The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Hepatic Energy Metabolism Underlying Differential Lipidomic Responses to High-Carbohydrate and High-Fat Diets in Male Wistar Rats

Simon N Dankel,[1,2] Bodil Bjørndal,[2] Carine Lindquist,[2,3] Mari L Grinna,[3] Christine Renate Rossmann,[4] Pavol Bohov,[2] Ottar Nygård,[1,2,3] Seth Hallström,[4] Elin Strand,[3] and Rolf K Berge[2,3]

[1]Mohn Nutrition Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway; [2]Department of Clinical Science, University of Bergen, Bergen, Norway; [3]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway; and [4]Division of Physiological Chemistry, Medical University of Graz, Graz, Austria

ABSTRACT

Background: Low-carbohydrate diets are suggested to exert metabolic benefits by reducing circulating triacylglycerol (TG) concentrations, possibly by enhancing mitochondrial activity.

Objective: We aimed to elucidate mechanisms by which dietary carbohydrate and fat differentially affect hepatic and circulating TG, and how these mechanisms relate to fatty acid composition.

Methods: Six-week-old, ∼300 g male Wistar rats were fed a high-carbohydrate, low-fat [HC; 61.3% of energy (E%) carbohydrate] or a low-carbohydrate, high-fat (HF; 63.5 E% fat) diet for 4 wk. Parameters of lipid metabolism and mitochondrial function were measured in plasma and liver, with fatty acid composition (GC), high-energy phosphates (HPLC), carnitine metabolites (HPLC-MS/MS), and hepatic gene expression (qPCR) as main outcomes.

Results: In HC-fed rats, plasma TG was double and hepatic TG 27% of that in HF-fed rats. The proportion of oleic acid (18:1n–9) was 60% higher after HF vs. HC feeding while the proportion of palmitoleic acid (16:1n–7) and vaccenic acid (18:1n–7), and estimated activities of stearoyl-CoA desaturase, SCD-16 (16:1n–7/16:0), and de novo lipogenesis (16:0/18:2n–6) were 1.5–7.5-fold in HC vs. HF-fed rats. Accordingly, hepatic expression of fatty acid synthase (*Fasn*) and acetyl-CoA carboxylase (*Acaca/Acc*) was strongly upregulated after HC feeding, accompanied with 8-fold higher FAS activity and doubled ACC activity. There were no differences in expression of liver-specific biomarkers of mitochondrial biogenesis and activity (*Cytc*, *Tfam*, *Cpt1*, *Cpt2*, *Ucp2*, *Hmgcs2*); concentrations of ATP, AMP, and energy charge; plasma carnitine/acylcarnitine metabolites; or peroxisomal fatty acid oxidation.

Conclusions: In male Wistar rats, dietary carbohydrate was converted into specific fatty acids via hepatic lipogenesis, contributing to higher plasma TG and total fatty acids compared with high-fat feeding. In contrast, the high-fat, low-carbohydrate feeding increased hepatic fatty acid content, without affecting hepatic mitochondrial fatty acid oxidation. *J Nutr* 2021;151:2610–2621.

Keywords: dietary carbohydrate, dietary fat, fatty acid composition, triacylglycerols, lipogenesis, mitochondria

Introduction

An elevated circulating concentration of triacylglycerols (TGs; hypertriglyceridemia) is an important risk factor for cardiovascular disease independently of LDL cholesterol (1), and is strongly linked to hyperinsulinemia and insulin resistance in the context of obesity, type 2 diabetes, and fatty liver (2, 3). Elevated circulating TG is associated with fatty liver, hyperinsulinemia, and insulin resistance even in lean people with normal glucose tolerance (4). Concurrent with a marked increase in overweight and obesity during the last decades, national advisers suggested that dietary fat intake should be decreased in an effort to reduce the incidence of atherosclerotic cardiovascular diseases. However, high carbohydrate, low-fat (HC) diets have been known for more than half a century to increase plasma TG, suggested to at least partly result from decreased clearance of TG from the circulation (5). Several studies have since confirmed a dose-dependent TG-lowering effect of lower carbohydrate intake (6). Additionally, low-carbohydrate, high-fat (HF) diets increase circulating concentrations of HDL cholesterol relative to HC diets, while HC diets tend to decrease LDL cholesterol, at least in the shorter term (6). However, we still lack mechanistic insight into how HF and HC diets differentially affect lipid

© The Author(s) 2021. Published by Oxford University Press on behalf of the American Society for Nutrition. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Manuscript received February 23, 2021. Initial review completed March 17, 2021. Revision accepted May 11, 2021.
First published online June 16, 2021; doi: https://doi.org/10.1093/jn/nxab178.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

metabolism and cardiovascular risk factors, including fatty acid (FA) composition in plasma and liver.

The liver plays a principal role in regulating whole-body lipid homeostasis. Chylomicron and VLDL remnants are taken up by the liver and, under normolipidemic conditions, TG is either oxidized or secreted as VLDL. However, excessive accumulation of hepatic fat unrelated to alcohol consumption [i.e., hepatic steatosis or nonalcoholic fatty liver disease (NAFLD)] is the most frequent liver disease in industrialized countries (7). Moreover, the development of this disease often parallels that of insulin resistance and is associated with obesity, metabolic syndrome, dyslipidemia, and type 2 diabetes (8, 9), and has been linked to mitochondrial malfunction (10). Mitochondria are active in β-oxidation of FAs, where carnitine-mediated FA transport is involved in the generation of metabolic energy from long-chain SFAs. Plasma concentrations of specific acylcarnitines are used to screen for mitochondrial FA β-oxidation disorders in newborns (11, 12), and reflect altered postprandial FA oxidation in type 2 diabetes (13). Situations with an excess uptake of lipids by the liver require an adaptive increase in mitochondrial oxidative capacity. Under these conditions, mitochondrial dysfunction will compromise metabolic performance in association with increased reactive oxygen species (ROS) generation. Hence, mitochondrial adaptations are important to protect against harmful effects of diet-induced oxidative stress, inflammation, and intrahepatic accumulation of lipids (14).

Plasma and hepatic TG concentrations are determined by a balance between hepatic uptake of FAs, hepatic lipogenesis, TG biosynthesis, and mitochondrial FA oxidation and secretion, on one hand, and plasma clearance on the other. The objective of the present study was to determine the effect of feeding isocaloric HC or HF diets on the concentrations of plasma and hepatic TG, lipid metabolism pathways, FA composition, and hepatic energy status, including measurement of plasma concentrations of choline, betaine, FA subtypes, and carnitine metabolites, using male Wistar rats at an early age.

Methods

The animal study was conducted according to the Guidelines for the Care and Use of Experimental Animals, and in accordance with the Norwegian legislation and regulations governing experiments using live animals. The Norwegian Food Safety Authority approved the protocol (permit number 2015–7367). All efforts were made to optimize the animal environment and minimize suffering. Male Wistar rats, 5 wk old, were purchased from Taconic (Ejby, Denmark). Upon arrival, the rats were labeled and placed in open cages, 4 in each cage, where they were allowed to acclimatize to their surroundings for 1 wk. During the acclimatization and experiment period, the rats had unrestricted access to feed pellets and tap water. The rats were kept in a 12-h light/dark cycle at a constant temperature (22° ± 2°C) and a relative humidity of 55% (±5%). Upon start of the experiment, the rat cages were block randomized to their respective interventions using Research Randomizer. During the experiment, there were 4 rats in each cage separated with a divider, which let them have sniffing contact. Diets were prepared in-house by mixing individual ingredients (Dyets, Inc.) (Table 1). The HC group (n = 8) received a diet with 18.5% of energy (E%) protein, 15.8 E% fat, and 65.7 E% carbohydrate; and the HF group (n = 8) received a diet with 18.2 E% protein, 66.8 E% fat, and 15.0 E% carbohydrate (Table 1). All rats were weighed daily and feed intake was determined weekly.

TABLE 1 Nutrient composition (E% and g/kg) of the HC and HF experimental diets[1]

	HC	HF	Catalog number[2]
Fat, E%	15.8	66.8	
Soy oil, g	19.4	26.4	104687
Lard, g	48.6	370	402400
Carbohydrate, E%	65.7	15.0	
Cornstarch, g	386	106	401200
Dyetrose, g	128.2	34.4	401477
Sucrose, g	97.1	26.4	404400
Fiber, g	48.6	66.1	401850
Protein, E%	18.5	18.2	
Casein, g	223	304	400600
Micronutrients, E%	0	0	
AIN-93G-MX mineral mix, g	34.0	46.3	210025
AIN-93-VX vitamin mix, g	9.7	13.2	310025
L-Cysteine, g	2.9	4.0	401340
Choline bitartrate, g	2.4	3.3	400750
tert-Butyl hydroquinone, g	0.014	0.019	
Total, g	1000	1000	

[1] E%, % of energy; HC, high-carbohydrate, low-fat diet; HF, low-carbohydrate, high-fat diet.
[2] Product from Dyets Inc.

