Atherosclerosis 279 (2018) 52–61

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Effect of low carbohydrate high fat diet on LDL cholesterol and gene expression in normal-weight, young adults: A randomized controlled study

Kjetil Retterstøl[a,c,*], Mette Svendsen[a,b], Ingunn Narverud[a,d], Kirsten B. Holven[a,d]

[a] Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, PB 1046, Blindern, 0317, Oslo, Norway
[b] Section for Preventive Cardiology, Department of Endocrinology, Obesity and Preventive Medicine, Oslo University Hospital, Norway
[c] Lipid Clinic, Department of Endocrinology, Obesity and Preventive Medicine, Oslo, University Hospital, Norway
[d] Norwegian National Advisory Unit on Familial Hypercholesterolemia, Oslo University Hospital, Rikshospitalet, Norway

HIGHLIGHTS

- Low carbohydrate/high fat (LCHF) diet for three weeks increased LDL-cholesterol (LDL-C) with 44% *versus* controls.
- The response to LCHF diet varied between the individuals from no more than 5% increase in LDL-C and up to a 107% increase.
- The unpredictable individual response to the LCHF diet suggest that LDL-C should be measured in people using an LCHF diet.

ARTICLE INFO

Keywords:
Fat
Diet
Cholesterol
LDL
Fatty acids
Saturated fatty acids
Cardiovascular risk factors
Atkins Diet

ABSTRACT

Background and aims: The effects of a low carbohydrate/high fat (LCHF) diet on health are debated. This study aims to explore the effects of a diet with less than 20 g carbohydrates per day (LCHF) on plasma low density lipoprotein cholesterol (LDL-C) in young and healthy adults. The secondary aim is the assessment of lipid profile and peripheral blood mononuclear cells (PBMC) gene expression.

Methods: This was a randomized controlled parallel-designed intervention study. Participants were either assigned to a three-week LCHF diet or a control group continuing habitual diet *ad libitum,* in both groups.

Results: In total, 30 healthy normal weight participants completed the study. Nine subjects did not complete it due to adverse events or withdrawn consent. In the LCHF diet group (n = 15), plasma LDL-C increased from (mean ± SD) 2.2 ± 0.4 mmol/l before intervention to 3.1 ± 0.8 after, while in the control group (n = 15), LDL-C remained unchanged: 2.5 ± 0.8 mmol/l ($p < 0.001$ between groups). There was a significant increase in apolipoprotein B, total cholesterol, high-density lipoprotein cholesterol, free fatty acids, uric acid and urea in the LCHF group *versus* controls. Plasma levels of triglycerides, lipoprotein (a), glucose, C-peptide or C-reactive protein (CRP), blood pressure, body weight or body composition did not differ between the groups. PBMC gene expression of sterol regulator element binding protein 1 (SREBP-1) was increased in the LCHF group *versus* controls ($p \leq 0.01$). The individual increase in LDL-C from baseline varied between 5 and 107% in the LCHF group.

Conclusions: An LCHF diet for three weeks increased LDL-C with 44% *versus* controls. The individual response on LCHF varied profoundly.

1. Introduction

The role of low carbohydrate high fat (LCHF) diets in relation to health effects is debated in the scientific literature [1,2] as well as the role of saturated fatty acids for the development of atherosclerosis despite the overwhelming evidence linking saturated fat to increased LDL-C levels [3–5]. Low carbohydrate diet has a long tradition for the purpose of weight reduction and data on the effects have gradually accumulated, in particular after Robert C. Atkin published his bestseller book *Dr. Atkin's Diet revolution* in 1972 [6]. Less than 20 g carbohydrates per day are allowed in the initially phase of the Atkin diet, typically resulting in a high intake of saturated fat. A high intake of saturated fat > 10 energy percent (E%) is not in accordance with major dietary guidelines [7–9] and causes concern regarding cardiovascular diseases

* Corresponding author. Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, PB 1046, Blindern, 0317, Oslo, Norway.
E-mail address: kjetil.retterstol@medisin.uio.no (K. Retterstøl).

https://doi.org/10.1016/j.atherosclerosis.2018.10.013
Received 21 April 2018; Received in revised form 22 September 2018; Accepted 16 October 2018
Available online 17 October 2018

(CVD). LCHF has become a trend diet with millions of users. In a study of more than 32,000 U.S. dieters, nearly 34% of respondents reported that the Atkins diet helped them loose and maintain weight [10]. Books promoting LCHF diets have been on the top of sales lists in the Nordic countries in the recent years. Longitudinal data for the period when LCHF diet was most trendy reported an increased intake of saturated fat in Finland and Sweden [11,12]. Butter and cream are typically promoted as healthy in LCHF literature, and in Norway, the use of butter increased by 24% during the period 2009–2012 [13]. Consumption of butter increased globally at a rate of 2–4% annually and sales of whole milk increased 11% the first six months of 2015 in the US, according to a report from Credit Suisse [14], indicating the consumers choice. In line with food consumption data, the steady decrease in mean serum low density lipoprotein cholesterol (LDL-C) in the populations over almost 40 years has recently been replaced by an increase in serum LDL-C observed both in Finland [11] and in northern Sweden [12].

There are no randomized controlled trials (RCT's) reporting outcomes on hard end points, such as CVD, after the use of LCHF diets. Several large association studies have, however, challenged the view that saturated fat increases the risk for CVD [15–17], while other studies find clear correlation with intake of SFA and risk of CVD [18,19]. Interestingly, previous studies on LCHF diets have mainly been performed in overweight subjects for the purpose of weight reduction [20]. A meta-analysis on the effect of low carbohydrate diets with 23 RCT reported a statistically significant higher LDL-C (0.1 mmol/l, 95% CI: 0.026–0.165) in the low-carbohydrate *versus* the low-fat diet groups [21]. Another meta-analysis of eleven RCT with 1369 participants using a diet with less than 20 E% carbohydrate also observed an increase in LDL-C (0.16 mmol/l, 95% CI: 0.003, 0.33) [22]. Notably, within these meta-analysis, there are single RCT reporting significant reduction [23] or increase [24] of LDL-C by the low-carbohydrate diets. There are many possible reasons for the difference in the effect on LDL-C, like the amount of saturated fatty acids or cholesterol in the diets. Further, weight reduction itself typically leads to beneficial changes in the lipid profile [25], particularly in the initial catabolic phase [26]. Indeed, LCHF diets are used not only by overweight, but by healthy normal-weight people as well [27]. Thus, we need more data on the effect of LCHF diet in normal-weight people.

An LDL-C increasing effect of saturated fat has been suggested to involve both lowering of LDL-receptor numbers [28] and the transcription factors, sterol regulatory element binding protein (SREBP) [29]. Together with proprotein convertase subtilisin/kexin type 9 (PCSK9), which modulates the degradation of LDL receptors [30], these factors are key regulators of the serum LDL-C levels.

The primary aim of the present study was to investigate the effect of three weeks on LCHF diet on serum LDL-C in healthy, normal-weight, young adults. Further, we aimed to study the effect of LCHF on other lipid and metabolic markers and expression of genes involved in lipid metabolism in peripheral blood mononuclear cells (PBMC) and circulating PCSK9 levels.

2. Materials and methods

2.1. Study participants

Participants were mostly recruited among students or employees at the Department of Nutrition Research at the University of Oslo after information meetings. The participants were included if they were willing to change their dietary intake according to the restrictions of a LCHF diet, provided informed consent and if they fulfilled the inclusion criteria after a physical examination by the study physician. Inclusion criteria were age 18 years or older, no chronic disease present, alcohol consumption less than 14 units per week and not following any particular dietary restrictions. Exclusion criteria were chronic diseases, regular use of medications (except oral contraceptives), blood pressure ≥140/90 mm Hg, pregnant or lactating women.

