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Original Research Article

The impact of short-term eucaloric low- and high-carbohydrate diets on liver triacylglycerol content in males with overweight and obesity: a randomized crossover study

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ABSTRACT

Background: Intrahepatic triacylglycerol (liver TG) content is associated with hepatic insulin resistance and dyslipidemia. Liver TG content can be modulated within days under hypocaloric conditions.

Objectives: We hypothesized that 4 d of eucaloric low-carbohydrate/high-fat (LC) intake would decrease liver TG content, whereas a high-carbohydrate/low-fat (HC) intake would increase liver TG content, and further that alterations in liver TG would be linked to dynamic changes in hepatic glucose and lipid metabolism.

Methods: A randomized crossover trial in males with 4 d + 4 d of LC and HC, respectively, with ≥2 wk of washout. ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) was used to measure liver TG content, with metabolic testing before and after intake of an LC diet (11E% carbohydrate corresponding to 102 ± 12 {mean ± standard deviation [SD]) g/d, 70E% fat} and an HC diet (65E% carbohydrate corresponding to 537 ± 56 g/d, 16E% fat). Stable [6,6-$^{2}H_2$]-glucose and [1,1,2,3,3-D5]-glycerol tracer infusions combined with hyperinsulinemic-euglycemic clamps and indirect calorimetry were used to measure rates of hepatic glucose production and lipolysis, whole-body insulin sensitivity and substrate oxidation.

Results: Eleven normoglycemic males with overweight or obesity (BMI 31.6 ± 3.7 kg/m^2) completed both diets. The LC diet reduced liver TG content by 35.3% (95% confidence interval: −46.6, −24.1) from 4.9% [2.4–11.0] (median interquartile range) to 2.9% [1.4–6.9], whereas there was no change after the HC diet. After the LC diet, fasting whole-body fat oxidation and plasma beta-hydroxybutyrate concentration increased, whereas markers of de novo lipogenesis (DNL) diminished. Fasting plasma TG and insulin concentrations were lowered and the hepatic insulin sensitivity index increased after LC. Peripheral glucose disposal was unchanged.

Abbreviations: ^{1}H-MRS, ^{1}H-magnetic resonance spectroscopy; CI, confidence interval; DNL, de novo lipogenesis; E%, energy percent; FA, fatty acid; FM, fat mass; FFM, fat-free mass; FGF 21, fibroblast growth factor 21; Fib-4, fibrosis-4; GIR, glucose infusion rate; GDF 15, growth differentiation factor 15; HC, high-carbohydrate/low-fat; HISI, hepatic insulin sensitivity index; HGP, hepatic glucose production; LC, low-carbohydrate/high-fat; MASLD, metabolic dysfunction-associated steatotic liver disease; Ra, rate of appearance; Rd, rate of disappearance; RER, respiratory exchange ratio; SCD, stearoyl-CoA desaturase; TG, triacylglycerol.

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Conclusions: Reduced carbohydrate and increased fat intake for 4 d induced a marked reduction in liver TG content and increased hepatic insulin sensitivity. Increased rates of fat oxidation and ketogenesis combined with lower rates of DNL are suggested to be responsible for lowering liver TG. This trial was registered at clinicaltrials.gov as NCT04581421.

Keywords: MASLD, fatty liver, high-fat diet, hepatic insulin sensitivity, de novo lipogenesis, ketogenesis

Introduction

Accumulation of intrahepatic triacylglycerol (liver TG) is recognized as a key contributor to the pathophysiology of the metabolic syndrome as it is closely associated with hepatic insulin resistance, impaired glucose metabolism, dyslipidemia [1–3], and systemic hyperinsulinemia [4,5]. It has therefore been suggested that nonalcoholic fatty liver disease should be termed metabolic-associated fatty liver disease [6,7] and, most recently, metabolic dysfunction-associated steatotic liver disease (MASLD) [8].

Reducing liver TG content is an appealing therapeutic goal because it targets both metabolic disturbances and may reduce the risk of progressive liver disease. Specific pharmacological treatments for MASLD are currently scarce, with weight reduction being the primary therapeutic approach [9,10]. Nevertheless, obtaining and particularly maintaining significant weight loss is challenging [11], although newer gut hormone-based pharmacological possibilities may hold promise in aiding weight loss maintenance [12]. Dietary strategies focusing on reducing liver TG are far from consensual [13]. Under hypocaloric conditions, dietary carbohydrate restriction is superior to fat restriction in reducing liver TG content (–30% compared with –9%) within 48 h [14]. Moreover, we have recently demonstrated that basal hepatic glucose production (HGP), systemic insulin, and TG concentrations can be reduced within days when carbohydrate intake is reduced (to 10E%) and compensated by increased fat intake (72E%), even under overfeeding conditions [15]. Thus, carbohydrate restriction may reduce liver TG content in a matter of days, independently of calorie restriction. In line with this, an extremely low-carbohydrate (4E%), high-protein (24E%) diet with 72E% fat reduced liver TG by 44% in participants with liver steatosis within days [16].

In our study, we investigated short-term changes in liver TG concentration with proton magnetic resonance spectroscopy (^{1}H-MRS) (primary outcome) after a diet with moderate carbohydrate restriction (11E% ~100 g/d) and compared it with changes after a high-carbohydrate diet (65E% ~500 g/d), while keeping protein content fixed at 16E% and energy requirements eucaloric. The study was a randomized crossover study in males with overweight or obesity but without type 2 diabetes. In addition, we investigated the metabolic effect of the diets on hepatic and whole-body insulin action and on parameters related to the regulation of hepatic glucose and lipid metabolism, including markers of ketogenesis, de novo lipogenesis (DNL) as well as several hepatokines and proteomic analyses of plasma for explorative analysis of the underlying metabolic processes. We hypothesized that 4 d of eucaloric low-carbohydrate/high-fat (LC) intake would decrease liver TG content while a high-carbohydrate/low-fat (HC) intake would increase liver TG content, and further that alterations in liver TG would be linked to changes in hepatic glucose and lipid metabolism.

Methods

We included males, aged 18–65 y, with a BMI of 25–40 kg/m^2. Exclusion criteria were contraindications to ^{1}H-MRS, diabetes (HbA1c $\geq$ 48 mmol/mol) or medication affecting glucose metabolism, fibrosis-4 (FIB-4) score >1.45, fasting plasma TG concentration $\geq$4.0 mmol·/L, high physical activity level (defined as extensive aerobic exercise or heavy weightlifting), excessive alcohol consumption (>168 g/wk), or daily use of nicotine products. Written informed consent was obtained from all participants before inclusion. Twelve participants were included, but 1 participant was excluded because of high blood hemoglobin and ferritin concentrations. The study received approval from the Ethical Committee for the Capital Region of Denmark (H-20010659) and carried out in accordance with the Declaration of Helsinki and registered at clinicaltrials.gov (NCT04581421).

Participants were recruited between November 2020 and May 2022, with experiments conducted from December 2020 to June 2022.