At sacrifice, rats were anesthetized by inhalation of 5% isoflurane (Schering-Plough). EDTA-blood was collected by cardiac puncture and immediately placed on ice. The plasma samples were centrifuged at 3100 rpm (2074 x g) for 15 min at 4°C, and liver was collected, weighed, and snap-frozen in liquid nitrogen before storage at −80°C until analysis.

Quantification of plasma and liver lipids

Liver lipids were extracted from frozen samples according to Bligh and Dyer (15), evaporated under nitrogen, and redissolved in isopropanol before analysis. Lipids from liver and plasma were measured enzymatically on a Hitachi 917 system (Roche Diagnostics GmbH) using the TG (Triglycerides GPO-PAP, 11,730,711) kit from Roche Diagnostics and the free cholesterol (Free Cholesterol FS, ref 113,609,910,930), nonesterified fatty acid (NEFA FS, ref 157,819,910,935), and phospholipid kit (Phospholipids FS, ref 157,419,910,930) from DiaSys (Diagnostic Systems GmbH). FA composition in plasma and liver extract was measured by GC-LC as described previously (16).

Calculation of double bond index

To measure the degree of FA unsaturation of the FA pool, the double bond index (DBI) was calculated by summing the concentration of FAs with 1 double bond, FAs with 2 double bonds multiplied by 2, FAs with 3 double bonds multiplied by 3, FAs with 4 double bonds multiplied by 4, FAs with 5 double bonds multiplied by 5, and FAs with 6 double bonds multiplied by 6, divided by the total concentration of FAs.

The project was funded by the University of Bergen and the Department of Heart Disease, Haukeland University Hospital, Bergen, Norway.
Author disclosures: The authors report no conflicts of interest.
Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
SND and BB contributed equally.
Address correspondence to SND (e-mail: simon.dankel@uib.no) or RKB (e-mail: rolf.berge@uib.no).
Abbreviations used: ACC, acetyl-CoA carboxylase; CS, citrate synthase; DBI, double bond index; FA, fatty acid; FAS, fatty acid synthase; FFA, free fatty acid; E%, energy percentage of total energy intake; HC, high-carbohydrate, low-fat diet; HF, high-fat low-carbohydrate diet; *Lipc*, lipase C; MLYCD, malonyl-CoA decarboxylase; *Mttp*, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; SCD, stearoyl-CoA desaturate; TG, triacylglycerol; *Ucp*, uncoupling protein.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

Quantification of carnitine metabolites and ketone body in plasma

L-Carnitine, trimethyllysine, γ-butyrobetaine, and acetylcarnitine were analyzed in plasma by HPLC-MS/MS, as described by Vernez et al. (17) with some modifications (18). The ketone body D-β-hydroxybutyrate was analyzed in plasma by the Colorimetric Assay Kit from Cayman Chemical Company (item no. 700,190).

Measurement of high-energy phosphates, hypoxanthine, and xanthine by HPLC

Freeze-clamped biopsies were taken from the liver and stored in liquid nitrogen prior to analysis. Sample preparation and measurement of high-energy phosphates (ATP, ADP, AMP), hypoxanthine, and xanthine by HPLC were performed as previously described (19, 20). Energy charge was calculated using the following formula: energy charge = (ATP + 0.5 ADP)/(AMP + ADP + ATP) and protein was determined by the Bicinchoninic Acid (BCA) method (BCA Protein Assay; Pierce).

Enzyme activities

Post-nuclear fractions from 100 mg homogenized liver tissue from each rat were prepared as described previously (21), and the amount of proteins was measured by BioRad protein assay kit (Bio-Rad Laboratories). The FA synthase (FAS) activity and the activities of citrate synthase (CS) and malonyl-CoA decarboxylase (MLYCD) were measured as previously described (22, 23).

Hepatic gene expression analysis

Tissue samples (20 mg frozen liver) were homogenized in RNeasy Lysis Buffer from Qiagen (catalog: 79,216) with 1% β-mercaptoethanol using Tissuelyser II (Qiagen) for 2 × 2 min at 25 Hz, and total cellular RNA was further purified using the RNeasy mini kit (Qiagen) including DNase digestion. cDNA was produced from 500 ng RNA using the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems). RT-PCR was performed on Sarstedt 384-well Multiply-PCR plates (Sarstedt, Inc.) using an ABI Prism 7900HT Sequence detection system from Applied Biosystems with the software SDS 2.3. Primers and probes to detect mRNA levels of genes of interest (**Supplemental Table 1**) were mixed with 2× Taqman buffer (Applied Biosystems). A standard curve using either an appropriate cDNA sample or universal rat reference RNA was performed for each probe. Ribosomal protein L14 (*Rpl4*) was used as a reference gene.

Statistical analysis

Data were analyzed using Prism Software (Graph-Pad Software) to determine statistical significance. The results are shown as means with SDs of 8 rats per group. Student's *t* test was used to evaluate statistical differences between the intervention groups. Spearman correlation coefficients were used when comparing 2 independent variables. *P* values < 0.05 were considered statistically significant.

Results

Effect on food intake, body and liver weights, hormones, and plasma glucose and insulin

During the 4-wk intervention, the estimated mean energy intake per rat per day was 1.2-fold in rats fed the HC compared with the HF diet (**Figure 1**A). Weight gain, liver weight, and plasma concentrations of appetite hormones [leptin, pancreatic polypeptide (PP), and amylin] were similar in rats fed either diet (Figure 1B–D). Circulating concentrations of gastric inhibitory peptide (GIP) were, however, 2.5-fold in the HC compared with the HF group (Figure 1D). There were no significant differences in plasma insulin, glucose, and insulin-glucose ratio with the HC compared with the HF diet, although glucose tended to be lower in the HF group ($P = 0.072$) (Figure 1E).

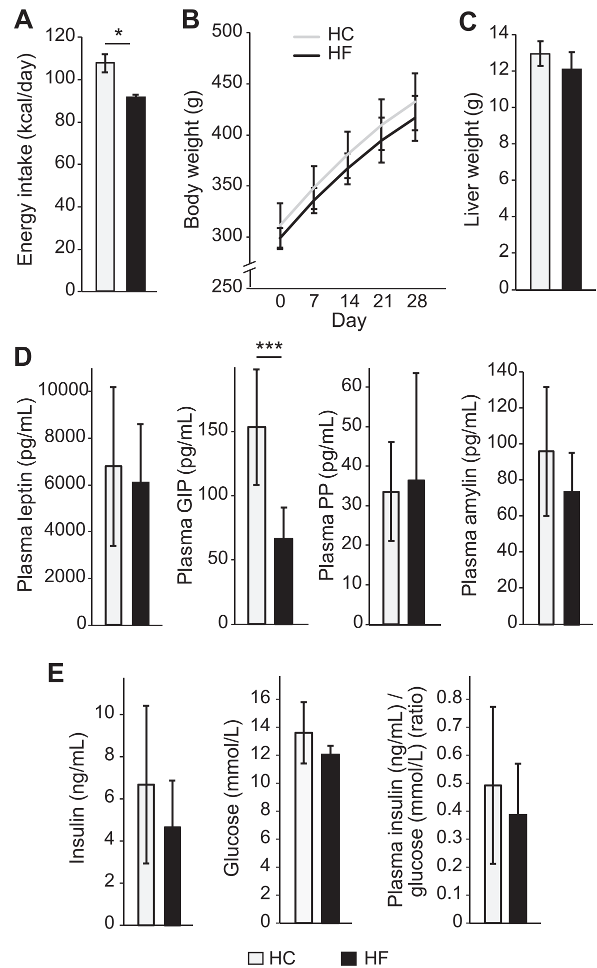

FIGURE 1 Changes in measures related to adiposity and glucose metabolism on HC and HF diets in male Wistar rats fed for 4 wk. (A) Mean caloric intake per day throughout the diet period. (B) Accumulated body-weight gain throughout the study period. (C) Liver weight after sacrifice. (D, E) Hormones and glucose measured in plasma at the end of the study period. Values are mean ± SDs, $n = 8$ per group, *Denotes group difference, $P < 0.05$. ***Denotes group difference, $P < 0.001$. GIP, gastric inhibitory protein; HC, high-carbohydrate, low-fat diet; HF, low-carbohydrate, high-fat diet; PP, pancreatic polypeptide.