2.2. Study design and diet

The study was a three week randomized controlled parallel-designed intervention study, performed in the period September 2011 to December 2012. The LCHF group was instructed to follow the guidelines described in Dr. Atkins' New Diet Revolution [6] aiming to limit carbohydrate intake, and otherwise eat *ad libitum*. The subjects should eat in accordance to their energy needs with the same level of physical activity during the study period to maintain weight. The amount of carbohydrate was restricted to 20 g per day, or no more than 5 E%. There were no specific requirements for energy distribution between the intake of protein and fat. The diet was self-selected, thus the type of fat was optional. The aforementioned restrictions meant in practice that participants could eat unlimited quantities of poultry, meat, fish, seafood, eggs and vegetable oils. A partial limited intake existed for some food items such as whole-fat cheeses containing carbohydrates and vegetables with low carbohydrate content, such as spinach, sprouts, mushrooms, cucumber and avocado. Use of sweeteners such as sucralose, saccharin, cyclamate, acesulfame K, aspartame and erythritol was allowed. Due to diet restrictions on carbohydrates, beer, wine and liquor containing carbohydrate alcohol were naturally limited in the LCHF group but there were no restriction on alcohol intake as such. The participants in the control group were instructed to continue with their ordinary diet.

The study was registered in ClinicalTrials.gov with the ID number: NCT01476436. The study protocol was approved by the regional ethical committee South-Eastern Norway with the ID number: 2011/1365.

2.3. Assessment of dietary intake and subjective feelings of the LCHF diet

Prior to study start, the participants performed a weighed dietary record for four days to assess their habitual diet. A weighed dietary record was also performed for three days during the LCHF diet period. The dietary records were calculated for energy and nutrients by KostBeregningsSystem version 7.1, University of Oslo, Norway and “MAT PÅ DATA” version 5.2 and nutrient calculations are based on the Norwegian Food Composition Table from 2006 [31].

2.4. Anthropometric measures

Body weight, fat free mass, fat mass and total body water were determined using a bioimpedance analyzer (Tanita TBF-300, Tanita Corp., Japan) at baseline and after three weeks of LCHF diet. The measurement was undertaken according to the manual and the subjects fasted for at least 8 h prior to study visits. During the measurement the subjects were in standing position with the arms and legs abducted from the body and instructed not to move or speak.

2.5. Blood pressure

Fasting systolic and diastolic blood pressure (BP) measurements were performed by trained personnel. Three measurements at 1-min intervals were recorded after 10 min of rest in a waiting room followed by another 5 min in an investigation room where the subject sat in a resting chair with the cuff mounted and the arm at the armrest. Validated oscillometric devices (Carescape V100, GE Healthcare, Norway) with suitable cuffs were used for the measurements. In the analyses, we used the mean of all three measurements.

2.6. Blood sampling and analysis

2.6.1. Routine laboratory analysis

Fasting blood samples were drawn at baseline and after three weeks. Serum was obtained from silica gel tubes (Becton Dickinson) kept in room temperature for at least 30 min, until centrifugation at 1500*g* for

12 min. Plasma was obtained from EDTA tubes (Becton Dickinson), immediately placed on ice and centrifuged within 10 min (1500 rpm, 4 °C, 15 min). The plasma samples were frozen and stored at −80 °C until further analysis. Routine measurements were analyzed at the central laboratory of Oslo University Hospital, Rikshospitalet. Plasma (P)-total cholesterol (TC) and P-triglyceride (TG) was measured with an enzymatic colorimetric assay, while P-LDL-C and P-high density lipoprotein cholesterol (HDL-C) was measured with a homogeneous enzymatic colorimetric assay. P-C reactive protein (CRP) was measured by particle reinforced immunoturbidimetric assay and serum-glucose was measured enzymatic with hexokinase. All analyses were carried out on Cobes 8000, c702. Apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) were measured by turbidometry on Cobas c501. The instruments, reagents and calibrator were delivered from Roche Diagnostics (Mannheim, Germany). All analyzes were accredited after International and European standard NS-EN ISO 15189.

2.7. Expression of lipid-related genes in peripheral blood mononuclear cells (PBMCs)

After blood collection, PBMCs were isolated by using the BD Vacutainer Cell Preparation tubes with sodium heparin according to the manufacturer (Becton Dickinson, San Jose, CA) and stored as pellets at −80° C until further mRNA isolation. Total RNA was isolated from all cell pellets using RNeasy mini kit (Qiagen, Hilden, Germany) with lysis buffer added β-mercaptoethanol, and treated with DNase I (Qiagen) according to the manufacturer's instructions and stored at −80 °C. RNA quantity and quality measurements were performed using ND 1000 Spectrophotometer (Saveen Werner Carlson Circle Tampa, FL) and Agilent Bioanalyser (Agilent Technology, Santa Clara, CA), respectively. Four hundred ng of RNA from all samples was reverse transcribed using High capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA). Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) was performed on an ABI PRISM 7900HT Sequence Detector System (Applied Biosystems, Foster City, CA) using custom designed TaqMan array microfluidic cards (Applied Biosystems). Product numbers of the inventoried TaqMan probe and primer sets used are provided in Table 3. The relative mRNA expression level for each transcript was calculated by the ΔΔ cycle threshold (Ct) method [32]. The reference genes glucuronidase β (GUSβ) and TATA box binding protein (TBP) were used for normalization. Briefly, the Ct values of each target gene was normalized to the mean Ct values of the two reference genes (=ΔCt). ΔΔCt was then calculated as $\Delta Ct^{\text{end of study}}$ minus $\Delta Ct^{\text{baseline}}$. The fold change in mRNA expression was calculated as $2^{-\Delta\Delta Ct}$.

2.8. Enzyme linked immunosorbant assay (ELISA)

Concentration of Proprotein convertase subtilisin/kexin 9 (PCSK9) was measured by ELISA (R&D Systems, Minneapolis, MN) using serum samples. The inter- and intra-assay coefficient of variation were < 10%.

2.9. Statistical analyses

Data are presented as mean ± standard deviation (SD) if normally distributed, or median (minimum-maximum) if not normally distributed. Wilcoxon signed rank test was used to assess change within groups and Mann Whitney *U* test was used to compare changes between groups when the data was not normally distributed. Paired *t*-test was used to assess change within groups and independent t-tests for comparisons between groups when the data was normally distributed. $p < 0.05$ was regarded as significant. IBM SPSS Statistics v 20 (Armonk, NY, USA) was used for statistical analysis. In the present study we used a conservative approach and hypothesized that Atkins diet would lead to an increase in LDL-C from 2.0 to 2.5 mmol/l, which is a less increase than what we observed in a small pilot study [33]. In the pilot study we observed a median (min-max) increase of LDL-C from 2.2 (1.8–3.4) mmol/l at baseline to 3.1 (1.9–6.2) mmol/l (41%) after 4 weeks on Atkins diet. With 80% power and a level of significance of 0.05 and a SD of 0.5 mmol/l for LDL-C the necessary sample size (for each sample separately) was 16.

3. Results

Forty-one subjects met to the screening visit and two did not fulfill the inclusion criteria. Thirty-nine participants, 32 women and seven men, were randomized, of whom 30 participants completed the study (Fig. 1). A total of 9 subjects withdrew from the study. Table 1 shows the baseline and end of study characteristics of the participants. The routine laboratory measurements for lipids, glucose and CRP were within the reference value in all participants at baseline (Table 1).

3.1. Body weight and body composition

No difference in weight, body mass index (BMI), waist circumference, total body fat mass or total body water was observed between the LCHF and control group after three weeks (Table 1). During the study period, weight and BMI were significantly reduced in both the LCHF and control group (1.2 kg and 2.0 kg and 0.4 kg/m^2 and 0.7 kg/m^2, respectively). Weight circumference was significant reduced in the LCHF group ($p = 0.045$) and fat mass was significant reduced in the control group (1 kg; $p = 0.007$) and non-significantly reduced in LCHF (0.4 kg, $p = 0.23$ (Table 1).

3.2. Changes in serum lipids and other biochemical markers

As seen in Table 1, plasma levels of TC, LDL-C, HDL-C, ApoB and ApoA1 and free fatty acids (FFA) increased significantly in the LCHF group compared to the control group after three weeks (Table 1), whereas fasting plasma levels of TG, glucose, glycated hemoglobin A1c (HbA1c), C-peptide, alanin aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatinine, uric acid and CRP remained unchanged between the groups after intervention.