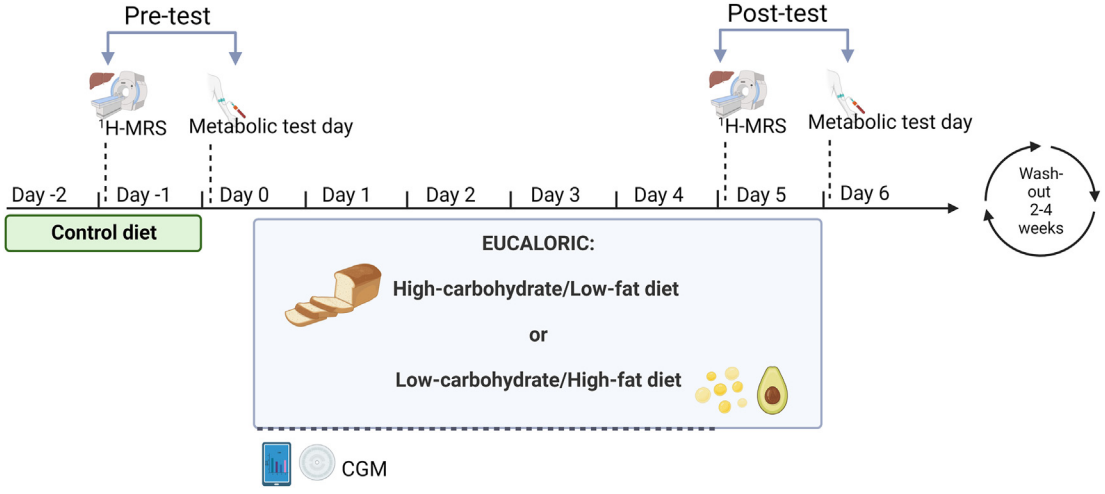

FIGURE 1. Illustration of the study design. ^{1}H-MRS, proton magnetic resonance spectroscopy; CGM, continuous glucose monitoring

TABLE 1
Baseline characteristics

Baseline characteristics (n = 11)	Mean ± SD
Age (y)	40 ± 8
Body weight (kg)	103.2 ± 18.1
BMI (kg/m^2)	31.6 ± 3.7
HbA_{1c} (mmol/mol)	34.6 ± 3.0
FIB-4 score	0.69 ± 0.24

Abbreviations: BMI, body mass index; FIB-4 score, fibrosis-4 score; HbA_{1c}, glycated hemoglobin A_{1c}; SD, standard deviation.

Experimental protocol

The experimental protocol is illustrated in Figure 1.

Study design

Participants were randomly assigned to the intervention order using block randomization with sealed envelopes ensuring balanced allocation (Figure 1). Each block had an equal number of participants starting either with an LC or an HC diet. Before each diet intervention, participants were provided with 2 d of a eucaloric control diet (Days 2 and 1). On Day 1, participants underwent ^{1}H-MRS in the morning after an overnight fast. On Day 0, the metabolic test day was initiated after overnight fasting. After completion of the metabolic test day, participants were provided with either the LC or HC diet and a continuous glucose monitor (Freestyle Libre) was mounted on the upper arm. The intervention diets were provided from the metabolic kitchen to participants and ingested in a free living setting at home. On Days 5 and 6, respectively, ^{1}H-MRS and the metabolic test day were repeated. After a minimum washout period of 2 wk, participants crossed over to the opposite diet intervention, and all investigations were repeated. Participants abstained from vigorous physical activity 2 d before and during the 5-d diet intervention.

Liver TG and liver glycogen

Liver TG content was determined by ^{1}H-MRS using a 3 Tesla (T) scanner (Philips Healthcare). Spoiled gradient echo (2D multi-echo fast field echo (M-FFE)) images were acquired to position ^{1}H-MRS voxels in the right liver lobe. First- and second-order shimming was adjusted automatically, using the vendor-supplied shimming routine. ^{1}H-MRS spectra were acquired from a 16 mL volume (2.5 × 2.5 × 2.5 cm^3) using the (Point RESolved Spectroscopy (PRESS) sequence with the following parameters; repetition time (TR) = 2 s, echo time (TE) = 29 ms, BW = 2 kHz, 1024 samples, 32 water-suppressed spectra, 32 water-unsuppressed spectra. To reduce partial volume effects, care was taken in voxel positioning avoiding visible intrahepatic blood vessels or bile ducts. ^{1}H-MRS spectra were quantified with the LC Model using an automated pipeline [17]. Fat fractions were estimated as previously described [18].

Liver glycogen content was determined in a subgroup of n = 4 with carbon MRS (^{13}C-MRS) on a 7T scanner (Philips Healthcare) using a transmit–receive ^{1}H–^{13}C surface coil (Rapid Biomedical GmbH). ^{13}C spectra were acquired by a pulse-and-acquire sequence (TR 200 ms; 3000 acquisitions, adiabatic excitation). The glycogen content was quantified by integration of the area under the glycogen peak ranging from 95 to 110 ppm.

Metabolic test day

The participants met after an overnight fast (10−12 h) at 07:30–08:00 employing passive transportation. Body composition was determined using dual-energy X-ray absorptiometry (Lunar DPX-IQ, GE Healthcare). In a subgroup of n = 5, body water content was measured using bioelectrical impedance analysis (InBody270, InBody Korea). After 30 min of rest in a supine position, oxygen uptake and carbon dioxide production were measured by indirect calorimetry (Vyntus CPX Canopy, Vyaire Medical). Catheters were then inserted into antecubital veins in both arms and a superficial dorsal hand vein, which was subsequently heated to obtain arterialized blood.

After fasting venous blood sampling, a bolus of [6,6-$^2H_2$] glucose tracer (3.2 mg/kg fat-free mass [FFM]) and [1,1,2,3,3-D5]-glycerol (0.15 mg/kg FFM) (Cambridge Isotope Laboratories) were administered, followed by infusions of labeled glucose and labeled glycerol at 0.073 mg/kg FFM/min and 0.15 mg/kg FFM/min, respectively. After 120 min, a low-dose hyperinsulinemic-euglycemic clamp was initiated with an insulin bolus (3 mU/kg body weight) (Actrapid, Novo Nordisk) followed by a constant infusion rate of 12 mU/m^2 body surface area/min for 120 min. After injection of the insulin bolus, infusion rates of labeled glucose and glycerol were reduced to 0.024 mg/kg FFM/min and 0.075 mg/kg FFM/min, respectively, and a 10% glucose solution enriched with 1.9% [6,6-$^2H_2$]-glucose was infused at a variable rate (GIR; glucose infusion rate) to maintain euglycemia, defined as the individual overnight fasting arterialized glucose concentration on the first preintervention test day. Arterialized blood was sampled before and every 20 min during the clamp, and for triplicate determination of tracer enrichments during the last 20 min of the basal period and the last 40 min of the clamp period.

Diet

Energy content and macronutrient composition of the habitual diet of the participants were determined based on a 3-d diet registration, where all food was weighed to 1 g of accuracy.

The daily energy requirement for each participant was based on the basal metabolic rate calculated using the modified Harris–Benedict equation [19] and multiplied by an individual physical activity level factor, assessed from a questionnaire on habitual activity patterns. The control diet was designed to reflect the composition of the average Danish population (49E% carbohydrate, 35E% fat, and 16E% protein) (Table 1). The eucaloric LC diet was designed to comprise 14E% carbohydrate and 70E% fat, and the HC diet to comprise 70E% carbohydrate and 14E% fat, while the amount of protein in both diets was maintained at 16 E%. In both LC and HC, the composition of dietary fat was distributed equally between saturated, monounsaturated, and polyunsaturated fatty acids (FAs).