Effect on plasma and liver lipids

The plasma TG concentration was >2-fold higher in the HC compared with HF-fed rats (**Figure 2**A). On the other hand, the hepatic TG concentration was almost 4-fold higher in HF compared with HC, and this was accompanied by almost twice as high hepatic concentrations of cholesterol and free FAs (FFAs) (Figure 2B). It is noteworthy that the plasma cholesterol and FFA concentrations were similar for the 2 experimental groups (Figure 2C). Moreover, the phospholipid concentrations in both plasma and liver were similar in rats fed HC and HF diets (Figure 2D). These results suggest that the carbohydrate in the HC diet is converted into fat that is distributed in the bloodstream as TG. Indeed, hepatic gene expression levels of microsomal triglyceride transfer protein (*Mttp*) were significantly increased in rats fed the HC diet compared with the HF diet (Figure 2E). Also, mRNA for hepatic lipase C (*Lipc*) was higher in the HC group (Figure 2E), suggesting a concomitant increase in hepatic lipoprotein uptake.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

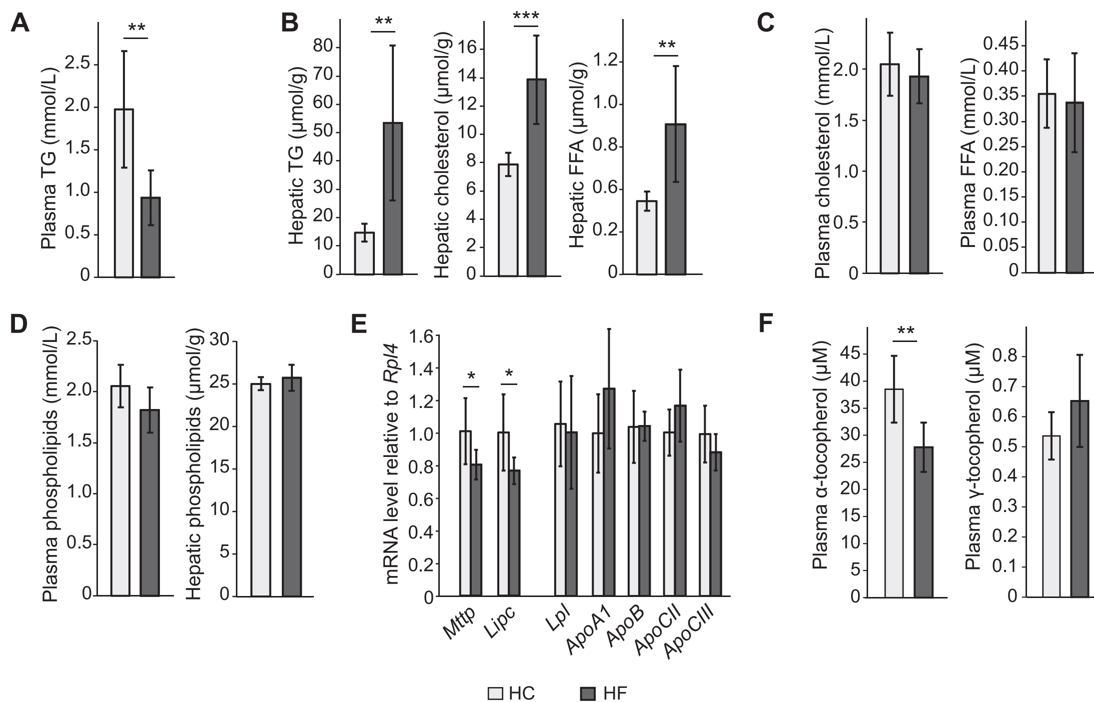

FIGURE 2 Plasma and hepatic lipid concentrations and related hepatic gene expression after 4-wk HC or HF feeding of male Wistar rats. (A–D) Plasma and/or hepatic TG, cholesterol, FFAs, and phospholipids were measured as indicated. (E) mRNA expression was measured in liver tissue by qPCR, normalized to *Rpl4* mRNA. (F) Vitamin E (α- and γ-tocopherols) was measured in plasma. Values are means ± SDs, $n = 8$ per group, *Denotes group difference, $P < 0.05$. **Denotes group difference, $P < 0.01$. ***Denotes group difference, $P < 0.001$. FFA, free fatty acid; HC, high-carbohydrate, low-fat diet; HF, low-carbohydrate, high-fat diet; *Lipc*, hepatic lipase C; *Lpl*, lipoprotein lipase; *Mttp*, microsomal triglyceride transfer protein; *Rpl14*, Ribosomal protein L14; TG, triacylglycerol.

Vitamin E is associated with VLDL secretion, and the plasma concentration of α-tocopherol was lower in the HF group, reflecting lower plasma TG compared with the HC group (Figure 2F). γ-Tocopherol and the hepatic gene expression of lipoprotein lipase (*Lpl*), *ApoB*, *ApoA1*, *ApoCII*, and *ApoCIII* remained constant between the 2 experimental groups (Figure 2E, F).

Hepatic lipogenesis, TG biosynthesis, and estimated desaturation and elongation indexes from FA composition in liver and plasma

The differential effects of the HC and HF diets on hepatic TG concentrations, although the plasma glucose concentration on the HC diet was marginally affected, prompted us to investigate hepatic FA synthesis, TG biosynthesis, and FA composition. Interestingly, hepatic fatty acid synthase (FAS) activity in HC-fed rats was almost 8-fold that in the HF-fed rats (**Figure 3**A), accompanied with twice as high expression of fatty acid synthase (*Fasn*) mRNA (Figure 3B). Moreover, the gene expression of the rate-limiting enzyme in lipogenesis, acetyl-CoA carboxylase (*Acaca/Acc*), was almost twice as high in HC- compared with HF-fed rats (Figure 3B), accompanied with >4-fold the enzyme activity (Figure 3A). Notably, the liver enzyme activities of citrate synthase (CS) and malonyl-CoA decarboxylase (MLYCD) were unchanged (Figure 3A), and gene expressions of diacylglycerol O-acyltransferase 1 (*Dgat1*), *Dagt2*, and mitochondrial glycerol-3-phosphate acyltransferase (*Gpam*) were also similar after HC and HF feeding (Figure 3B).

The hepatic concentration of total FAs was close to twice as high in HF- compared with HC-fed rats (Figure 3C). Although the hepatic proportions of SFAs (Figure 3C) and 18:0 were similar, there were significantly higher proportions of the individual SFAs capric (10:0), lauric (12:0), myristic (14:0), pentadecyclic (15:0), and palmitic (16:0) acid in HC- compared with HF-fed rats (**Table 2**). The proportions of MUFAs (Figure 3C) and *cis*-7 hexadecenoic acid (16:1n–9) in the liver (Table 2) were not different between groups, but proportions of the individual FAs palmitoleic acid (16:1n–7) and *cis*-vaccenic acid (18:1n–7) were 7- and 2.6-fold in HC- to that in HF-fed rats (Table 2). Notably, the relative amount of hepatic oleic acid (18:1n–9), the most abundant individual FA, was 67% higher in HF-fed rats (Table 2). The estimated activity of stearoyl-CoA desaturate (SCD)-16 (16:1n–7/16:0) in liver was 6.5-fold after HC vs. HF feeding (Figure 3D). A positive correlation with hepatic TG was seen for SCD-16 activity in the HF-fed rats (rho = 0.655, $P = 0.08$) but not in the HC-fed rats (rho = −0.253) (data not shown). The estimated SCD-18 activity (18:1n–9/18:0) in liver was 66% higher in HF-fed rats (Figure 3D), and showed a stronger positive correlation with hepatic TG concentrations after HF (rho = 0.952, $P < 0.001$) than after HC (rho = 0.714, $P = 0.046$) feeding (Figure 3E).