Large variations in the biochemically responses from baseline after a LCHF diet were observed, particularly regarding the increase in LDL-C, ranging from 5 to 107% (Fig. 2).

3.3. Dietary intake and compliance

A total of 18 participants performed a weighed dietary record of their habitual diet and 10 participants performed a weighed dietary record of their LCHF diet (Table 2). The E% from total fat, saturated fat, monounsaturated fat, polyunsaturated fat, protein, carbohydrate and dietary cholesterol differed significantly between the habitual diet and the LCHF diet (all $p < 0.001$). The mean ± SD (min-max) intake of reported carbohydrates was 16 ± 3 (9–20) g per day in the LCHF group showing good compliance with the dietary restrictions during the LCHF diet period.

3.4. Expression of LDL receptor and other lipid-related genes and circulating PCSK9 level

One of the suggested mechanisms by which saturated fat increase LDL-C levels is through modulation of the LDL receptor. Thus, the PBMC gene expression of LDL receptor and other relevant genes involved in lipid metabolism was measured (Table 3). The gene expression of the transcription factor SREBP-1 was significantly different between the LCHF and control group after three weeks ($p = 0.01$) (Table 4), whereas there were no significant differences between groups in the change of expression of the other lipid-related genes. There were several within-group changes in gene expression in both the control and LCHF group (Table 4). We found no difference in circulating levels of

Table 1
Anthromometry and biochemical measurements.

	n	LCHF	n	LCHF	p^1	n	Control	n	Control	p^2	p^3
		Baseline		End-of-study			Baseline		End-of-study		
Descriptives											
Age, years	15	24.1 ± 3.8				15	26.7 ± 5.7				0.14
Female sex, n (%)	15	14 (93.3)				15	11 (73.3)				0.33[b]
Weight, kg	15	61.0 ± 8.6	15	59.8 ± 8.4	**0.014**	15	63.6 ± 7.8	15	61.6 ± 8.0	< **0.001**	0.20
BMI, kg/m^2	15	21.7 ± 1.3	15	21.3 ± 1.4	**0.02**	15	21.9 ± 1.6	15	21.3. ± 1.6	< **0.001**	0.15
Waist circumference, cm	8	72.2 ± 4.6	13	71.7 ± 4.9	**0.045**	9	73.8 ± 6.6	10	75.8 ± 7.1	0.43	0.18
Fat mass, kg (SD)	15	14.2 ± 3.8	15	13.8 ± 3.4	**0.23**	15	13.8 ± 5.1	15	12.8 ± 4.2	**0.007**	0.21
Total body water, kg (SD)	15	34.2 ± 5.9	15	33.7 ± 5.2	0.07	15	36.4 ± 6.5	15	35.8 ± 6.6	**0.003**	0.69
Systolic blood pressure, mmHg	11	114 ± 9.3	14	116 ± 10.1	0.93	12	112 ± 9.3	10	120 ± 11.8	0.38	0.55
Diastolic blood pressure, mmHg	11	70 ± 8.9	14	72 ± 8.7	0.86	12	72 ± 7.7	10	66 ± 10	0.45	0.42
Blood biochemistry											
Total cholesterol, mmol/l	15	4.1 ± 0.4	15	5.3 ± 1.0	< **0.001**	15	4.6 ± 0.7	15	4.6 ± 0.7	0.78	< **0.001**
HDL cholesterol, mmol/l	15	1.6 ± 0.3	15	1.9 ± 0.4	< **0.001**	15	1.8 ± 0.3	15	1.8 ± 0.4	0.79	< **0.001**
LDL cholesterol, mmol/l	15	2.2 ± 0.4	15	3.1 ± 0.8	< **0.001**	15	2.5 ± 0.8	15	2.5 ± 0.8	0.87	< **0.001**
Triglycerider, mmol/l	15	0.7 (0.5–1.5)	15	0.7 (0.4–1.5)	0.94[a]	14	0.8 (0.4–1.4)	15	0.8 (0.4–2.1)	0.84[a]	0.85[c]
Non-HDL-C, mmol/l	15	2.5 ± 0.5	15	3.4 ± 0.9	< **0.001**	15	2.8 ± 0.9	15	2.9 ± 0.8	0.66	< **0.001**
TRL cholesterol, mmol/l	15	0.34 ± 0.12	15	0.29 ± 0.15	0.29	14	0.33 ± 0.15	15	0.36 ± 0.20	0.65	0.31
ApoA1, g/L	15	1.6 ± 0.2	15	1.9 ± 0.3	< **0.001**	14	1.6 ± 0.2	15	1.7 ± 0.2	0.51	**0.001**
ApoB, g/L	15	0.7 ± 0.1	15	0.9 ± 0.2	< **0.001**	14	0.8 ± 0.3	15	0.8 ± 0.3	0.26	< **0.001**
Lipoprotein(a), pmol/l	15	84 (60–794)	14	135 (60–804)	0.52[a]	14	126 (60–777)	15	151 (60–724)	0.53[a]	0.38[c]
Glucose, mmol/l	15	4.8 ± 0.3	15	4.7 ± 0.4	0.33	14	4.9 ± 0.4	15	4.9 ± 0.3	1.00	0.43
C-peptide, nmol/l	14	0.5 ± 0.2	14	0.4 ± 0.1	**0.01**	14	0.5 ± 0.2	15	0.5 ± 0.2	0.46	0.06
HbA1c, %	11	5.3 ± 0.4	15	5.2 ± 0.2	0.62	11	5.4 ± 0.3	13	5.2 ± 0.2	**0.002**	**0.03**
CRP, mg/l	11	1.6 (0.6–7.8)	13	0.9 (0.6–3.5)	0.40[a]	15	0.6 (0-0.6-3.0)	15	0.7 (0.6–3.5)	0.40[a]	0.24[c]
FFA, mmol/l	15	0.4 (0.1–0.6)	14	0.8 (0.4–1.3)	**0.001**[a]	15	0.3 (0.2–0.9)	15	0.4 (0.2–0.9)	0.07[a]	**0.002**[c]
Urat, umol/l	11	234 ± 59	14	249 ± 58	**0.004**	12	302 ± 69	15	283 ± 78	0.44	**0.05**
Urea, mmol/l	11	4.5 ± 1.1	15	6.5 ± 1.6	0.01	12	5.2 ± 1.3	15	5.4 ± 1.1	0.51	0.04
Creatinine, µmol/L	15	72 ± 12	14	74 ± 13	0.12	15	75 ± 15	15	73 ± 14	0.23	0.06
ALAT, U/L	15	22 ± 16	15	23 ± 10	0.82	14	21 ± 9.4	15	20 ± 5.6	0.78	0.75
ASAT, U/L	15	25 ± 4.6	15	27 ± 13	0.57	15	25 ± 5.2	15	26 ± 5.6	0.46	0.80
PCSK9, µg/l	15	220 ± 55	15	164 ± 61	**0.001**	15	231 ± 74	13	184 ± 70	**0.02**	0.18

Apo, apolipoprotein; g/L, grams per litre; HDL, high density lipoprotein; kg, kilograms; LDL, low density lipoprotein; Lp(a), lipoprotein (a); m, meters; mg, milligram; mmol/l, millimoles per litre; µmol/l, micromole per litre; nmol/l, nanomoles per litre; NS, non significant; PCSK9, proprotein convertase subtilisin/kexin type 9; HbA1c, glycated hemoglobin A1c; ALAT, alanin aminotransferase; ASAT, aspartate aminotransferase; ApoB; apolipoprotein B, Apo A1; apolipoprotein A1, FFA, serum free fatty acid; CRP, serum C-reactive protein; non-HDL-C (TC minus HDL-C); TRL cholesterol (triglyceride-rich lipoprotein cholesterol; TC minus LDL-C minus HDL-C).[a]Wilcoxon paired t-test; [b]Fischers exact test.
p^1: within change LCHF group. Wilcoxon or paired *t*-test, p^2: within change control group; Wilcoxon or paired *t*-test, p^{3c}: Between group difference; independent *t*-test or Mann-Whitney *U* test. Significance is defined as $p < 0.05$, marked in bold.