During the intervention, participants weighed themselves at home. Per design, the diets were eucaloric, but the daily energy provision was increased in case of reported body mass reductions ≥0.8 kg/d. Diets were provided to the participants, and they were not allowed to ingest other food or drink items, black coffee/tea beyond 4 cups/d, or alcohol. The composition of habitual and intervention diets was analyzed using VITAKOST software.

Plasma analyses

Blood samples were collected in heparin-coated syringes and transferred to EDTA-coated tubes, except for TG and FA analyses, which were only EDTA-treated. All blood samples were immediately centrifuged at 12,000 rpm for 5 min at 4°C. Plasma glucose concentrations were measured using ABL800 FLEX (Radiometer Medical). Plasma concentrations of FA (NEFA C kit, Wako Chemicals GmbH) and TG (GPO-PAP kit, Roche Diagnostics), and plasma total-, LDL-, and HDL-cholesterol were measured using enzymatic colorimetric methods on an autoanalyzer (Pentra C400 analyzer). Plasma concentrations of insulin and C-peptide were measured by Immulite 2000

(Siemens Healthcare). Plasma beta-hydroxybutyrate concentration was measured by a colorimetric method (Sigma-Aldrich) using Multiskan FC (Thermo Fisher Scientific). Plasma enrichment of labeled glucose and glycerol was measured using liquid chromatography mass spectrometry (ThermoQuest Finnegan AQA), as previously described [20]. For analyses of plasma FA composition, lipids were extracted using chloroform/methanol, methylated using 12% BF_3 in methanol, and separated and identified by high-performance liquid chromatography with ultraviolet detection and consecutive gas chromatographic analysis [21]. Plasma total amino acids (L-amino acid assay kit, Abcam), glucagon (Mercodia), fibroblast growth factor 21 (FGF21), growth differentiation factor 15 (GDF15), and follistatin (DF2100, DGD150, and DFN00, respectively, R&D Systems) were measured colorimetrically following the manufacturer's instructions.

Plasma proteome analyses

Sample preparation was performed on an Agilent Bravo Liquid Handling Platform as previously described [22]. Samples were injected into a liquid chromatography mass spectrometry (Exploris 480, Thermo Fisher Scientific) operated in a data-independent mode.

The MS raw files were processed with Spectronaut version 17 (Biognosys).

Data was processed using the Clinical Knowledge Graph and Jupyter [23].

Calculations

The rate of appearance (Ra) for glucose and glycerol was calculated relative to FFM and fat mass (FM), respectively, from the last 20 min of the basal period and the last 40 min of the clamp, using Steele's steady state equation [24]. The HISI was calculated as c (constant)/glucose $Ra_{basal} \times [C\text{-peptide}]_{basal}$. Basal insulin clearance was estimated as $[C\text{-peptide}]_{basal}/[insulin]_{basal}$. Whole-body insulin sensitivity was calculated as glucose rate of disappearance (Rd = GIR + Ra) relative to FFM with adjustment for average plasma insulin concentration in the last 40 min of the clamp (glucose Rd/I) [25,26]. Clamp insulin clearance was calculated as insulin infusion rate/($[insulin]_{clamp} - ([C\text{-peptide}]_{clamp} \times [insulin]_{basal}/[C\text{-peptide}]_{basal})$) [27]. Adipose tissue insulin resistance was calculated as $[insulin]_{basal} \times$ glycerol Ra_{basal} (Lipo-IR) [26]. From measurements of plasma FA composition, the lipogenic index was calculated as C16:0/C18:2n-6 [28] and hepatic stearoyl-CoA desaturase (SCD) (Δ9) index as C18:1n-9/C:18:0. Respiratory exchange ratio (RER) was calculated as VCO_2/VO_2.

Statistics

Data are presented as means (±SD) whereas data with a skewed distribution, as visually determined through histograms, are presented as medians (interquartile range). Changes in relative and absolute liver TG are reported as means (95% confidence interval [CI]). Mixed effect models were used to test for intervention (pre and post) and diet (HC and LC) effects, and post hoc testing (precompared with the post for each diet and post-HC compared with post-LC) was applied in case of significant intervention × diet effects. Logarithmic transformation was used for data with a skewed distribution. Model control was visually examined using QQ and residual plots. Tukey correction was used for the primary outcome. The significance level was set at $P < 0.05$. Based on data from a previous study on a low-carbohydrate diet [29], we estimated that 12 participants were sufficient to detect a 30% difference in ^{1}H-MRS estimated liver TG, assuming an SD of 30% with a significance level of $\alpha = 5\%$ and power of 85%. Following the exclusion of a participant based on pathological high ferritin levels, 11 participants were included for analysis. The COVID-19 pandemic impacted our recruitment timeline, and we were unable to recruit an additional participant to replace the excluded individual. Analyses were performed in SPSS ('IBM SPSS, version 25, IBM Corp) and GraphPad Prism (version 9, GraphPad).

For proteomic analysis, intensities were log_{10}-transformed and unpaired *t*-tests were used to compare post-LC with post-HC. Multiple hypothesis correction was applied using the Benjamini–Hochberg method, with adjusted *P* values (*Q* values). $P < 0.05$ was considered significant.

Results

Participants included in the study were overweight to obese men with low risk of liver fibrosis and without diabetes, based on the Fib-4 index and $HbA_{1c,}$ respectively (Table 1). Exact registered compositions of the ingested diets were 11 ± 0.3E% carbohydrates, 70 ± 0.8E% fat, and 17 ± 0.7E% protein for the LC diet and 65 ± 1.1 E% carbohydrates, 16 ± 0.4 E% fat, and 16 ± 1.1 E% protein for the HC diet (Table 2).

TABLE 2
Dietary composition and energy consumption

Dietary composition ($n = 11$)	HC	LC	Control	Habitual
Energy (kJ/d)	13987 ± 1579	15278 ± 1836	14844 ± 1203	12120 ± 2371
Relative intake				
Protein, E%	16.1 ± 0.4	16.6 ± 0.7	18.0 ± 0.2	17.8 ± 6.0
Carbohydrate, E%	65.4 ± 1.1	11.4 ± 0.3	48.4 ± 0.3	40.9 ± 8.1
Fat, E%	15.6 ± 1.1	70.3 ± 0.8	31.7 ± 0.4	38.5 ± 6.9
Saturated fatty acids	36 ± 2.9	35 ± 1.1	42 ± 1.1	51 ± 8.5
Monounsaturated fatty acids	29 ± 2.0	35 ± 0.6	43 ± 0.6	37 ± 6.8
Polyunsaturated fatty acids	36 ± 2.7	30 ± 0.6	15 ± 1.2	14 ± 3.7
Absolute intake				
Protein (g/d)	133 ± 17	149 ± 15	157 ± 13	122 ± 30
Carbohydrate (g/d)	537 ± 56	102 ± 12	422 ± 34	293 ± 79
Dietary fiber (g/d)	51 ± 7	39 ± 4	53 ± 8	25 ± 7
Fat (g/d)	59 ± 9	291 ± 37	124 ± 10	124 ± 37
Cholesterol (mg/d)	205 ± 28	558 ± 65	459 ± 41	363 ± 244

Data are shown for the high-carbohydrate/low-fat diet (HC), low-carbohydrate/high-fat diet (LC), the control diet (provided before each intervention), and for the 3 d habitual weighed dietary registration. E%: percentage of total energy intake. For the fatty acid type, data are shown as percentage of known fatty acids, as data were obtainable for ~70%–80% of the total fat in the intervention diets. Values are means ± SD.