HC feeding led to higher plasma total FA concentrations compared with HF feeding, and the total FAs correlated to plasma TG concentrations in both groups (Spearman's rho >0.95, $P < 0.001$) (Figure 3F). The proportion of plasma SFAs, in contrast to liver, was lower in HC-fed rats (Figure 3G), which can be attributed to lower concentrations of 18:0 (Table 2). On the other hand, the proportion of MUFAs was higher in the HC group (Figure 3G), which is reflected by the higher proportions of oleic acid (18:1n–9) (1.4-fold), 7-hexadecenoic acid (16:1n–9) (2-fold), vaccenic acid (18:1n–7) (2.8-fold), and palmitoleic acid (16:1n–7) (7.5-fold) (Table 2). A marginal difference was observed for the proportion of palmitic acid, lauric acid and 20–24:0, whereas the proportions of myristic and pentadecyclic

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

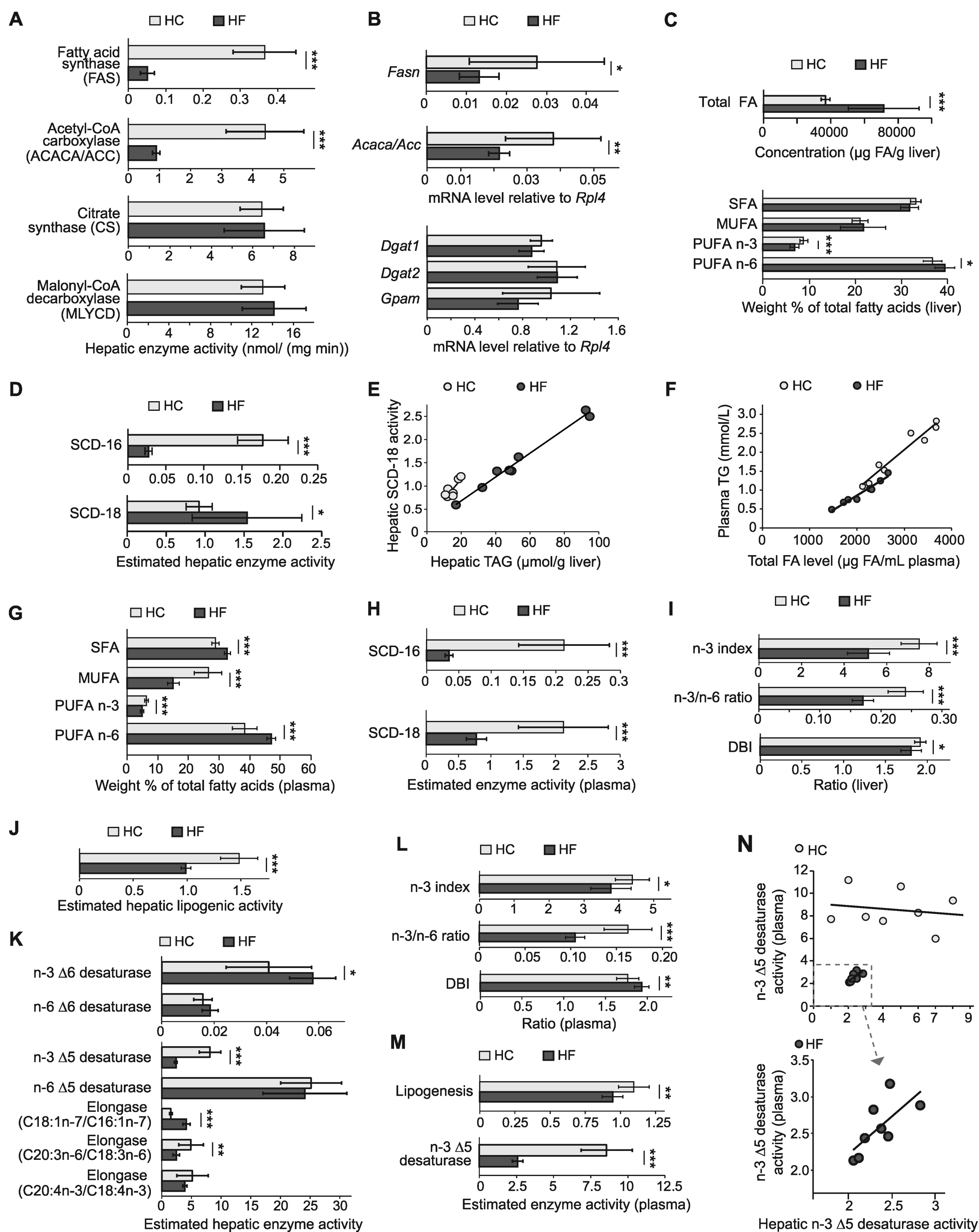

FIGURE 3 Plasma and hepatic enzyme activities, lipid composition, and related hepatic gene expression in male Wistar rats fed HC or HF diets for 4 wk. (A) Hepatic enzyme activities were measured in post-nuclear fractions isolated from homogenized liver tissue, normalized to amount of total protein. (B) mRNA expression was measured in liver tissue by qPCR, normalized to *Rpl4* mRNA. (C) Relative hepatic concentrations of main FA classes expressed as weight % of total FAs in the liver. (D, E) Estimated hepatic stearoyl-CoA dehydrogenase activity based on the hepatic ratios of 16:1n–7/16:0 and 18:1n–9/18:0, and correlation with hepatic TG. (F) Concentrations and relation of TG and total FAs in plasma. (G) Relative concentrations in plasma of main FA classes expressed as weight % of total FAs in plasma. (H) Estimated plasma stearoyl-CoA dehydrogenase (SCD) activity based on the circulating ratios of 16:1n–7/16:0 and 18:1n–9/18:0. (I–N) n–3 and n–6 FA concentrations and DBI in the liver (I) and plasma (L), and estimated enzyme activity related to lipogenesis and FA desaturation and elongation measured in the liver (J, K, N) and plasma (L, M) based on ratios of FA concentrations. Values are means ± SDs, $n = 8$ per group, *Denotes group difference, $P < 0.05$. *Acaca/Acc*, acetyl-CoA carboxylase; DBI, double bond index; *Dgat*, diacylglycerol O-acyltransferase; FA, fatty acid; *Fasn*, fatty acid synthase; *Gpam*, mitochondrial glycerol-3-phosphate acyltransferase; HC, high-carbohydrate, low-fat diet; HF, low-carbohydrate, high-fat diet; TG, triacylglycerol.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

TABLE 2 Fatty acid composition of plasma and liver (g/100 g total fatty acids) after 4-wk feeding of male Wistar rats with HC and HF diets[1]