Table 2
Dietary intake on habitual diet and on LCHF diet.

	Habitual diet (n = 18)	LCHF diet (n = 10)	p[a]
Energy, kcal	2007 ± 468[b]	2062 ± 361	0.751
Protein, E%	17.2 ± 3.0	25.6 ± 4.5	< **0.001**
Fat, E%	30.6 ± 5.1	70.8 ± 4.8	< **0.001**
Saturated fat[c], E%	11.0 ± 1.9	29.0 ± 5.6	< **0.001**
Monounsaturated fat, E%	10.7 ± 2.7	24.8 ± 2.9	< **0.001**
Polyunsaturated fat, E%	5.8 ± 1.3	11.3 ± 2.3	< **0.001**
Carbohydrate, E%	45.8 ± 4.6	3.0 ± 0.4	< **0.001**
Fiber, E%	2.7 ± 0.7	2.5 ± 0.7	0.376
Cholesterol[d], mg	261 ± 70	1072 ± 267	< **0.001**

Statististical significance is defined as $p < 0.05$ and marked with bold text.
[a] Independent sample *t*-test.
[b] Mean ± SD.
[c] Missing value for 1 participant on habitual diet.
[d] Missing value for 1 participant on habitual diet and 1 participant on LCHF diet LCHF, low carbohydrate high fat; E%, energy percent.

PCSK9 between groups, however, PCSK9 decreased in both groups ($p < 0.05$ for both) compared to baseline (Table 1).

3.5. Adverse events

There were two serious adverse events during the study, both occurred in the LCHF group. One healthy participant experienced chest pain three days after starting on the LCHF diet. ST-elevation was observed at the electro cardiogram at admission to hospital and severely elevated serum levels of troponins and creatine kinase-MB were observed.

Coronary angiography was, however, normal. Despite extensive investigation including magnetic resonance and computer tomography scans, heart biopsy and metabolic investigations of acylcarnitines and organic acids in serum plus whole genome exome sequencing, including mitochondrial DNA sequencing, of the patient and both parents, the cause of possible myocardiopathy remained undetermined. The patient was excluded from the study and partly recovered after standard medical treatment and restarting eating carbohydrate. Another subject experienced autoimmune thyroiditis during LCHF. Seven participants did not complete the study due to the non-serious adverse events headache and/or fatigue or withdrawn consent.

4. Discussion

An important finding of this study is that after three weeks on an LCHF diet, mean plasma LDL-C increased by 44% compared to the control group. Clinical relevant and statistically significant increases were also observed for plasma levels of TC, apoB, apoA1, HDL-C, FFA, urea and uric acid. The reported intake of saturated fatty acids in the subject on LCHF diet was two to three fold higher than in the controls. Interestingly, a large variation in the individual response on LDL-C was observed ranging from 5% increase to 107% increase after three weeks

Table 3
List of lipid-related genes analyzed in peripheral blood mononuclear cells before and after LCHF intervention.

Gene symbol	Full name	ThermoFisher Scientific's product number
ABCA1	ATP-binding cassette, sub family A, member 1	Hs01059118_m1
ABCG1	ATP-binding cassette, sub family G, member 1	Hs00245154_m1
ACACA	acetyl-CoA carboxylase alpha	Hs01046047_m1
ACAT1	acetyl-CoA acetyltransferase 1	Hs00608002_m1
ACOX1	acyl-Coenzyme A oxidase 1	Hs00244515_m1
ACSS2	acyl-CoA synthetase short chain family member 2	Hs00218766_m1
CD36	CD 36 molecule	Hs01567185_m1
CETP	cholesteryl ester transfer protein	Hs00163942_m1
CPT1A	carnitine palmitoyltransferase 1A	Hs00912671_m1
CRAT	carnitine O-acetyltransferase	Hs00912963_m1
ELOVL4	elongation of very long chain fatty acids-like 4	Hs00224122_m1
FASN	fatty acid synthase	Hs01005622_m1
HMGCR	3-hydroxy-3methylglutaryl-CoA reductase	Hs00168352_m1
LDLR	low density lipoprotein receptor	Hs00181192_m1
LRP1	low density lipoprotein receptor related protein 1	Hs00233856_m1
NPC1	Niemann-Pick C1 intracellular cholesterol transporter 1	Hs00975249_m1
NPC1L1	Niemann-Pick C1 like intracellular cholesterol transporter 1	Hs00203602_m1
NR1H3	nuclear receptor subfamily 1 group H member 3	Hs00172885_m1
PLIN2	perilipin 2	Hs00605340_m1
PLTP	phospholipid transfer protein	Hs01067287_m1
PPARA	peroxisome proliferator activated receptor alpha	Hs00947536_m1
PPARD	peroxisome proliferator activated receptor delta	Hs04187066_g1
PPARG	peroxisome proliferator activated receptor gamma	Hs01115512_m1
SCARB1	scavenger receptor class B member 1	Hs00969821_m1
SCD	stearoyl-CoA desaturase	Hs01682761_m1
SREBF1	sterol regulatory element binding transcription factor 1	Hs01088691_m1
UCP2	uncoupling protein 2	Hs01075227_m1

The ThermoFisher's product number (TaqMan Microfluidic Cards) refers to information concerning the probe used to detect the target gene, e.g. the specific sequence of the probe. http://www.thermofisher.com/no/en/home/brands/applied-biosystems.html.

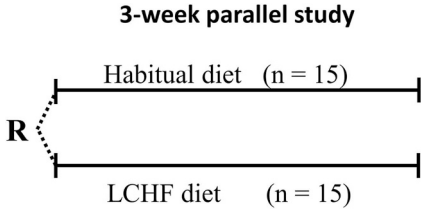

Fig. 1. Flow chart of the study design.
R denotes randomization; habitual diet denotes the control arm; LCHF diet denotes low carbohydrate high fat diet.

on an LCHF diet. The gene expression of the transcription factor SREBP-1 was significantly different between the groups and the LDL receptor gene was non-significantly reduced in the LCHF group.

An interesting finding in the present study was the striking differences between individuals in the response to an LCHF diet, in particular for plasma levels of LDL-C. A variation in intake of saturated fat among individuals might possibly explain the different response in LDL-C, however, all subjects in the LCHF group reported a very high intake of saturated fat with a minimum intake of saturated fat of 20 E%. It is therefore difficult to explain the variation in LDL-C by the variation in saturated fat intake alone. Further, it is unlikely that changes in body weight can explain the large individual response, since BMI did not change much during the intervention for any of the subjects. Taken together, this suggests that large inter-individual differences in sensitivity to LCHF exist. It is known that some genotypes increase the sensitivity to the dietary composition, such as different isoforms of apoA I, apoA IV, apoB and apoE reported to be important for the variation in the response to fatty acid composition and cholesterol in the diet [34,35]. LDL receptor activity is important for plasma LDL-C concentration. In the present study, we observed a 30%, but not statistically significant decrease, in LDL receptor gene expression in the LCHF group.

However, we observed a reduction in circulating PCSK9 within both groups. Since PCSK9 and LDL receptor are coordinately regulated at a transcriptional level [36] the decrease in circulating PCSK9 may be caused by a reduced synthesis of the LDL receptor protein. It is not known how diet may influence on the new treatment with PCSK9 inhibitors. However, some inter-individual variation in effect on LDL-C reduction has been demonstrated and our results may suggest a role of diet [37]. The clearance of both LDL-C and HDL-C from the circulation seems to be delayed with high dietary intake of saturated fatty acids [38] possibly due to reduced hepatic LDL receptor mRNA as shown in animal studies [39]. However, in contrast, a reduced intake of saturated fat was associated with increased levels of LDL receptors in PBMCs in healthy men and women [28].

The results in the present study support the finding in animal studies that saturated fat reduce LDL receptor mRNA in humans after a LCHF diet for three weeks. However, our gene expression studies are hypothesis-driven and not corrected for multiple testing and should thus be interpreted with care.