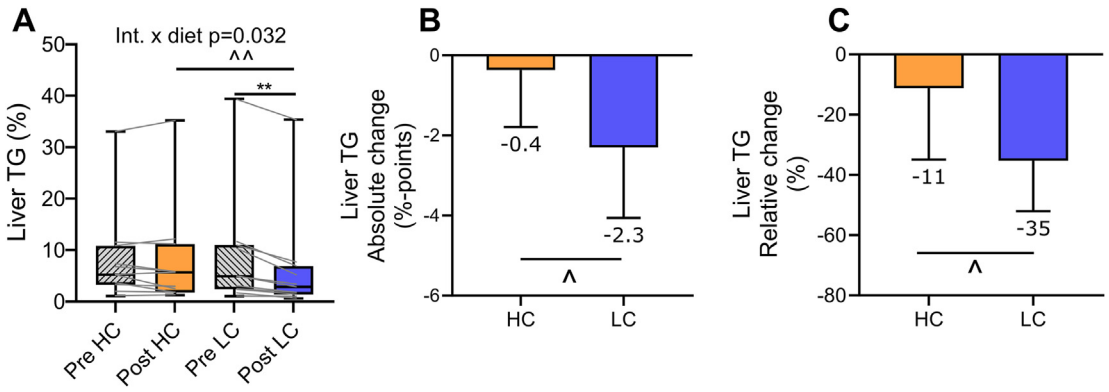

FIGURE 2. Liver triacylglycerol content decreased during a low-carbohydrate/high-fat diet. (A) Liver TG content measured by ^{1}H-MRS in the overnight-fasted state before and 4 d after high-carbohydrate/low-fat diet (65E% carbohydrates, 16E% fat, and 16E% protein) (HC) and low-carbohydrate/high-fat diet (11E% carbohydrates, 70E% fat, and 17E% protein) (LC) (box plot with median, IQR, and min and max value, $^{**}P < 0.01$ pre-LC compared with post-LC, $^{\wedge\wedge}P < 0.01$ post-HC compared with post-LC). (B) Absolute and (**C**) relative change in liver TG content during HC and LC (mean ± SD, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$ HC compared with LC). In (A), a mixed effect model was performed to test for the effects of intervention (pre and post) and diet (HC and LC). In (B) and (C), paired *t*-tests were performed to test for differences between diets; $n = 11$. IQR, interquartile range; TG, triacylglycerol.

Four days of LC diet reduced liver TG content

Liver TG content at the first preintervention ^{1}H-MRS was 5.2% [2.5–11.5] and 5 of 11 participants had liver TG >5.5%. The response in liver TG differed significantly between the 2 diets (intervention × diet $P = 0.032$, Figure 2A). After 4 d of an LC diet with 11E% carbohydrates and 70E% fat led to a significant decrease in liver TG content compared with preintervention ($P = 0.002$), and liver TG content decreased in all participants. No change was found after 4 d of HC diet with 65E% carbohydrates and 16E% fat compared with preintervention (Figure 2A). Liver TG decreased after LC by 2.3% points (95% CI: −3.5, −1.1), corresponding to a 35.3% (−46.6, −24.1) relative reduction compared with preintervention (Figure 2B and C). Thus, liver TG content was 2.9% [1.4–6.9] post-LC and 5.7% [1.8–11.2] post-HC (Figure 2A). Preintervention liver TG contents were similar before the first and second diet interventions indicating sufficient washout.

Along with the lowering of liver TG content after LC, a minor weight loss was seen compared with preintervention (–1.0 ± 0.7 kg, $P < 0.001$, from 103.0 ± 18.5 to 102.0 ± 18.3 kg) (Supplemental Table 1). This occurred despite an increase in average total energy provision of 9% (+1291 ± 1076 kJ/d) in the LC group (Table 2). The increased energy provision during LC led to a modest increase in absolute protein provision compared with the HC diet. However, the relative protein intake remained similar between the 2 interventions (Table 2). During HC, the participants were weight stable, and no adjustment in energy provision was necessary (Supplemental Table 1). Notably, weight loss during LC did not correlate with changes in liver TG content ($r = 0.28$, $P = 0.403$). No change in body composition was observed during LC or HC; FM and visceral fat content estimated by dual-energy X-ray absorptiometry scan remained unchanged compared with preintervention (Supplemental Table 1). To reveal any potential differences in hydration state, bioimpedance measurements were added to the last 5 participants, but no differences were observed (Supplemental Table 1).

Increased fat oxidation and ketogenesis and lowered DNL after 5 days of LC diet

In the overnight-fasted state, whole-body RER decreased after LC (0.76 ± 0.03 to 0.72 ± 0.03) and increased after HC (0.76 ± 0.03 to 0.79 ± 0.05) compared with preintervention (Figure 3A). The resting metabolic rate remained unchanged (Figure 3B). Along with the increase in whole-body fat oxidation, fasting plasma concentration of beta-hydroxybutyrate increased by 73% after LC compared with preintervention (0.20 ± 0.05 to 0.32 ± 0.10 mmol·/L) but remained unchanged after HC (Figure 3C).

Fasting plasma TG concentration decreased by 35% after LC (1.5 [1.0–1.9] to 0.9 [0.7–1.1] mmol·/L) but did not change after HC (Figure 3D). A reduction in plasma total cholesterol level was observed for both interventions (Table 3). After HC, plasma LDL- and HDL-cholesterol concentrations were lowered, with no change after LC (Table 3). Fasting plasma concentration of palmitoleic acid (C16:1n-7), representing the desaturated end product of DNL, decreased by 67% after LC but remained unchanged after HC (Figure 3E). Accordingly, the lipogenic index was lowered by 37% after LC (Figure 3F). In line with a lower requirement for desaturation of DNL-derived FAs, a decrease in the hepatic SCD (Δ9) index was observed after LC (Figure 3G).

Fasting plasma concentration of GDF15 was higher at post-LC compared with post-HC (Figure 3H), whereas FGF21 and follistatin concentrations remained unchanged after both diets (Supplemental Table 2). The proteomic analysis was carried out with an exploratory purpose. Proteomics analysis revealed 309 hits, whereof several proteins associated with lipid metabolism and the immune system were upregulated and downregulated. Of interest, apolipoprotein CIII was downregulated, and apolipoprotein A-IV was upregulated comparing post-LC with post-HC (Supplemental Figure 1).