	Plasma (% of total fatty acids)				Liver (% of total fatty acids)			
	HC	HF	HC:HF	Group difference *P* value	HC	HF	HC:HF	Group difference *P* value
10:0	0.009 (0.002)	0.007 (0.002)	1.32	0.064	0.006 (0.001)	0.004 (0.001)	1.56	0.007
12:0	0.027 (0.007)	0.02 (0.005)	1.35	0.051	0.013 (0.003)	0.005 (0.001)	2.41	<0.001
14:1n–5	0.035 (0.018)	0.004 (0.001)	8.32	<0.001	0.03 (0.008)	0.002 (0.001)	15.0	<0.001
14:0	0.55 (0.126)	0.28 (0.061)	1.95	<0.001	0.45 (0.068)	0.24 (0.034)	1.83	<0.001
15:0	0.18 (0.031)	0.1 (0.010)	1.77	<0.001	0.12 (0.023)	0.09 (0.007)	1.35	0.002
16:1n–9	0.3 (0.085)	0.15 (0.024)	2.01	<0.001	0.23 (0.037)	0.24 (0.045)	0.97	0.695
16:1n–7	3.97 (1.41)	0.55 (0.112)	7.27	<0.001	3.34 (0.740)	0.47 (0.104)	7.05	<0.001
16:1n–7t	0.017 (0.005)	0.011 (0.003)	1.59	0.007	0.014 (0.002)	0.027 (0.005)	0.53	<0.001
16:1	0.06 (0.021)	0.016 (0.002)	3.80	<0.001	0.06 (0.015)	0.02 (0.003)	3.06	<0.001
16:0	18.5 (0.604)	15.6 (0.737)	1.19	<0.001	18.7 (0.787)	17.5 (1.20)	1.07	0.027
17:0	0.17 (0.023)	0.23 (0.006)	0.73	<0.001	0.2 (0.020)	0.23 (0.006)	0.88	0.002
18:3n–6	0.32 (0.069)	0.2 (0.012)	1.59	<0.001	0.2 (0.050)	0.33 (0.062)	0.62	0.001
18:4n–3	0.042 (0.015)	0.013 (0.002)	3.23	<0.001	0.018 (0.008)	0.029 (0.004)	0.64	0.008
18:2n–6	17.1 (1.73)	16.6 (1.68)	1.03	0.626	12.7 (1.16)	17.7 (1.62)	0.72	<0.001
18:3n–3	0.86 (0.185)	0.41 (0.107)	2.11	<0.001	0.44 (0.077)	0.5 (0.099)	0.87	0.166
18:1n–9	17 (2.63)	12 (1.59)	1.42	<0.001	11.6 (1.506)	18.3 (4.465)	0.64	0.001
18:1n–7	4.22 (0.743)	1.55 (0.186)	2.71	<0.001	4.88 (0.584)	1.91 (0.207)	2.56	<0.001
18:1t	0.088 (0.013)	0.096 (0.011)	0.91	0.165	0.093 (0.016)	0.092 (0.003)	1.01	0.878
18:1	0.14 (0.015)	0.098 (0.012)	1.44	<0.001	0.17 (0.020)	0.097 (0.005)	1.73	<0.001
18:0	8.43 (1.55)	15.7 (1.32)	0.54	<0.001	12.7 (1.01)	13 (2.92)	0.98	0.786
20:4n–6	19.3 (4.63)	28.1 (2.56)	0.69	<0.001	22 (1.61)	17.9 (4.04)	1.23	0.020
20:5n–3	0.87 (0.163)	0.27 (0.029)	3.27	<0.001	0.62 (0.154)	0.26 (0.021)	2.43	<0.001
20:3n–9	0.23 (0.029)	0.24 (0.022)	0.95	0.352	0.21 (0.028)	0.27 (0.034)	0.77	0.001
20:3n–6	0.7 (0.076)	0.55 (0.068)	1.27	0.001	0.9 (0.150)	0.76 (0.097)	1.18	0.048
20:4n–3	0.1 (0.011)	0.1 (0.009)	0.98	0.704	0.077 (0.006)	0.11 (0.009)	0.70	<0.001
20:2n–6	0.32 (0.033)	0.34 (0.030)	0.93	0.174	0.31 (0.035)	0.56 (0.072)	0.55	<0.001
20:1n–11	0.031 (0.007)	0.03 (0.005)	1.05	0.304	0.034 (0.006)	0.05 (0.006)	0.69	<0.001
20:1n–9	0.2 (0.031)	0.2 (0.030)	1.00	0.992	0.16 (0.015)	0.37 (0.093)	0.44	<0.001
20:1n–7	0.25 (0.052)	0.17 (0.076)	1.45	0.032	0.11 (0.016)	0.1 (0.020)	1.12	0.222
20:0	0.063 (0.01)	0.08 (0.008)	0.79	0.002	0.048 (0.005)	0.054 (0.005)	0.90	0.038
21:5n–3	0.016 (0.007)	0.004 (0.001)	4.09	<0.001	0.006 (0.002)	0.007 (0.002)	0.88	0.416
22:5n–6	0.21 (0.053)	0.48 (0.176)	0.43	0.001	0.31 (0.048)	0.76 (0.231)	0.41	<0.001
22:6n–3	3.53 (0.444)	3.57 (0.598)	0.99	0.886	6.89 (0.741)	4.89 (0.999)	1.41	<0.001
22:4n–6	0.35 (0.055)	0.71 (0.144)	0.48	<0.001	0.35 (0.036)	1.42 (0.28)	0.25	<0.001
22:5n–3	0.74 (0.156)	0.56 (0.050)	1.33	0.007	0.76 (0.148)	0.96 (0.106)	0.80	0.009
22:2n–6	0.019 (0.003)	0.015 (0.004)	1.26	0.026	0.007 (0.001)	0.01 (0.002)	0.69	<0.001
22:1n–11	0.002 (0.0001)	0.001 (0.0003)	1.83	<0.001	0.001 (0.0003)	0.001 (0.0005)	0.63	0.115
22:1n–9	0.013 (0.001)	0.015 (0.002)	0.89	0.036	0.082 (0.021)	0.054 (0.015)	1.50	0.010
22:1n–7	0.039 (0.007)	0.03 (0.008)	1.31	0.027	0.014 (0.002)	0.008 (0.002)	1.79	<0.001
22:0	0.11 (0.01)	0.14 (0.008)	0.76	<0.001	0.12 (0.014)	0.09 (0.028)	1.30	0.028
23:0	0.079 (0.01)	0.11 (0.013)	0.74	<0.001	0.096 (0.010)	0.077 (0.021)	1.26	0.031
24:1n–9	0.23 (0.05)	0.22 (0.030)	1.07	0.481	0.19 (0.025)	0.08 (0.023)	2.44	<0.001
24:0	0.24 (0.024)	0.28 (0.021)	0.89	0.017	0.35 (0.043)	0.21 (0.066)	1.66	<0.001

[1]Values are means (±SD), $n = 8$. HC, high-carbohydrate, low-fat diet; HF, high-fat, low-carbohydrate diet.

acid were close to twice as high in the HC group (Table 2). As observed in liver, the estimated SCD-16 activity in plasma was 6-fold in rats fed HC compared with the HF diet (Figure 3H). The estimated SCD-18 activity in plasma was also higher after HC feeding (2.7-fold) (Figure 3H), which was opposite to that observed in liver (Figure 3D).

Compared with HF, HC feeding resulted in a higher proportion of hepatic omega-3 (n–3) PUFAs (Figure 3C) and individual n–3 FAs [e.g., EPA (20:5n–3) and DHA (20:6n–3)] (Table 2). This is reflected by a 50% higher hepatic n–3 index in the HC group (Figure 3I), but the proportion of hepatic α-linolenic acid (18:3n–3) was similar (Table 2). The proportions of hepatic n–6 PUFAs (Figure 3C) and the individual FAs gamma-linolenic acid (18:3n–6) and linoleic acid (18:2n–6) (Table 2) were, however, significantly lower in the HC group. This was reflected by a higher hepatic n–3 to n–6 ratio and DBI (which reflects the degree of unsaturation of the FAs) (Figure 3I) as well as 1.5-fold the estimated lipogenic activity (16:0/18:2n–6) in the HC compared with the HF group (Figure 3J). The estimated hepatic activity of n–3 $\Delta 6$ desaturase was lower with the HC diet, whereas that of n–3 $\Delta 5$ desaturase was markedly higher with the HC diet (Figure 3K). The n–6 $\Delta 5$ desaturase and

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

Δ6 desaturase were not significantly affected (Figure 3K). The estimated activity of hepatic elongase given by 18:1n–7/16:1n–7 was markedly lower in rats fed the HC compared with the HF diet, whereas hepatic elongase given as 20:3n–6/18:3n–6 was higher in the HC group (Figure 3K). The estimated activity of n–3 elongase measured as 20:4n–3/18:4n–3 was similar between the 2 groups (Figure 3K).

In agreement with changes in the liver, the proportions of plasma n–3 PUFAs (Figure 3G) and of the individual FAs EPA and eicosatetraenoic acid (18:4n–3), but not DHA, were significantly higher in plasma of rats fed the HC diet (Table 2), and accordingly the n–3 index was also significantly higher (Figure 3L). Moreover, the plasma concentrations of n–6 PUFAs were lower in HC-treated rats (Figure 3G), mostly due to lower arachidonic acid (20:4n–6) (Table 2), resulting in a higher plasma n–3 to n–6 ratio (Figure 3L) and a higher estimated plasma lipogenic activity (Figure 3M). The proportion of plasma gamma-linolenic acid was higher in rats fed the HC diet, whereas that of linoleic acid was similar compared with the HF diet (Table 2). As observed for the liver, the estimated n–3 Δ5 desaturase activity based on plasma values was markedly higher with the HC compared with the HF diet (Figure 3M). Notably, the estimated plasma and hepatic n–3 Δ5 desaturase activities showed a positive correlation only in the HF group (rho = 0.881, $P = 0.004$) (Figure 3N).

Overall, the observed differences in hepatic and plasma FA composition between groups could not readily be ascribed to different FA concentrations in the diets, shown by clearly divergent concentrations of most individual FAs in feed relative to plasma and liver (e.g., on average, close to 10-fold the content of individual FAs in the HF vs. HC feed but 65% the FA content in plasma and merely double the FA content in liver of HF- compared with HC-fed rats) (**Supplemental Table 2**). Taken together, our data point to potential markers of a dietary carbohydrate–dependent increase in plasma TG and total FAs, including plasma concentrations of myristic, pentadecyclic, palmitoleic and *cis*-vaccenic acid, EPA and DPA (22:5n–6), and related estimates of SCD-16 activity, n–7 elongase index, n–3 Δ5 desaturase index and de novo lipogenesis.