Previous studies with LCHF diets have, to our knowledge, not shown a strong effect on the serum lipid profile as reported here. Our study population consisted of healthy normal-weight subjects, different from the study populations used in most previous studies with obese subjects aiming to reduce weight during the dietary intervention period. It is important to gain knowledge of how LCHF diet influence cardiovascular risk factors in normal-weight subjects since the use of LCHF diets is popular and not restricted to weight reduction in obesity only. Population surveys in Norway have reported that 29% of adult women and 21% of men had been on a “low carbohydrate diet” the last 12 months [27]. Therefore, the use of a LCHF diet with modest or no weight reduction as studied in the present study, provide important data.

LCHF diets may be used to reduce glycemia. Thus, the combined use of LCHF diet and anti-diabetic drugs, such as sodium-glucose transport protein 2 (SGLT2) may not be uncommon. However, life-threatening atypical normo-glycemic diabetic ketoacidosis was recently reported with the use of SGLT2 inhibition in combination with LCHF diet [40]. The authors discuss that the loss of glucose in urine may increase the secretion of glucagon, which subsequently induce the ketosis. Further,

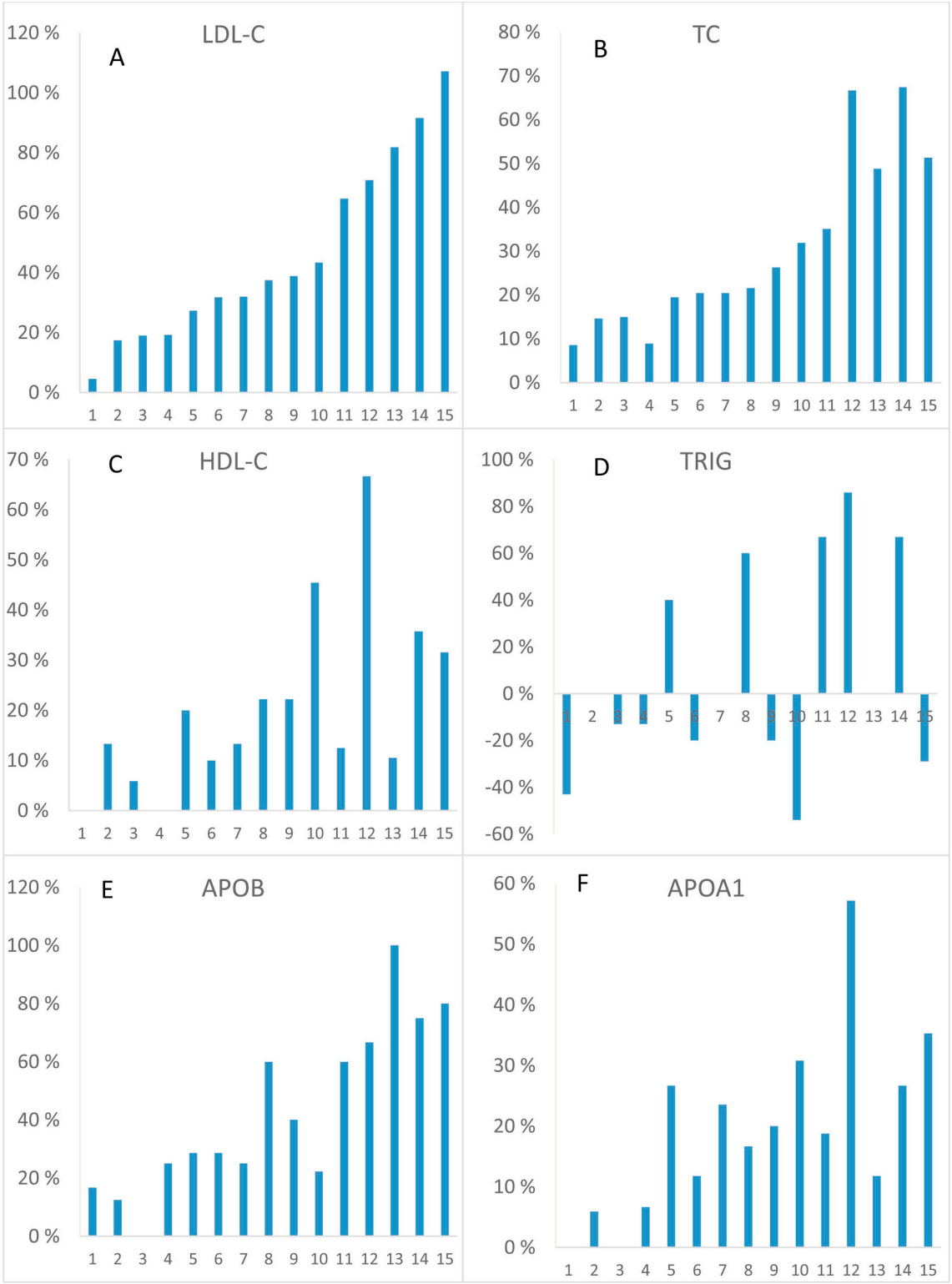

Fig. 2. Each bar represents one subject's percent change in plasma lipids, as indicated by the text in each panel.
The fasting plasma levels at baseline were compared to fasting plasma levels after using LCHF for 3 weeks expressed as percent change. Subjects were ranked in order after the percent increase in LDL-C, from the least change to the left side in the panel and the largest change to the right side of the panel. All subjects were ranked in the same order according to the percent increase of LDL-C to visualize the association of individual change in LDL-C and changes in other lipid parameters. LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TRIG, triglycerides; APOB, apolipoprotein B; APOA1, apolipoprotein A1.

SGLT2 inhibitors may slightly increase LDL-C (0.1 mmol/l or less) [41]. Taken together, further research is needed on how this class of drugs affects lipid metabolism.

This study has both strengths and limitations. The strength is the randomized controlled study design and the high compliance of the study participants. A weakness is the short duration of the study. However,

Table 4
Expression of lipid-related genes in peripheral blood mononuclear cells (PBMCs) in the low carbohydrate high fat (LCHF) arm and in the control arm of the study..

