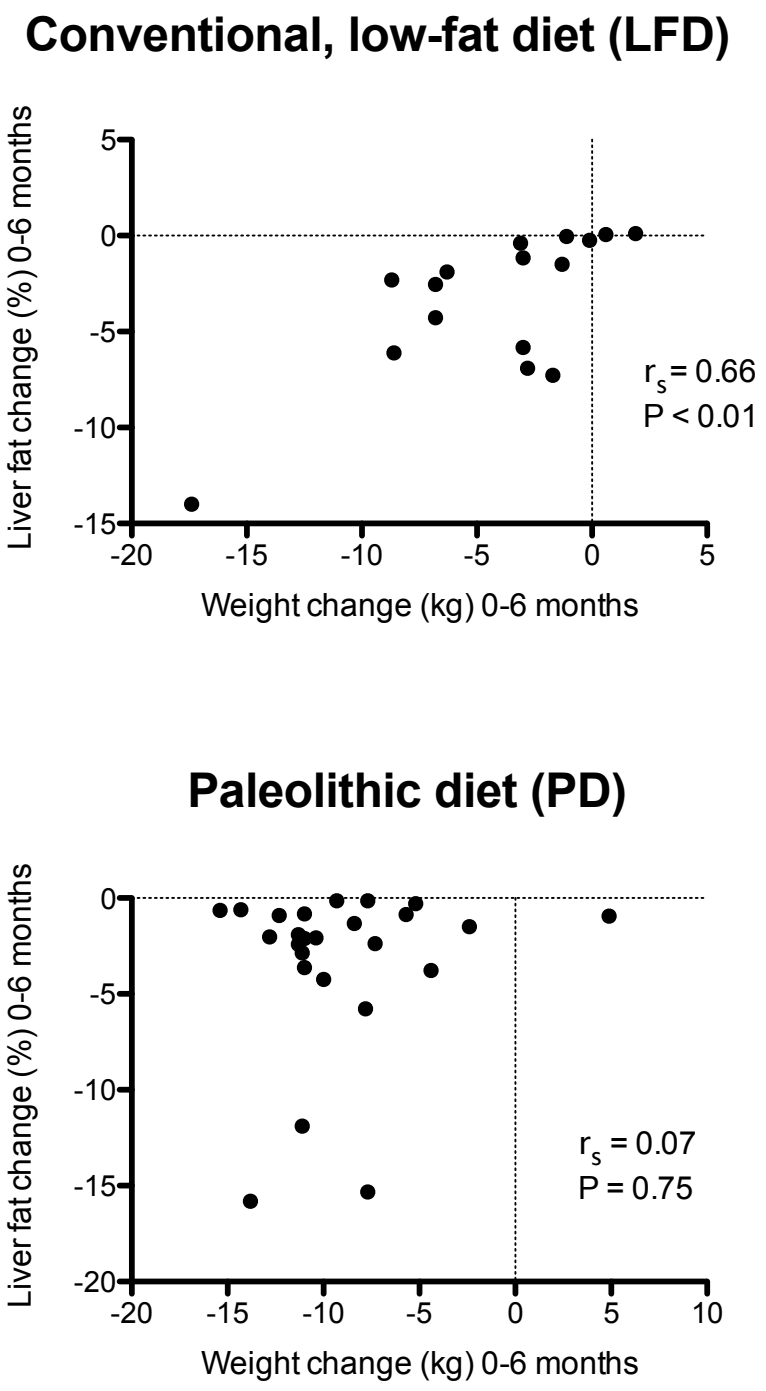

a
0
-20
-40
-60
-80
% change in liver fat
**
PD
LFD
0
6
24
Months
b
4
2
0
-2
-4
-6
% change in liver IR index
PD
LFD
0
6
24
Months
c
50
40
30
20
10
0
-10
-20
-30
% change in HOMA-IR
PD
LFD
0
6
24
Months

Fig. 3


Conventional, low-fat diet (LFD)
Liver fat change (%) 0-6 months
5
0
-5
-10
-15
-20
-15
-10
-5
0
5
Weight change (kg) 0-6 months
r_s = 0.66
P < 0.01
Paleolithic diet (PD)
Liver fat change (%) 0-6 months
0
-5
-10
-15
-20
-20
-15
-10
-5
0
5
10
Weight change (kg) 0-6 months
r_s = 0.07
P = 0.75