Mitochondrial function and FA oxidation

Carnitine is involved in the generation of metabolic energy from long-chain FAs, by mediating their transport across the mitochondrial membrane. The concentrations of plasma carnitine and its precursors γ-butyrobetaine and trimethyllysine were similar in rats fed HC and HF diets (**Figure 4**A). Moreover, concentrations of short-chain acylcarnitines (acetylcarnitine, propionylcarnitine, and valeroylcarnitine), medium acylcarnitine (octanoylcarnitine), and long-chain acylcarnitines (lauroylcarnitine, myristoylcarnitine, palmitoylcarnitine) were also similar in the diet groups (Figure 4A). The ratio of acetylcarnitine to palmitoylcarnitine (AC2/AC16) could be a biomarker of mitochondrial FA oxidation, as blocking FA oxidation was associated with reduced plasma AC2 concentrations in mice (23). The AC2 to AC16 ratio was also similar in rats fed HC and HF diets (Figure 4B). These results suggest that, in the 2 experimental groups, there were no differences in *1*) biosynthesis of carnitine and/or increased consumption or *2*) mitochondrial FA oxidation and/or biogenesis. Hepatic gene expression data further support this conclusion, as there were no differences in carnitine palmitoyltransferase 1 (*Cpt1*), *Cpt2*, peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Ppargc1a*), mitochondrial transcription factor A, (*Tfam*), and cytochrome C (*Cycs*) mRNA (Figure 4C). Although gene expression of 3-hydroxy-3-methylglutaryl-CoA synthase (*Hmgcs2*) was significantly lower in the HF compared with the HC group, hepatic enzyme activities of CPT2 and HMGCS2 were similar between the 2 groups (data not shown), suggesting that there was no difference in mitochondrial FA oxidation capacity. A similar degree of FA oxidation was also supported by the similar hepatic gene expression of fatty acid binding protein (*Fabp1*), fatty acid transporter (*Fatp1*), *Pltp*, *Cd36*, and uncoupling protein 2 (*Ucp2*) (Figure 4C), and no differences were observed for hepatic energy status measured as ATP, ADP, and AMP concentrations, energy charge, and AMP to ATP and ATP to AMP ratios (Figure 4D). Furthermore, the hepatic content of the adenine degradation products xanthine and hypoxanthine was similar in the HC- and HF-fed rats (Figure 4E). Finally, peroxisomes are also organelles involved in FA chain shortening and FA oxidation. The gene expression of the rate-limiting enzyme in peroxisomal FA oxidation, acyl-CoA oxidase (*Acox1*), was similar in the rats fed HC and HF diets (Figure 4C).

Discussion

In the present study we investigated mechanisms involved in the TG-raising effect of dietary carbohydrates, by comparing high- and low-carbohydrate isocaloric diets in Wistar rats. Results after 4 wk of feeding showed that the differential effects were greater on lipid metabolism than on glucose homeostasis, reflected by differences in plasma and liver FA composition as well as the activities and expression of Δ5 desaturase (D5D), Δ6 desaturate (D6D), Δ9 desaturase (stearoyl-CoA desaturase), and estimated de novo lipogenesis. Because no differences were seen in mitochondrial FA oxidation, our data indicate that the HC diet raised circulating TG via lipogenic pathways in the liver, involving production of specific FAs. The data provide new detailed insight into the relations between hepatic lipid metabolism and circulating TG and FAs in response to dietary carbohydrates and fats, as summarized in **Figure 5**.

The most striking finding after 4 wk of feeding was, however, a twice as high plasma concentration of TG in HC- compared with HF-fed rats. Previous lower-carbohydrate, high-fat studies in rats show conflicting results with regard to lowering plasma TG (24), whereas this effect is well documented in human studies (6) and was also seen in the rat study by Ferramosca et al. (25). Different mechanisms have been proposed by which dietary carbohydrates increase circulating TG, including reduced TG clearance and increased hepatic de novo lipogenesis (26). The present study supports that carbohydrate in the HC diet was converted into fat and distributed in the bloodstream as TG and specific fatty acids. The activities and expression of FAS and ACC were severalfold higher in the HC-fed compared with the HF-fed rats, along with higher hepatic expression of *Mttp*, and lower plasma α-tocopherol in HF-fed rats. Furthermore, the data substantiate the role of the liver in synthesizing and subsequently distributing specific lipid species to other tissues via the circulation as VLDL-TG, especially in response to HC feeding.

Cellular uptake of lipoproteins and increased mitochondrial activity and β-oxidation of FAs have been implicated in regulation of plasma TG concentrations in rats (27). However, except for *Lipc* and *Hmgcs2*, we found no significant differences between HC- and HF-fed rats in hepatic expression levels of *Lpl*, *ApoB*, *ApoA1*, *ApoCII*, *ApoCIII*, *Cpt1*, *Cpt2*, and *Ucp2*; energy status; concentrations of ATP, ADP, and AMP; ratios

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

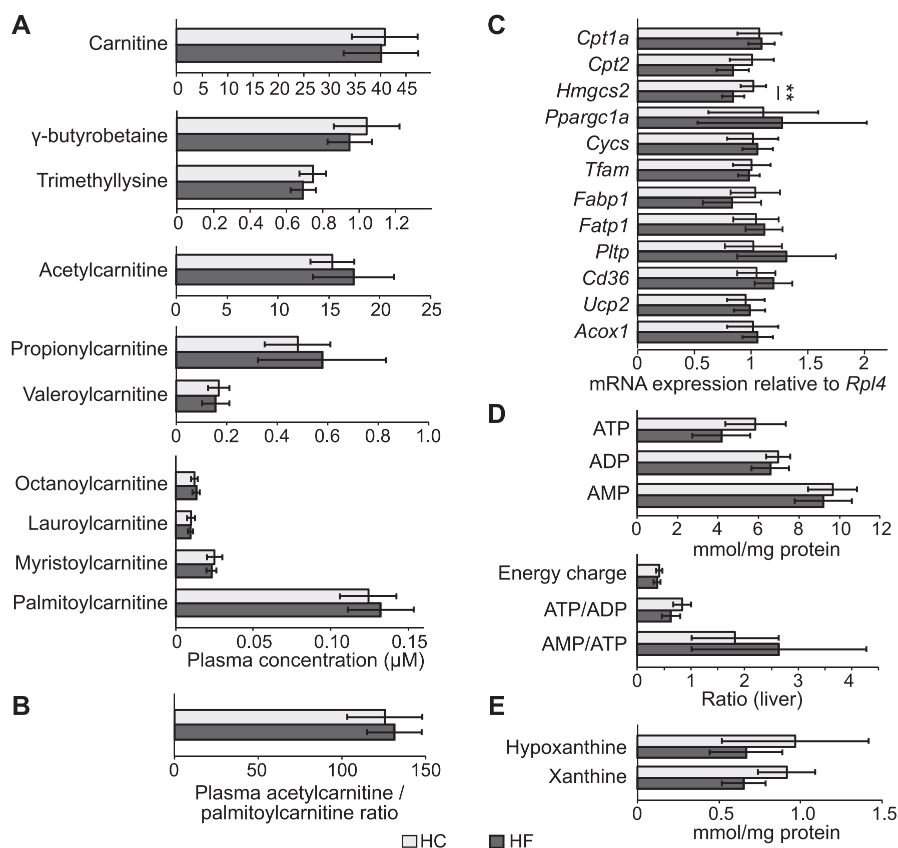

FIGURE 4 Circulating concentrations of carnitines and hepatic mRNA levels and energy status after 4 wk of HC or HF feeding in male Wistar rats. (A, B) Carnitine, carnitine precursors, and acylcarnitines measured in plasma. (C) mRNA expression was measured in liver tissue by qPCR, normalized to *Rpl4* mRNA. (D, E) Adenosine phosphate concentrations (D) and their degradation products (E) in the liver. Energy charge was calculated by the formula (ATP + 0.5 ADP)/(AMP + ADP + ATP). Values are means ± SDs, $n = 8$ per group, **Denote s group difference, $P < 0.05$. *Cpt*, carnitine palmitoyltransferase; *Cycs*, cytochrome C (somatic); *Fabp1*, fatty acid binding protein; HC, high-carbohydrate, low-fat diet; HF, low-carbohydrate, high-fat diet; *Hmgcs2*, 3-hydroxy-3-methylglutaryl-CoA synthase; *Ppargc1a*, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; *Tfam*, mitochondrial transcription factor A.

of AMP to ATP and ATP to AMP; concentrations of plasma carnitine as well as its precursors (short-, medium- and long-chain acylcarnitine); or the acetylcarnitine to palmitoylcarnitine ratio. Moreover, mitochondrial biogenesis and peroxisomal FA oxidation were similar between the groups. These data indicate that changes in mitochondrial activity and FA β-oxidation did not contribute substantially to the differences in circulating TG concentrations between the 2 diet groups. A recent study found that intake of fructose and not glucose decreases hepatic fatty oxidation and mitochondrial activity (28). Carbohydrates from other sources than fructose might therefore explain the lack of effect of the HC diet on measures of FA oxidation and energy status in the present study.