Target gene	n	Baseline	End of study	$_p$a	Fold change	$_p$c
		(2ˆ-dCt)	(2ˆ-dCt)		(from baseline)[b]	
ABCA1						
LCHF	11	0.30 (0.27–0.42)	0.40 (0.24–0.51)	0.42	0.91 (0.52–1.38)	0.86
Control	10	0.27 (0.25–0.31)	0.25 (0.20–0.32)	0.45	0.85 (0.73–1.16)	
ABCG1						
LCHF	10	0.42 (0.34–0.44)	0.35 (0.32–0.47)	0.17	0.88 (0.73–1.03)	0.22
Control	11	0.31 (0.28–0.40)	0.32 (0.26–0.41)	1.00	1.00 (0.92–1.14)	
ACACA						
LCHF	11	0.52 (0.44–0.54)	0.39 (0.30–0.44)	**0.02**	0.84 (0.59–0.89)	1.00
Control	10	0.43 (0.35–0.50)	0.35 (0.28–0.35)	**0.02**	0.74 (0.57–0.97)	
ACAT1						
LCHF	11	0.99 (0.85–1.33)	0.97 (0.92–1.02)	0.13	0.95 (0.75–1.03)	0.48
Control	11	0.89 (0.79–1.34)	0.94 (0.81–1.19)	0.29	0.90 (0.88–1.06)	
ACOX1						
LCHF	11	1.07 (0.72–1.14)	0.85 (0.79–1.03)	0.66	1.03 (0.75–1.16)	0.09
Control	11	1.16 (1.03–1.33)	0.86 (0.76–0.92)	< **0.01**	0.75 (0.66–0.89)	
ACSS2						
LCHF	11	0.62 (0.51–0.76)	0.65 (0.50–0.73)	0.93	1.01 (0.77–1.25)	0.56
Control	11	0.61 (0.52–0.69)	0.52 (0.48–0.62)	0.16	0.92 (0.79–0.97)	
CD36						
LCHF	11	5.36 (3.73–6.20)	4.62 (3.18–5.46)	0.66	0.99 (0.57–1.33)	0.65
Control	11	5.51 (4.03–6.35)	4.52 (4.04–5.20)	**0.03**	0.78 (0.71–1.00)	
CETP						
LCHF	11	0.04 (0.03–0.07)	0.03 (0.03–0.05)	0.72	0.95 (0.69–1.51)	0.65
Control	11	0.04 (0.02–0.05)	0.03 (0.03–0.05)	0.26	0.80 (0.73–1.11)	
CPT1A						
LCHF	11	2.19 (1.63–2.25)	2.19 (1.88–2.84)	0.29	1.09 (0.92–1.30)	0.27
Control	11	1.98 (1.70–2.22)	1.79 (1.66–2.07)	0.72	0.96 (0.83–1.14)	
CRAT						
LCHF	11	1.04 (0.90–1.20)	0.96 (0.79–1.09)	0.16	0.96 (0.78–1.04)	0.52
Control	11	1.16 (0.95–1.25)	0.93 (0.83–1.05)	**0.01**	0.86 (0.80–0.92)	
ELOVL4						
LCHF	11	0.05 (0.04–0.08)	0.06 (0.06–0.09)	0.14	1.29 (1.17–1.62)	0.56
Control	11	0.04 (0.03–0.06)	0.05 (0.04–0.07)	0.09	1.21 (1.01–1.53)	
FASN						
LCHF	11	1.27 (1.19–1.40)	1.14 (1.04–1.22)	**0.01**	0.91 (0.78–0.97)	0.81
Control	10	1.29 (1.26–1.46)	1.22 (1.15–1.32)	0.06	0.85 (0.80–0.99)	
HMGCR						
LCHF	11	1.34 (1.14–1.48)	1.20 (1.04–1.43)	0.33	0.90 (0.82–1.09)	0.52
Control	11	1.47 (1.39–1.78)	1.30 (1.17–1.46)	***0.03***	0.86 (0.81–0.95)	
LDLR						
LCHF	10	0.30 (0.22–0.48)	0.20 (0.16–0.26)	0.06	0.73 (0.43–1.00)	0.72
Control	9	0.36 (0.29–0.41)	0.28 (0.22–0.29)	0.11	0.77 (0.68–0.79)	
LRP1						
LCHF	11	6.24 (4.17–7.13)	4.77 (4.35–5.64)	0.37	0.85 (0.72–1.31)	0.44
Control	11	5.71 (5.41–6.52)	4.62 (3.90–5.90)	**0.02**	0.81 (0.66–0.94)	
NPC1						
LCHF	11	0.91 (0.84–1.00)	0.90 (0.67–0.96)	0.16	0.80 (0.74–1.17)	0.85
Control	11	1.06 (0.98–1.20)	0.91 (0.78–1.11)	**0.02**	0.82 (0.77–0.96)	
NPC1L1						
LCHF	6	0.01 (0.01–0.02)	0.01 (0.01–0.02)	0.75	0.97 (0.48–1.33)	0.10
Control	3	0.01 (0.01–0.01)	0.002 (0.002–0.002)	0.11	0.28 (0.27–0.49)	
NR1H3						
LCHF	10	0.14 (0.13–0.14)	0.14 (0.11–0.19)	0.80	1.01 (0.91–1.20)	0.17
Control	11	0.18 (0.15–0.20)	0.16 (0.13–0.17)	0.06	0.85 (0.79–0.91)	

(*continued on next page*)

Table 4 (*continued*)

Target gene	n	Baseline	End of study	$_p$a	Fold change	$_p$c
		(2^-dCt)	(2^-dCt)		(from baseline)[b]	
PLIN2						
LCHF	11	1.12 (1.05–1.44)	1.40 (1.23–1.55)	0.08	1.13 (1.02–1.38)	0.50
Control	9	0.94 (0.80–1.47)	1.17 (0.94–1.32)	0.68	1.05 (0.94–1.17)	
PLTP						
LCHF	11	0.14 (0.09–0.15)	0.08 (0.08–0.10)	0.05	0.77 (0.61–1.00)	1.00
Control	11	0.10 (0.07–0.13)	0.06 (0.06–0.12)	***0.02***	0.79 (0.67–0.89)	
PPARA						
LCHF	11	0.56 (0.52–0.68)	0.57 (0.50–0.63)	0.53	1.00 (0.78–1.08)	0.44
Control	11	0.62 (0.52–0.72)	0.62 (0.59–0.66)	0.93	1.11 (0.85–1.22)	
PPARD						
LCHF	11	1.97 (1.71–2.24)	1.66 (1.38–1.76)	**0.02**	0.76 (0.69–0.97)	0.61
Control	10	2.09 (1.76–2.28)	1.54 (1.34–2.05)	0.05	0.72 (0.67–0.82)	
PPARG						
LCHF	10	0.02 (0.01–0.03)	0.01 (0.01–0.02)	0.17	0.71 (0.41–1.22)	0.92
Control	11	0.02 (0.01–0.02)	0.01 (0.01–0.02)	**0.03**	0.70 (0.53–0.91)	
SCARB1						
LCHF	10	0.26 (0.17–0.32)	0.18 (0.17–0.21)	0.05	0.82 (0.60–1.03)	1.00
Control	11	0.22 (0.19–0.26)	0.17 (0.17–0.19)	**0.03**	0.82 (0.66–0.95)	
SCD						
LCHF	11	0.22 (0.21–0.37)	0.18 (0.15–0.25)	**0.02**	0.73 (0.60–0.84)	0.48
Control	11	0.26 (0.17–0.34)	0.20 (0.17–0.24)	**0.01**	0.76 (0.68–0.83)	
SREBF1						
LCHF	10	1.37 (1.03–1.71)	1.60 (1.28–2.23)	0.19	1.37 (0.93–1.60)	**0.01**
Control	11	1.74 (1.47–2.22)	1.43 (1.31–1.59)	0.05	0.83 (0.67–1.01)	
UCP2						
LCHF	11	39.13 (34.64–50.30)	34.14 (30.43–40.75)	0.53	0.95 (0.73–1.11)	0.80
Control	11	44.10 (31.92–49.25)	38.48 (30.29–49.06)	1.00	1.00 (0.89–1.15)	

Data are presented as median (25th −75th percentile). Significance is defined as $p < 0.05$, marked in bold text.
[a]*p*-value: within group difference using Wilcoxon Signed Rank Test. [b]Data are given as $2^{-\Delta\Delta Ct}$ (normalized for reference genes and baseline). [c]*p*-value: between group difference using Mann Whitney *U* Test.
ABCA1, ATP-binding cassette, sub family A, member 1; ABCG1, ATP-binding cassette, sub family G, member 1; ACACA, acetyl-CoA carboxylase alpha; ACAT1, acetyl-CoA acetyltransferase 1; ACOX1, acyl-Coenzyme A oxidase 1; ACSS2, acyl-CoA synthetase short chain family member 2; CD36, Cluster of Differentiation 36 molecule; CETP, cholesteryl ester transfer protein; Ctr, control; CPT1A, carnitine palmitoyltransferase 1A; CRAT, carnitine O-acetyltransferase; ELOVL4, elongation of very long chain fatty acids-like 4; FASN, fatty acid synthase; HMGCR, 3-hydroxy-3methylglutaryl-CoA reductase, LCHF, low-carbohydrate high-fat diet; LDLR; low density lipoprotein receptor; LRP1, low density lipoprotein receptor related protein 1; NPC1, Niemann-Pick C1 intracellular cholesterol transporter 1; NPC1L1, Niemann-Pick C1 like intracellular cholesterol transporter 1; NR1H3, nuclear receptor subfamily 1 group H member 3; PBMC, peripheral mononuclear cells; PLIN2, perilipin 2; PLTP, phospholipid transfer protein; PPARA, peroxisome proliferator activated receptor alpha; PPARD, peroxisome proliferator activated receptor delta; PPARG, peroxisome proliferator activated receptor gamma; SCARB1, scavenger receptor class B member 1; SCD, stearoyl-CoA desaturase; SREBF1, sterol regulatory element binding transcription factor 1; UCP2, uncoupling protein 2.

consuming a diet with less than 20 g per day of carbohydrates requires a tremendous change of most people's diet, challenges not easy to overcome in studies of longer durations. Seven out of 39 participants discontinued the study due to the non-serious adverse events headache and/or fatigue but there were also two serious adverse events. The cause of the two serious adverse events, myocardiopathy and autoimmune thyroiditis after few days on LCHF diet, remains undetermined despite extensive investigation. Other study limitations are the small study population albeit the *p*-values on primary end points were strong. The use of PBMC as model for gene expression analysis has previously been found to correlate with gene expression in liver [42], however such data must be interpreted with caution. Another limitation was the lack of reliable biomarkers for the dietary intake data and the dietary assessments data was not collected for the whole population. Further, the effect was observed in young, healthy, normal weight subjects and cannot be generalized to the population as whole. We measured HDL-C levels but it would have been useful also to measure HDL function [43,44]. A strength was that all samples in the present study were obtained from fasting individuals.