Increased basal hepatic insulin sensitivity after 5 days of LC diet

By design, there was a pronounced difference in the glycemic load of the diets with lower daytime, SD, and glycemic variation (CV%) around the mean sensor glucose concentration, but also lower nocturnal interstitial glucose concentrations during LC compared with HC (Figure 4A–C), resulting in lower 24 h mean sensor glucose concentrations of 5.2 ± 0.2 mmol·l^{-1} compared with 5.8 ± 0.4 mmol·l^{-1} ($P <$ 0.001). The interventions did not result in changes in the fasting venous plasma glucose concentrations on the metabolic test day (Table 3). Fasting plasma insulin concentration was 24% lower after LC compared with preintervention, whereas it was unaltered after HC

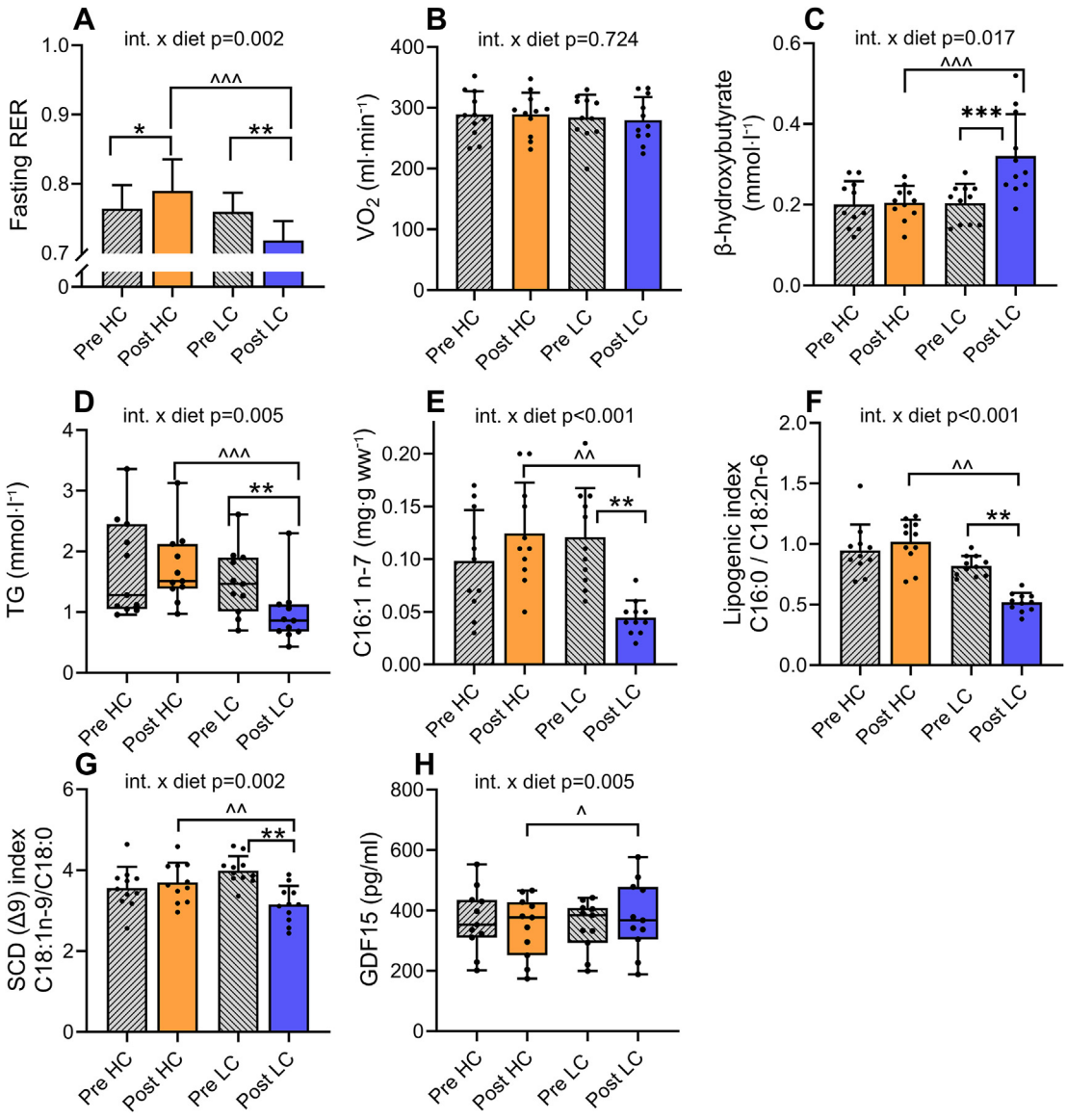

FIGURE 3. Indices of increased whole-body fat oxidation, increased ketogenesis, and lowered de novo lipogenesis after a low-carbohydrate/high-fat diet. (A) Whole-body respiratory exchange ratio (RER). (B) The resting metabolic rate is determined as the resting oxygen consumption rate (VO_2). (C) Fasting plasma beta-hydroxybutyrate concentration. (D) Fasting plasma triacylglycerol concentration. (E) Fasting plasma concentration of palmitoleic acid (C16:1 *n*–7), representing the desaturated end product of de novo lipogenesis. (F) Lipogenic index, calculated as the ratio of C16:0/C18:2 *n*–6. (G) Stearoyl-CoA desaturase (SCD) (Δ-9 desaturase) index. (H) Fasting plasma GDF15 concentration. All values are obtained in the overnight-fasted state before and 5 d after high-carbohydrate/low-fat diet (65E% carbohydrates, 16E% fat, and 16E% protein) (HC) and low-carbohydrate/high-fat diet (11E% carbohydrates, 70E% fat, and 17E% protein) (LC). Data are presented as means ± SDs, except for fasting plasma TG and GDF15 concentrations, which are presented as box plots with medians, IQRs, and min and max values. In (A)–(H), a mixed effect model was performed to test for effects of intervention (pre and post) and diet type (HC and LC). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ precompared with post for the indicated diets, $^{\wedge}P < 0.05$ $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ post-HC compared with post-LC; $n = 11$. GDF 15, growth differentiation factor 15; IQR, interquartile range; TG, triacylglycerol.

(Table 3). Fasting plasma C-peptide only tended to differ in response to the 2 diets (intervention × diet $P = 0.053$) (Table 3). However, the estimate of basal insulin clearance (C-peptide/insulin) was not significantly changed in response to the 2 diets (Table 3).

In agreement with the maintained fasting plasma glucose concentrations, basal Ra of glucose was unchanged in response to the diets (Table 3). However, the hepatic insulin sensitivity index (HISI) increased by 24% following LC compared with preintervention, whereas HISI was unchanged after HC (Figure 4D). Fasting plasma glucagon only tended to differ in response to the 2 diets, but the glucagon to C-peptide ratio was higher after LC (Table 3). Plasma concentrations of total amino acids were unaltered after both diets (Supplemental Table 2). The overnight-fasted liver glycogen content measured by ^{1}C-MRS in a subgroup of 4 participants did not seem to change after LC but was 93% increased after HC compared with preintervention (Supplemental Figure 2).

Fasting plasma FA concentration increased by 49% after LC, accompanied by a higher fasting plasma glycerol concentration (by 22%) with no changes after HC (Table 3).