Although total feed intake differed slightly between the HC and HF diets, the caloric intake was similar due to a higher energy density in the HF diet, resulting in similar weight gain and changes in appetite hormones. In this controlled dietary context, we observed no clear difference in fasting plasma insulin, glucose, and the insulin to glucose ratio in the HC-fed compared with HF-fed rats, albeit a tendency of lower glucose tolerance after HC feeding. Other studies have reported a modest difference in these fasting parameters when comparing HC and HF diets in rats (25), and that insulin resistance is associated with elevated hepatic TG synthesis in humans (29). Indeed, hyperinsulinemia is a strong inducer of hepatic lipogenesis in rats (30), and higher postprandial as well as fasting insulin due to the higher carbohydrate intake might have contributed to the higher circulating TG concentrations after HC feeding. We also cannot rule out an influence of differences in hepatic insulin sensitivity in response to the HC and HF feeding.

While HC feeding showed higher plasma TG and total FAs, including TG-bound FAs, rats fed the HF diet showed ∼2–3 times the concentration of TG and total FAs in the liver. This was accompanied with increased hepatic concentrations of FFAs and cholesterol, but not of phospholipids. Most likely, in line with other rat studies (31), these metabolic responses involved altered lipoprotein assembly and/or VLDL secretion, rather than accumulation of exogenous FAs directly from the FA-rich diet since the hepatic gene expression of *Fabp1*, *Fabp4*, and *Pltp* was similar between the 2 experimental groups. Of note, unlike the key lipogenic genes *Fasn* and *Acc*, some genes involved in TG synthesis were also not altered (e.g., *Fatb1*, *Gpam*, *Dgat1*, and *Dgat2*).

While our study shows that the liver responds with different mechanisms upon HC and HF feeding, the potential clinical implications of these differences need careful evaluation. Although the degree of liver steatosis was not documented in our experiment, an estimated 5% (wt:wt) TG in the liver of the HF-fed rats suggests some degree of fatty liver, based on the diagnostic criterion in humans (TG ≥5% of the liver weight) (32). Some previous studies have shown that

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

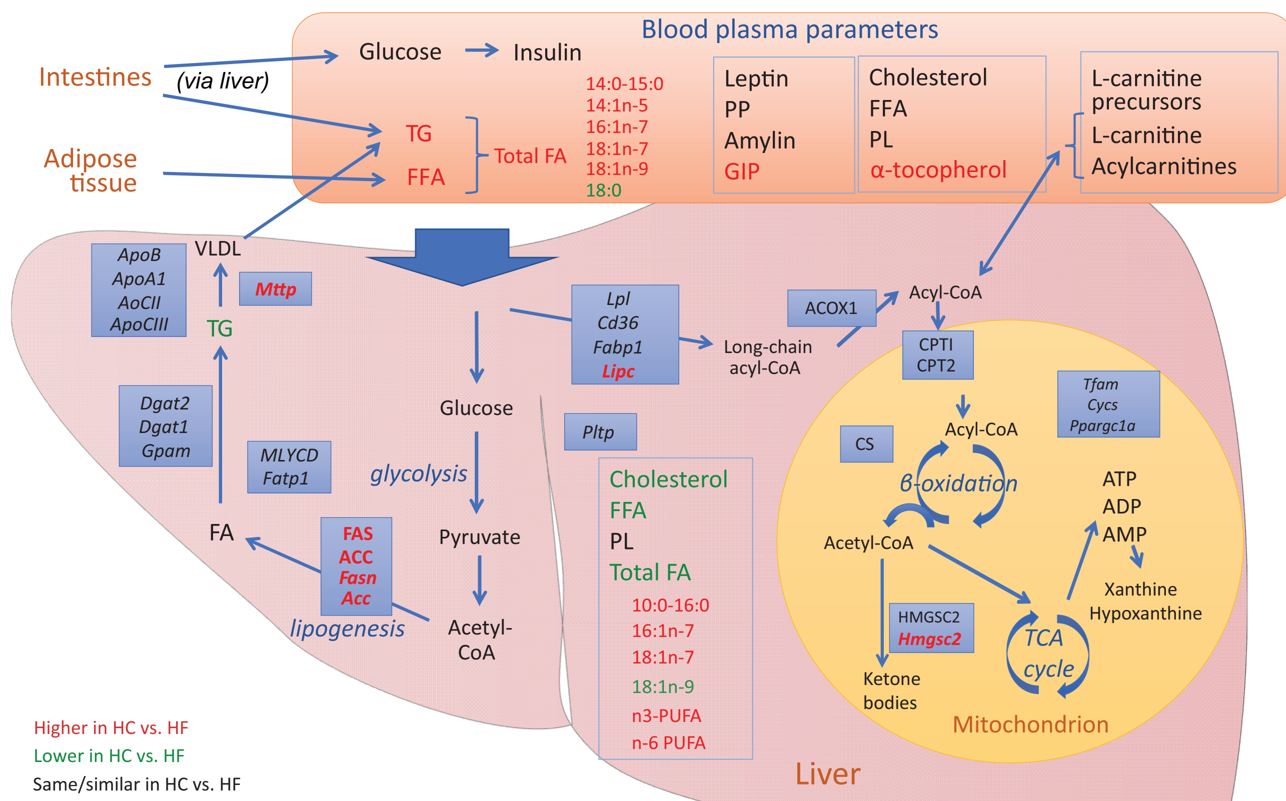

FIGURE 5 Summary of metabolic responses to 4-wk low-carbohydrate high-fat (HF) compared to high-carbohydrate low-fat (HC) feeding in male Wistar rats. Red text indicates higher and green lower values in the HC compared to the HF group, and black text no significant difference. For individual fatty acids the text colors indicate differences in relative values (weight %, i.e., gram fatty acid/100 g total fatty acids). Capitalized letters indicate protein activity, italicized letters gene expression. FFA, free fatty acids; FA, fatty acids; HF, low-carbohydrate high-fat diet; HC, high-carbohydrate low-fat diet; GIP, gastric inhibitory protein; PL, phospholipids; PP, pancreatic polypeptide; PUFA, polyunsaturated fatty acids; TAG, triacylglycerol; VLDL, very-low density lipoprotein.

high-fat diets result in fatty liver in rats (33). However, a 14-wk study found no effect of high saturated-fat intake on fatty liver in conjunction with enhanced *Ucp1* expression in brown adipocytes (34). Importantly, metabolic effects of high-fat diets depend on the level of concomitant carbohydrate intake. Even a slight intake of carbohydrate in a high-fat dietary context has been shown to increase fatty liver and insulin resistance in mice (35), rats (36), and humans (37). These effects may particularly depend on the amount of dietary fructose (38). Hence, the 15 E% carbohydrate in the HF diet in the present study may have synergized with the dietary fat to promote higher hepatic fat accumulation compared with the HC diet. Whether a high-fat diet, low or high in carbohydrates, promotes fatty liver in rats may furthermore depend on physiological and nutritional context, such as testosterone levels (31) and intake of n–3 FAs (39). In any case, the detailed insight from the present study strengthens the biological basis needed to understand the pathogenic potentials of excess intake of carbohydrates and/or fat, which remains a highly controversial topic.

By lipidomics, our study shows carbohydrate-induced elevations in circulating concentrations of specific lipids. The profile of FAs in the blood is the result of, e.g., lipids supplied from the diet, lipolytic activity of adipose tissue, and FA biosynthesis. Because the liver is central in the regulation of lipid homeostasis, FA profiling can be expected to reflect changes within this organ. We found that the total SFA concentration was similar in plasma and liver between the 2 experimental groups. However, in both plasma and liver, the individual SFAs palmitic, myristic, and pentadecyclic acid were present in lower proportions of the total FAs in rats fed the HF compared with the HC diet. These data indicate that diets higher in carbohydrate increase circulating concentrations of SFAs, in particular myristic and palmitic acid, whose dietary intake is known to increase LDL-cholesterol concentrations in humans (40, 41). Notably, we found that the proportion of margaric acid (17:0) in plasma and liver was higher in HF- compared with HC-fed rats. This odd-chain FA, largely derived from biosynthesis rather than dietary intake or the gut microbiota, was found to be positively associated with glucose tolerance (42).