In conclusion, mean plasma LDL-C increased markedly after three weeks on a classical LCHF diet. The metabolic stress caused by this diet may be used as a model for research on the regulation of LDL-C levels in human since little is known about the molecular mechanisms behind the large individual differences. In clinical practice, the unpredictable response to the LCHF diet suggests that LDL-C should be measured in people using a LCHF diet.

Conflicts of interest

There are no conflicts of interest related to the content of this manuscript. K.R. has received grants or honoraria for meeting and lectures the last three years from Amgen, Chiesi, Sanofi, Mills DA, MSD (Norway), Oslo Economics, Takeda outside the submitted work and has received honoraria for participation in meetings for Norwegian Directorate of Health and the Norwegian Medical Association outside the submitted work. K.B.H has received grants from TINE SA, Mills DA, Olympic Seafood, Amgen, Sanofi, Kaneka, and Pronova outside the submitted work. M.S. has received honoraria for meeting and lectures from Pronova and Navamedic. I.N. has nothing to report.

Financial support

We thank The Mills Vita Heart Foundation, The Throne Holst Foundation, Aktieselskabet Freia Chocolade Fabriks Medisinske Fond, Norsk Fond for Klinisk Kjemi, Dr. Fürst medisinske laboratoriums fond til klinisk kjemisk og klinisk fysiologisk forskning for financial support to this study. The funding bodies had no role in the conduct of the research and/or preparation of the article, study design, collection, analysis or interpretation of data, neither in the writing of the report; or in the decision to submit the article for publication.

Author contributions

K.B.H., M.S. and K.R conceived of the idea and planned the experiments. K.R. conducted the clinical part of the study, K.B.H. conducted the biochemical and genetic analysis and was responsible for the statistical work, I.N. conducted the genetic analysis and M.S. was responsible for the nutritional analyses and the dietary intervention. All authors did statistical work, discussed and interpreted the results and made substantial contributions to the final manuscript.

Acknowledgements

In relation to efforts to understand a serious adverse event mentioned in this manuscript we would like to thank Dr. Berit Woldseth for metabolic investigations, Dr. Einar Gude for clinical investigations and Dr. Kaja Kristine Selmer and Dr. Lars J. Retterstøl for genetic investigations. Further, we would like to thank professor Anette Hjartåker for providing data on weighed dietary record of the participants' habitual diet.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.10.013.