TABLE 3

Measures of lipid and glucose metabolism in the overnight-fasted state (basal) and during the hyperinsulinemic-euglycemic clamp before and after 5 days high-carbohydrate/low-fat (65E% carbohydrates, 16E% fat, and 16E% protein) (HC) and low-carbohydrate/high-fat (11E% carbohydrates, 70E% fat, and 17E% protein) (LC) intake

$n = 11$	Pre-HC	Post-HC	Pre-LC	Post-LC	Int.	Diet	Int. × diet	Post hoc test		
Basal (fasting)								Pre- vs. post-HC	Pre- vs. post-LC	Post-HC vs. post-LC
p-Fatty acids, μmol/L	470 [369–533]	412 [344–530]	502 [307–590]	688 [529–827]	0.364	0.007	0.017	0.445	0.039	0.001
p-Glycerol, μmol/L	66.3 ± 18.9	59.7 ± 17.4	73.4 ± 26.2	82.7 ± 14.4	0.176	0.004	0.031	0.168	0.061	<0.001
Glycerol Ra, μmol/min · kg/FM	6.2 ± 2.3	6.0 ± 1.5	6.7 ± 1.5	7.8 ± 1.2	0.249	0.102	0.131	—	—	—
LIPO-IR (μmol/min · kg/FM · pmol/L)	323 [209–474]	388 [157–621]	318 [160–547]	418 [185–535]	0.699	0.878	0.716	—	—	—
p-Insulin, pmol/L	53.5 [37.8–123.8]	51.3 [46.6–139.3]	48.0 [39.8–130.1]	37.0 [28.0–75.0]	0.089	0.127	0.029	0.648	0.011	0.014
p-C-peptide, pmol/L	547 [310–674]	496 [436–671]	484 [402–790]	394 [314–595]	0.174	0.583	0.053	—	—	—
p-Glucose, mmol/L	5.1 ± 0.3	5.1 ± 0.28	5.2 ± 0.26	5.2 ± 0.26	0.689	0.380	0.595	—	—	—
p-Glucagon, pmol/L	7.8 [5.3–14.0]	5.6 [4.1–10.6]	7.0 [5.9–11.9]	10.0 [8.0–14.4]	0.801	0.200	0.059	—	—	—
Glucagon/C-peptide	0.0167 ± 0.007	0.0127 ± 0.005	0.0156 ± 0.005	0.0240 ± 0.011	0.309	0.031	0.012	0.516	0.061	0.012
Glucose Ra, μmol/min · kg/FFM	16.0 ± 1.48	16.3 ± 2.09	16.1 ± 2.39	15.0 ± 1.35	0.386	0.283	0.081	—	—	—
C-peptide/insulin	9.3 ± 0.3.2	9.5 ± 3.4	9.4 ± 2.9	11.4 ± 4.2	0.079	0.100	0.154	—	—	—
p-HDL cholesterol, mmol/L	0.93 ± 0.12	0.81 ± 0.12	0.90 ± 0.14	0.87 ± 0.12	<0.001	0.604	0.007	<0.001	0.108	0.055
p-LDL cholesterol, mmol/L	3.6 ± 1.9	3.1 ± 1.3	3.3 ± 0.98	3.3 ± 0.83	0.041	0.833	0.021	0.002	0.984	0.145
p-Total cholesterol, mmol/L	5.4 ± 1.2	4.8 ± 1.5	5.1 ± 1.05	4.8 ± 0.91	0.005	0.355	0.306	—	—	—
Clamp										
p-Fatty acids, μmol/L	99 [59–157]	94 [61–158]	112 [75–193]	214 [132–337]	0.023	0.001	0.002	0.290	<0.001	<0.001
p-Glycerol, μmol/L	38 [28–48]	32 [24–44]	33 [25–48]	44 [33–54]	0.853	0.091	0.014	0.658	0.427	0.007
Glycerol Ra, μmol/min × kg/FM	3.3 ± 0.41.3	3.2 ± 0.8	3.2 ± 0.6	3.8 ± 1.2	0.374	0.440	0.293	—	—	—
p-Insulin, pmol/L	211 [178–303]	220 [158–311]	200 [183–276]	181 [169–236]	0.070	0.136	0.051	—	—	—
p-C-peptide, pmol/L	396 [249–639]	447 [260–664]	405 [235–589]	302 [212–621]	0.251	0.009	0.033	0.393	0.026	0.002
GIR, mg/min · kg/FFM	2.33 ± 0.73	2.46 ± 0.87	2.32 ± 0.90	1.98 ± 0.86	0.344	0.095	0.045	0.382	0.043	0.010
GIR/insulin, mg/min · kg/FFM pmol/L	0.011 ± 0.006	0.013 ± 0.008	0.011 ± 0.006	0.011 ± 0.006	0.310	0.440	0.293	—	—	—
Glucose Ra, μmol/min · kg/FFM	3.1 ± 1.6	2.8 ± 1.6	3.5 ± 2.4	3.2 ± 1.3	0.036	0.032	0.280	—	—	—
Glucose Rd, μmol/min · kg/FFM	16.3 ± 3.9	16.5 ± 4.8	16.6 ± 4.9	14.2 ± 4.0	0.126	0.148	0.078	—	—	—
RER	0.82 ± 0.04	0.84 ± 0.03	0.80 ± 0.02	0.76 ± 0.02	0.138	<0.001	0.002	0.117	0.003	<0.001

Abbreviations: FFM, fat-free mass; GIR, glucose infusion rate; HDL, high-density lipoprotein; LDL, low density lipoprotein; LIPO-IR, adipose tissue lipolysis insulin resistance index (basal glycerol Ra × basal insulin); Rd, rate of disappearance.

Basal glucose and glycerol rate of appearance (Ra) are obtained from the last 20 min of the basal infusion period. All parameters obtained during the clamp represent mean values of the last 40 min of insulin stimulation. Values are means ± SD, or medians [interquartile ranges] in case of non-normal distribution. Mixed effect models were performed to test for effects of intervention (pre and post) and diet type (HC and LC), with *P* values shown for the main effects of intervention (Int.) and Diet and the interaction between Int. × Diet. In case of significant interaction, post hoc tests for pre-HC compared with post-HC, pre-LC compared with post-LC, and post-LC compared with post-HC were performed (*P* values listed in the 3 right columns) ($n = 11$).

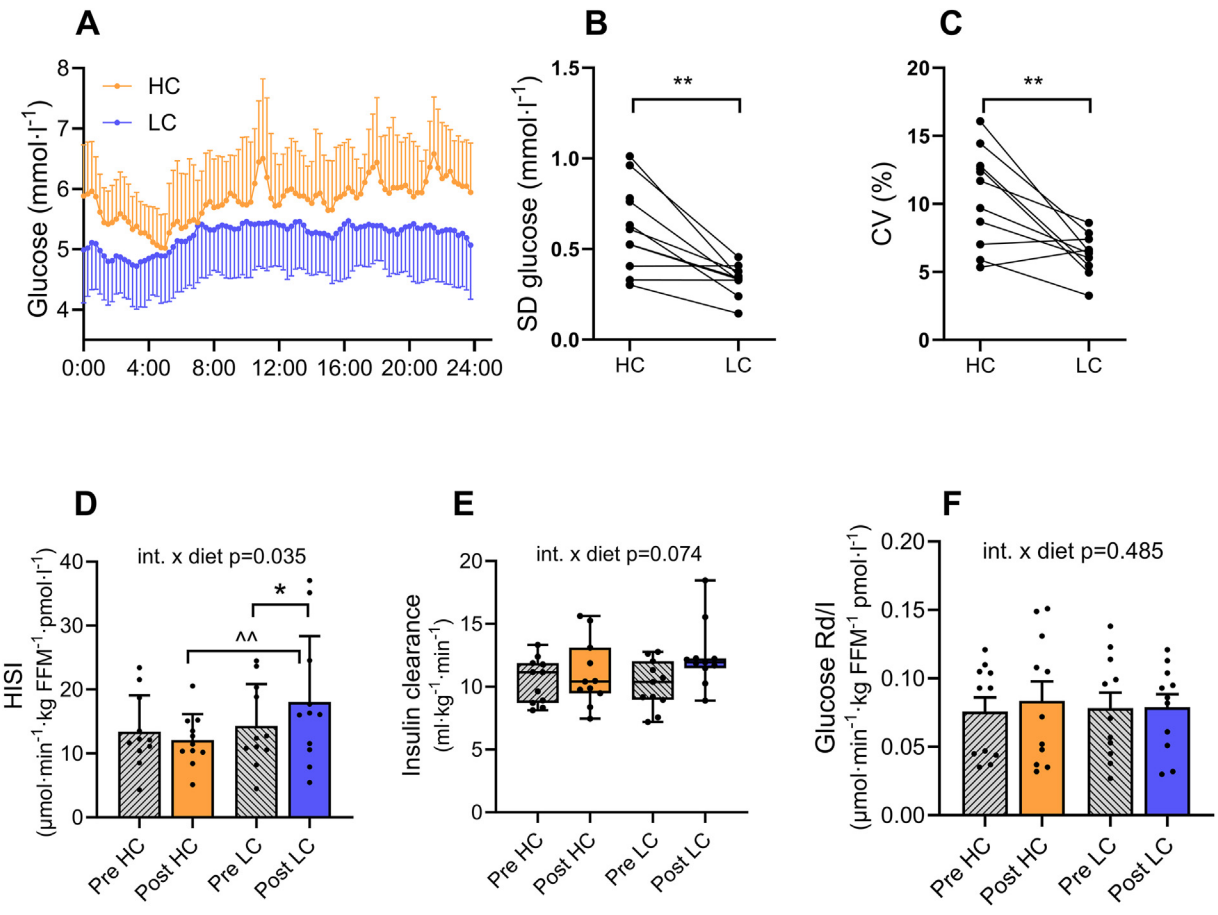

FIGURE 4. Lower mean glucose and glycemic variability during low-carbohydrate/high-fat diet and improved hepatic insulin sensitivity after low-carbohydrate/high-fat diet. (A) Mean interstitial glucose concentrations obtained from intermittently scanned continuous glucose monitoring (isCGM). Values are means ± SD. (B) Individual mean SD. (C) Individual mean glycemic variation (CV%). Values from (**A**) to (C**)** are obtained during 5 d high-carbohydrate/low-fat (65E% carbohydrates, 16E% fat, and 16E% protein) (HC) and low-carbohydrate/high-fat (11E% carbohydrates, 70E% fat, and 17E% protein) (LC) diets. (D) Hepatic insulin sensitivity index (HISI) measured in the overnight fasting state. (E) Insulin clearance measured during hyperinsulinemic-euglycemic clamp. (F) Whole-body insulin sensitivity is expressed as the rate of disappearance of glucose relative to clamp plasma insulin concentrations (Rd/I) during hyperinsulinemic-euglycemic clamp. Values from (D) to (F) are obtained before and after 5 d high-carbohydrate/low-fat (HC) and low-carbohydrate/high-fat (LC) diets. In (D) and (F), values are means ± SD. In (E), data are presented as box plots with median, IQR, and min and max values. In (B) and (**C**) (**$P < 0.01$), a paired *t*-test was performed to test for effect of diet type (HC compared with LC). In (D)–(F)**,** a mixed effect model was performed to test for effects of intervention (pre and post) and diet type (HC and LC). *$P < 0.05$ precompared with post within indicated diet group, ^$P < 0.05$ ^^$P < 0.01$, post-HC compared with post-LC; $n = 11$. IQR, interquartile range; SD, standard deviation.

Maintenance of hepatic, whole-body, and adipose tissue insulin action with LC and HC diets

During the hyperinsulinemic-euglycemic clamp, insulin infusion resulted in average plasma insulin concentrations of 211 and 200 pmol/L at the preintervention clamps before HC and LC, respectively (Table 3). Clamp plasma insulin tended to differ in response to the 2 diets (Table 3) concomitant with a different response to the 2 diets in endogenous insulin secretion; clamp plasma C-peptide, which was 25% lower after LC (Table 3). Insulin clearance during the clamp tended to differ in response to the 2 diets with a 22% numerical increase after LC (Figure 4E). Plasma FA concentration during the clamp was, in line with the fasting FA concentration, higher after LC compared with preintervention (Table 3), whereas unchanged after HC. Notably, the relative suppression of plasma FA during insulin infusion was maintained after both interventions.

During the clamp, glucose Ra was similar to preintervention after both LC and HC (Table 3) despite the lower plasma insulin and higher plasma FA concentration during the post-LC clamp. Whole-body insulin sensitivity, expressed as glucose Rd relative to clamp plasma insulin, did not change after either diet (Figure 4F).

Ra of glycerol in the fasted state was not changed in response to the 2 diets and neither was the LIPO-IR index (Table 3) indicating maintained sensitivity of basal adipose tissue lipolytic activity. Likewise, during the clamp, there were no effects of the diets on glycerol Ra or the insulin-mediated relative suppression of glycerol Ra (Table 3).

Association between liver TG levels and metabolic parameters

Liver TG content at baseline ranged from 1.4% to 36.2% and was inversely correlated with clamp insulin clearance rate ($r = -0.73$, $P = 0.010$) and HISI ($r = -0.61$, $P = 0.044$). Furthermore, liver TG content correlated with BMI ($r = 0.81$, $P = 0.003$), visceral fat content ($r = 0.77$, $P = 0.006$), fasting plasma FA ($r = 0.65$, $P = 0.029$), fasting plasma glycerol ($r = 0.67$, $P = 0.023$), and fasting plasma C-peptide ($r = 0.64$, $P = 0.035$) (data not shown).

Discussion

In this study, liver TG content was reduced by 35% in 11 male participants with overweight/obesity within just 4 d of implementing a eucaloric LC diet with 11E% carbohydrates, 70E% fat, and 17E% protein. The reduction in liver TG content following the LC diet was associated with several key metabolic shifts, including an increase in whole-body FA oxidation, and an elevation in hepatic ketogenesis, as evidenced by a 73% rise in beta-hydroxybutyrate levels as well as indications of diminished DNL as seen by a decrease in plasma concentrations of the desaturated end product.

Our results demonstrate that a modest reduction (to ~100 g/d) in dietary carbohydrates replaced by fat is enough to lower liver TG content in individuals with excess body fat. The acute efficacy of a low-carbohydrate diet has previously been demonstrated in individuals with obesity, where a hypocaloric low-carbohydrate diet outperformed a hypocaloric high-carbohydrate diet in reducing liver TG content within 48 h [14], and an isocaloric low-carbohydrate diet reduced liver TG content after only 24 h in individuals with fatty liver disease [16]. However, after 7% weight loss, both the hypocaloric low-carbohydrate and the hypocaloric high-carbohydrate diet showed similar decreases in liver TG content [14], which underscores the impact of weight loss on reductions in liver TG content. Long-term studies investigating eucaloric moderate carbohydrate restriction spanning from 6 to 12 wk in individuals with fatty liver disease and/or type 2 diabetes demonstrated a lowering of liver TG content (18–39E% reduction) when restricting carbohydrate intake to 34–40E% in the absence of weight loss [30–32] further highlighting the metabolic advantages of carbohydrate restriction during eucaloric conditions. In our study, all participants experienced a significant reduction in liver TG content after 4 d of the LC diet despite large differences in liver TG at preintervention (5 with/6 without MASLD). These findings suggest that a eucaloric low-carbohydrate high-fat diet is effective in reducing liver TG content, regardless of whether the participant has MASLD or diabetes.