References

[1] J. Mann, R. McLean, M. Skeaff, L.T. Morenga, Low carbohydrate diets: going against the grain, Lancet 384 (2014) 1479–1480.
[2] D. Mozaffarian, D.S. Ludwig, The 2015 US dietary guidelines: lifting the ban on total dietary fat, Jama 313 (2015) 2421–2422.
[3] J.I. Pedersen, P.T. James, I.A. Brouwer, R. Clarke, I. Elmadfa, M.B. Katan, P.M. Kris-Etherton, D. Kromhout, B.M. Margetts, R.P. Mensink, K.R. Norum, M. Rayner, M. Uusitupa, The importance of reducing SFA to limit CHD, Br. J. Nutr. 106 (2011) 961–963.
[4] B.A. Ference, H.N. Ginsberg, I. Graham, K.K. Ray, C.J. Packard, E. Bruckert, R.A. Hegele, R.M. Krauss, F.J. Raal, H. Schunkert, G.F. Watts, J. Boren, S. Fazio, J.D. Horton, L. Masana, S.J. Nicholls, B.G. Nordestgaard, B. van de Sluis, M.R. Taskinen, L. Tokgozoglu, U. Landmesser, U. Laufs, O. Wiklund, J.K. Stock, M.J. Chapman, A.L. Catapano, Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (2017) 2459–2472.
[5] F.M. Sacks, A.H. Lichtenstein, J.H.Y. Wu, L.J. Appel, M.A. Creager, P.M. Kris-Etherton, M. Miller, E.B. Rimm, L.L. Rudel, J.G. Robinson, N.J. Stone, L.V. Van Horn, Dietary fats and cardiovascular disease: a presidential advisory from the american heart association, Circulation 136 (2017) e1–e23.
[6] R.A. Atkins, Dr. Atkins' Diet Revolution: the High Calorie Way to Stay Thin Forever, Bantam, 1972.
[7] B.E. Millen, S. Abrams, L. Adams-Campbell, C.A. Anderson, J.T. Brenna, W.W. Campbell, S. Clinton, F. Hu, M. Nelson, M.L. Neuhouser, R. Perez-Escamilla, A.M. Siega-Riz, M. Story, A.H. Lichtenstein, The 2015 dietary guidelines advisory committee scientific report: development and major conclusions, Adv. Nutr. (Bethesda, Md.) 7 (2016) 438–444.
[8] L. Van Horn, J.A. Carson, L.J. Appel, L.E. Burke, C. Economos, W. Karmally, K. Lancaster, A.H. Lichtenstein, R.K. Johnson, R.J. Thomas, M. Vos, J. Wylie-Rosett, P. Kris-Etherton, Recommended dietary pattern to achieve adherence to the American Heart Association/American College of Cardiology (AHA/ACC) guidelines: a scientific statement from the american heart association, Circulation 134 (2016) e505–e529.
[9] J. Aranceta Bartrina, V.V. Arija Val, E. Maiz Aldalur, E. Martinez de Victoria Munoz, R.M. Ortega Anta, C. Perez-Rodrigo, J. Quiles Izquierdo, A. Rodriguez Martin, B. Roman Vinas, G. Salvador Castell, J.A. Tur Mari, G. Varela Moreira, L. Serra Majem, Dietary Guidelines for the Spanish population (SENC, diciembre 2016); the new graphic icon of healthy food, Nutr. Hosp. 33 (2016) 1–48.
[10] The truth about dieting, Consum. Rep. 67 (2002) 26–31.
[11] B.K. Vartiainen E, J ym Sundvall, Väestön kolesterolitaso on vuosikymmenien laskun jälkeen kääntynyt nousuun, Suom. Laakaril. 67 (2012) 2364–2369.
[12] I. Johansson, L.M. Nilsson, B. Stegmayr, K. Boman, G. Hallmans, A. Winkvist, Associations among 25-year trends in diet, cholesterol and BMI from 140,000 observations in men and women in Northern Sweden, Nutr. J. 11 (2012) 40.
[13] Utviklingen i norsk kosthold, The Norwegian Directorate of Health. IS-2680, (2017), p. 49 Oslo, Norway.
[14] S. Natela, G. Keating, Fat: the New Health Paradigm, Credit Suisse Research Institute AG, Zurich, Switzerland, 2015 CH-8070 www.credit-suisse.com/researchinstitute , Accessed date: 9 March 2018.
[15] R. Chowdhury, S. Warnakula, S. Kunutsor, F. Crowe, H.A. Ward, L. Johnson, O.H. Franco, A.S. Butterworth, N.G. Forouhi, S.G. Thompson, K.T. Khaw, D. Mozaffarian, J. Danesh, E. Di Angelantonio, Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis, Ann. Intern. Med. 160 (2014) 398–406.
[16] R.J. de Souza, A. Mente, A. Maroleanu, A.I. Cozma, V. Ha, T. Kishibe, E. Uleryk, P. Budylowski, H. Schunemann, J. Beyene, S.S. Anand, Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies, BMJ (Clinical Res. ed.) 351 (2015) h3978.
[17] M. Dehghan, A. Mente, X. Zhang, S. Swaminathan, W. Li, V. Mohan, R. Iqbal, R. Kumar, E. Wentzel-Viljoen, A. Rosengren, L.I. Amma, A. Avezum, J. Chifamba, R. Diaz, R. Khatib, S. Lear, P. Lopez-Jaramillo, X. Liu, R. Gupta, N. Mohammadifard, N. Gao, A. Oguz, A.S. Ramli, P. Seron, Y. Sun, A. Szuba, L. Tsolekile, A. Wielgosz, R. Yusuf, A. Hussein Yusufali, K.K. Teo, S. Rangarajan, G. Dagenais, S.I. Bangdiwala, S. Islam, S.S. Anand, S. Yusuf, Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study, Lancet (London, England) 390 (2017) 2050–2062.
[18] D. Mozaffarian, R. Micha, S. Wallace, Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials, PLoS Med. 7 (2010) e1000252.
[19] P. Lagiou, S. Sandin, M. Lof, D. Trichopoulos, H.O. Adami, E. Weiderpass, Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study, BMJ (Clinical Res. ed.) 344 (2012) e4026.
[20] A. Paoli, A. Rubini, J.S. Volek, K.A. Grimaldi, Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets, Eur. J. Clin. Nutr. 67 (2013) 789–796.
[21] T. Hu, K.T. Mills, L. Yao, K. Demanelis, M. Eloustaz, W.S. Yancy Jr., T.N. Kelly, J. He, L.A. Bazzano, Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: a meta-analysis of randomized controlled clinical trials, Am. J. Epidemiol. 176 (Suppl 7) (2012) S44–S54.
[22] N. Mansoor, K.J. Vinknes, M.B. Veierod, K. Retterstol, Effects of low-carbohydrate diets v. low-fat diets on body weight and cardiovascular risk factors: a meta-analysis of randomised controlled trials, Br. J. Nutr. 115 (2016) 466–479.
[23] I. Shai, D. Schwarzfuchs, Y. Henkin, D.R. Shahar, S. Witkow, I. Greenberg, R. Golan, D. Fraser, A. Bolotin, H. Vardi, O. Tangi-Rozental, R. Zuk-Ramot, B. Sarusi, D. Brickner, Z. Schwartz, E. Sheiner, R. Marko, E. Katorza, J. Thiery, G.M. Fiedler, M. Bluher, M. Stumvoll, M.J. Stampfer, Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet, N. Engl. J. Med. 359 (2008) 229–241.
[24] G.D. Brinkworth, M. Noakes, J.D. Buckley, J.B. Keogh, P.M. Clifton, Long-term effects of a very- low-carbohydrate weight loss diet compared with an isocaloric low-fat diet after 12 mo, Am. J. Clin. Nutr. 90 (2009) 23–32.
[25] T. Tzotzas, P. Evangelou, D.N. Kiortsis, Obesity, weight loss and conditional cardiovascular risk factors, Obes. Rev. : Official J. Int. Assoc. Study Obesity 12 (2011) e282–289.
[26] V.L. Veum, J. Laupsa-Borge, O. Eng, E. Rostrup, T.H. Larsen, J.E. Nordrehaug, O.K. Nygard, J.V. Sagen, O.A. Gudbrandsen, S.N. Dankel, G. Mellgren, Visceral adiposity and metabolic syndrome after very high-fat and low-fat isocaloric diets: a randomized controlled trial, Am. J. Clin. Nutr. 105 (2017) 85–99.
[27] A.B. Bugge, Eat Yourself Sound, Slim, Strong, Smart, Sexy…- Is There a Diet for Everything? National Institute for Consumer Research, Oslo, Norway, 2012, p. 371.
[28] V.A. Mustad, T.D. Etherton, A.D. Cooper, A.M. Mastro, T.A. Pearson, S.S. Jonnalagadda, P.M. Kris-Etherton, Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women, J. Lipid Res. 38 (1997) 459–468.
[29] J. Lin, R. Yang, P.T. Tarr, P.H. Wu, C. Handschin, S. Li, W. Yang, L. Pei, M. Uldry, P. Tontonoz, C.B. Newgard, B.M. Spiegelman, Hyperlipidemic effects of dietary saturated fats mediated through PGC- 1beta coactivation of SREBP, Cell 120 (2005) 261–273.
[30] M. Abifadel, M. Varret, J.P. Rabes, D. Allard, K. Ouguerram, M. Devillers, C. Cruaud, S. Benjannet, L. Wickham, D. Erlich, A. Derre, L. Villeger, M. Farnier, I. Beucler, E. Bruckert, J. Chambaz, B. Chanu, J.M. Lecerf, G. Luc, P. Moulin, J. Weissenbach, A. Prat, M. Krempf, C. Junien, N.G. Seidah, C. Boileau, Mutations in PCSK9 cause autosomal dominant hypercholesterolemia, Nat. Genet. 34 (2003) 154–156.
[31] Matportalen, The Norwegian Food Composition Table vol. 2018, The Norwegian Food Safety Authority, Oslo, 2017.
[32] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[33] M. Baumann, M.Z. Espeland, A.S. Kvaerner, M.P. Bogsrud, K. Retterstol, Lipid profile of healthy persons with low-carbohydrate diet, Tidsskrift for den Norske laegeforening : tidsskrift for praktisk medicin, ny raekke 133 (2013) 1193–1196.

[34] R.E. Peacock, A. Hamsten, J. Johansson, P. Nilsson-Ehle, S.E. Humphries, Associations of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis and high density lipoprotein subclasses, Clin. Genet. 46 (1994) 273–282.
[35] E.M. Ooi, T.W. Ng, G.F. Watts, P.H. Barrett, Dietary fatty acids and lipoprotein metabolism: new insights and updates, Curr. Opin. Lipidol. 24 (2013) 192–197.
[36] P. Costet, B. Cariou, G. Lambert, F. Lalanne, B. Lardeux, A.L. Jarnoux, A. Grefhorst, B. Staels, M. Krempf, Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c, J. Biol. Chem. 281 (2006) 6211–6218.
[37] F. Raal, R. Scott, R. Somaratne, I. Bridges, G. Li, S.M. Wasserman, E.A. Stein, Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial, Circulation 126 (2012) 2408–2417.
[38] B. Lamarche, P. Couture, Dietary fatty acids, dietary patterns, and lipoprotein metabolism, Curr. Opin. Lipidol. 26 (2015) 42–47.
[39] J.C. Fox, H.C. McGill Jr., K.D. Carey, G.S. Getz, In vivo regulation of hepatic LDL receptor mRNA in the baboon. Differential effects of saturated and unsaturated fat, J. Biol. Chem. 262 (1987) 7014–7020.
[40] K. Bonikowska, P. Magnusson, A. Sjoholm, [Life-threatening ketoacidosis in patients with type 2 diabetes on LCHF diet], Lakartidningen (2018) 115.
[41] E. Flores, C.G. Santos-Gallego, N. Diaz-Mejia, J.J. Badimon, Do the SGLT-2 inhibitors offer more than hypoglycemic activity? Cardiovasc. Drugs Ther. 32 (2018) 213–222.
[42] M. Bouwens, L.A. Afman, M. Muller, Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid beta-oxidation: functional role of peroxisome proliferator activated receptor alpha in human peripheral blood mononuclear cells, Am. J. Clin. Nutr. 86 (2007) 1515–1523.
[43] C.G. Santos-Gallego, J.J. Badimon, R.S. Rosenson, Beginning to understand high-density lipoproteins, Endocrinol Metab. Clin. N. Am. 43 (2014) 913–947.
[44] C.G. Santos-Gallego, C. Giannarelli, J.J. Badimon, Experimental models for the investigation of high-density lipoprotein-mediated cholesterol efflux, Curr. Atherosclerosis Rep. 13 (2011) 266–276.