The dietary approach was carefully controlled in our study to maintain a eucaloric state, and body weight was maintained during the HC diet. Nevertheless, during the LC intervention, wherein the participants were provided with an initial identical caloric content as in the HC regimen, there was a modest decrement in body weight amounting to -1.0 ± 0.2 kg, despite a compensatory augmentation in total energy provision. However, because participants were free living at home, we cannot completely rule out the possibility of selective underreporting of intake during the LC diet which could result in negative energy balance during this diet only. Importantly, the weight reduction observed during LC was independent of changes in resting metabolic rate or hydration status and did not correlate with reductions in liver TG. A decline in body weight during a low-carbohydrate high-fat diet has been observed in other studies of short-term low-carbohydrate intake [16,33,34] and has been attributed to underestimation of energy provision, but this is less likely to be the explanation in our study where weight loss did not manifest during the HC diet with identical initial calorie content. Interestingly, despite an increase in dietary carbohydrate intake of 23% (corresponding to +240 g carbohydrate daily) during the HC diet with 65E% carbohydrates, 16E% fat, and 16E% protein, liver TG content remained unchanged.

Liver TG content is the result of a balance between the uptake of plasma FA and DNL on one side and beta-oxidation in the hepatic mitochondria and the secretion of VLDL-TG into the circulation on the other side. Our findings after LC revealed an increase in whole-body fat oxidation, as indicated by a reduced RER which likely also includes the liver. This was supported by elevated plasma beta-hydroxybutyrate concentrations as a marker of increased hepatic oxidation/ketogenesis. Support for this notion is previous findings of a correlation between plasma beta-hydroxybutyrate and hepatic FA oxidation [35]. To evaluate DNL, we measured plasma concentrations of the FA syntheses marker C16:1n7 palmitoleate, representing the desaturated palmitate end product of DNL [36], which was reduced during LC. Taken together, increased FA oxidation, increased ketogenesis, and reduced DNL in the liver contribute to the lowering of the liver TG content with eucaloric LC intake. Notably, hepatic FA availability increased during LC, evidenced by higher plasma FA concentrations. This was likely due to lower plasma insulin concentration rather than diminished insulin action in adipose tissue, as the LIPO-IR index (glycerol Ra relative to insulin) remained stable.

Despite the high fat intake during LC and the increase in fasting FA concentrations, the fasting plasma TG concentration was reduced, most likely a result of diminished VLDL-TG secretion from the liver [37]. The lower plasma level of VLDL-TG is consistent with the plasma proteomics results revealing a significant reduction in apolipoprotein CIII. Interestingly, the LC diet led to a marginal reduction in plasma total cholesterol levels. Although previous studies have shown positive effects on liver TG content with a high intake of monounsaturated fats diet [32], we deliberately ensured that the high-fat intake of the LC diet was evenly distributed across saturated, monounsaturated, and polyunsaturated fats. Hence the reduction in liver TG content following the LC diet was observed even though the saturated fat intake was relatively high (~24% of energy intake, equivalent to ~100 g/d), suggesting that factors other than FA composition contributed to this outcome. Notably, the dietary elevation in saturated fat and cholesterol consumption during this short period of LC did not yield adverse effects on the lipoprotein profile. Altogether, our findings suggest that enhanced fat oxidation and a lowering of DNL were the primary mechanisms responsible for lowering liver TG during an LC diet whereas FA availability and secretion of VLDL-TG were not contributing importantly.

Hepatic insulin clearance is a main regulator of systemic insulin levels and has been demonstrated to be negatively associated with liver TG content [38–40]. Accordingly, we found baseline hepatic insulin clearance to be inversely correlated to liver TG content. Nevertheless, the lower fasting plasma insulin concentrations observed after the LC diet could be due to the reduced insulin secretion, likely caused by the lower carbohydrate intake in the LC diet. In addition, the decrease in liver TG content may have contributed to a trend toward differences in insulin clearance in response to the 2 diets, potentially further lowering fasting plasma insulin concentrations after LC. Despite the lower insulin concentrations after LC, HGP (glucose Ra) was maintained, indicating increased insulin sensitivity of the liver. Simultaneously, peripheral insulin sensitivity measured by the euglycemic-hyperinsulinemic clamp, remained unaltered after the LC diet. In line with this, we have previously demonstrated improved hepatic insulin sensitivity and maintained peripheral insulin sensitivity after 6 wk of a eucaloric diet reduced in carbohydrates (20E%) and increased in fat (64E%) in overweight to obese participants [41].

We investigated the effects of the diets on the hepatokines FGF21 and follistatin, because both proteins may contribute to metabolic improvements [42–45], but we did not observe changes in circulating FGF21 or follistatin. We also quantified GDF15 levels due to its established role in appetite regulation in rodent studies [46–48] and its known influence on improving insulin sensitivity in hepatic and adipose tissues in mice [46]. Interestingly, we observed increased GDF15

concentration after the LC diet in accordance with recent findings after weight loss induced by ketogenic diets [49].

This study explores the acute effects of altering carbohydrate and fat intake on liver TG content and metabolism. It emphasizes mechanistic adaptations and acute molecular responses, limiting conclusions with regard to results representative of long-term implications and broad dietary recommendations. The minor weight loss observed during LC is a limitation when interpreting the results, but importantly it did not correlate with the observed reduction in liver TG. Experiments were performed during the COVID-19 pandemic limiting data analysis to $n = 11$ instead of the planned $n = 12$ based on power calculations on liver TG (primary endpoint). Because the effect of the LC diet on liver TG was larger than expected, the study was adequately powered concerning the primary endpoint but inadequate power may be a limitation for other outcomes.

In conclusion, glucose-tolerant males with overweight/obesity and liver TG content spanning from normal to overt MASLD, a eucaloric LC (11E% carbohydrates, 70E% fat, and 17E% protein) (LC) diet resulted in a 35% reduction in liver TG content within 4 d. We demonstrated that this was likely caused by increased fat oxidation and ketogenesis in combination with the lowering of DNL. The LC diet reduced plasma TG concentrations and enhanced basal hepatic insulin sensitivity while whole-body and adipose tissue insulin sensitivities were maintained and no detrimental changes in lipoprotein profiles were observed. The LC diet resulted in 1% body weight loss, which did not correlate with the reduction in liver TG. Notably, our study also demonstrated that a 4-d diet with 65E% carbohydrates, 16E% fat, and 16E% protein did not adversely affect liver TG or overall measured parameters for glucose and lipid metabolism.

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Author contributions

The authors' responsibilities were as follows – AL, NJWA, SM, JJH, BK, AML, KNB-M: conceptualization; AL, MMR, NJWA, MP, GvH, HRS, EAR, BK, AML, KNB-M: methodology; AL, MMR, KAS, MP, AS, EAR, BK, AML, KNB-M: investigation; AL, MMR, MP, LD, HRS, BK, AML, KNB-M: formal analysis; AL, MMR, BK, AML, KNB-M: visualization; AL, MMR, EAR, BK, AML, KNB-M: writing original draft; AL, MMR, KAS, NJWA, MP, LD, AS, LM, JØ, SM, GvH, HRS, EAR, BK, AML, KNB-M: writing-review and editing; NJWA, LM, JØ, GvH: resources; and all authors: read and approved the final manuscript.

Conflict of interest

AML has taken a position at Novo Nordisk A/S as of October 2023. HRS has received honoraria from Lundbeck AS and royalties as a book editor. All other authors report no conflicts of interest.

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Data availability

Data described in the manuscript will be made available upon request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.06.006.

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