Moreover, MUFA changes reflect de novo lipogenesis pathways, where SCD catalyzes the synthesis of palmitoleic acid (16:1n–7; dependent on SCD-16) and oleic acid (18:1n–9; dependent on SCD-18). Increased estimated SCD activity and a significant correlation between SCD activity and severity of steatosis has been found in people with NAFLD (43). In the present study, particularly the estimated SCD-16 index was higher both in liver and plasma of HC- compared with HF-fed rats, and this index showed strong positive correlations with hepatic TG and total FA concentrations within both diet groups. Strikingly, in HC-fed rats, the proportion of palmitoleic acid was 7.5-fold and 6.5-fold that of HF-fed rats in plasma and liver, respectively. These data are consistent with previous research in humans showing higher concentrations of palmitoleic acid on high-carbohydrate diets. Importantly, increased circulating concentrations of palmitoleic acid have been observed in different disease states (44). Nonetheless, increased palmitoleic acid concentrations may reflect a normal regulatory mechanism in the response to dietary carbohydrates, since this FA was

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

previously found to act as an adipose-derived "lipokine" with stimulatory effects on insulin action in muscle and suppressive effects on hepatosteatosis (45).

Similar to palmitoleic acid, the proportion of circulating *cis*-vaccenic acid (18:1n–7), a downstream product of de novo lipogenesis, was higher in the HC-fed rats. Circulating concentrations of this FA show an inverse relation with insulin resistance and incident type 2 diabetes (46). On the other hand, studies of NAFLD patients have also shown a trend of increased circulating oleic acid (18:1n–9) (47). We found a 40% higher proportion of circulating oleic acid in the HC group, which showed less hepatic TG but higher circulating TG and total FAs. Conversely, the HF group showed a 70% higher proportion of oleic acid in the liver. Hence, the distribution of oleic acid reflected that of TG and total FAs. Another striking difference was seen for myristoleic acid (14:1n–5), which, although circulating at relatively low concentrations, was 8.5- and 15-fold in plasma and liver, respectively, for the HC compared with the HF group. This FA is a desaturated product of the saturated myristic acid dependent on SCD-1 activity. The possible metabolic effects of myristoleic acid are largely unknown, but cytotoxic and necrotic effects have implicated this FA as a potential compound in the treatment of prostate cancer (48).

The major PUFA in the Western diet is linoleic acid, with lower amounts of the FA from the n–3 series (e.g., α-linolenic acid). FAs are used as a source of energy in the body, but they are also metabolized by desaturation and elongation to longer and more unsaturated FAs with specific properties. Interestingly, the proportion of total n–3 FAs, and of the long-chain n–3 FA EPA, was lower in liver and plasma in HF- compared with HC-fed rats. This is possibly due to a markedly reduced n–3 Δ5 desaturase activity index, which is reflective of reduced conversion of 20:4n–3 to EPA in the liver. The proportion of DHA in HF-fed rats was also lower in liver, but not in plasma, whereas that of docosapentaenoic acid (22:5n–3) was higher in the liver compared with HC-fed rats. This cannot be attributed to a lower n–3 Δ6 desaturase index as this was increased in the liver and reduced in plasma. Interestingly, both EPA and the n–3 Δ5 desaturase index showed a similar pattern to hepatic TG and total FAs. Contrary to n–3, the hepatic proportion of n–6 FAs was increased in the HF- compared with the HC-fed rats, including the long-chain n–6 fatty acid DPA, which also followed hepatic TG concentrations. Finally, the estimated de novo lipogenesis pathway index 16:0/18.2n–6 was lower both in liver and plasma in HF-fed rats, in line with much lower activities of FAS and ACC compared with HC-fed rats. This index also correlated to the hepatic TG and total FA concentrations.

The differential concentrations of plasma and hepatic individual fatty acids between the diet groups could partly be a direct reflection of differences in dietary FA intake. Also, a different fat source in the HF diet (e.g., more soybean oil in replacement of lard) would be expected to affect hepatic and plasma FA composition, at least to some degree. Although the lard-based feed in the HF group contained up to 10-fold the FA content compared with the HC group, plasma in HF-fed rats contained ~65% of the FAs and the liver around double the FAs compared with HC-fed rats. Previous studies support such a disconnect between intake of total SFAs and circulating SFA concentrations, and have shown a carbohydrate-dependent increase in specific circulating FAs, including palmitoleic acid (49), in line with our study. Myristoleic acid was another FA with a marked relative increase after HC feeding in our study despite no differences in the feed. Overall, the data support the relevance of the metabolic activities that we estimated from the relative FA concentrations, such as SCD-16, SCD-18, and others that showed severalfold differences between groups and between liver and plasma. Another contributor to the different FA compositions could be the gut microbiota, via diet-dependent absorption of specific FAs formed by different microbes (50). Alternatively, the gut microbiome may have modulated FA metabolism via differential effects on microbiota-derived metabolites, such as acetate, which has been shown to affect hepatic lipid desaturation/elongation (51).

Our study has limitations. While the effect of the HC diet on circulating TG is consistent with several previous murine and human studies, it remains to be determined if the effects on specific lipid species are translatable to humans. Moreover, possible sex differences could not be assessed as we here only studied male rats. Females show higher hepatic mitochondrial respiratory capacity and FA oxidation in response to feeding compared with males, conferring sex-specific protection against hepatic steatosis (52, 53). Studies have also shown sex-specific diet responses in FA composition in plasma and liver (54, 55). Finally, despite the lack of differences in measured parameters related to hepatic FA oxidation and mitochondrial function, we cannot rule out that the diets affected FA oxidation and/or energy expenditure in other organs, or that the effects would be different in females.

In conclusion, dietary carbohydrates are effectively converted to specific FAs in the liver via upregulation and activation of lipogenic enzymes, most notably FAS and ACC, corresponding to higher plasma TG and total FA concentrations but lower hepatic TG and total FAs. The opposite pattern with the high-fat, 15.9 E% carbohydrate diet was reflected in markedly higher estimated SCD-16 and SCD-18 activity in plasma, and higher SCD-16 activity in the liver. The estimated n–3 Δ5 desaturase activity was severalfold higher in both liver and plasma comparing the HC and HF diets. Taken together, our study reveals that male Wistar rats exposed to diets based on carbohydrates or fats differ greatly in their plasma and hepatic FA concentrations. These differences may largely depend on higher activity of hepatic enzymes involved in lipogenesis and not on lower mitochondrial FA oxidation.

Acknowledgments

We thank Randi Sandvik, Liv Kristine Øysæd, and Kari Helland Mortensen for expert technical assistance. The authors' responsibilities were as follows—SND, BB, and RKB: interpreted the data and wrote the manuscript; BB, ES, CL, MLG, ON, and RKB: designed the study; CL, MLG, BB, and ES: planned and carried out the feeding experiment; CL and MLG: performed qPCR analyses; CRR, PB, and SH: carried out metabolite analyses; RKB: is responsible for the design, writing, and final content; and all authors: reviewed and revised the manuscript and read and approved the final manuscript.

References

1. Marston NA, Giugliano RP, Im KA, Silverman MG, O'Donoghue ML, Wiviott SD, Ference BA, Sabatine MS. Association between triglyceride lowering and reduction of cardiovascular risk across multiple lipid-lowering therapeutic classes. Circulation 2019;140:1308–17.
2. Reaven G. Insulin resistance and coronary heart disease in nondiabetic individuals. Arterioscler Thromb Vasc Biol 2012;32:1754–9.
3. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovasc Diabetol 2018;17:122.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

4. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107: 450–5.
5. Nestel PJ, Carroll KF, Havenstein N. Plasma triglyceride response to carbohydrates, fats and caloric intake. Metabolism 1970;19: 1–18.
6. Fechner E, Smeets ETHC, Schrauwen P, Mensink RP. The effects of different degrees of carbohydrate restriction and carbohydrate replacement on cardiometabolic risk markers in humans—a systematic review and meta-analysis. Nutrients 2020;12:991.
7. Bedogni G, Nobili V, Tiribelli C. Epidemiology of fatty liver: an update. World J Gastroenterol 2014;20:9050–4.
8. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. Nature 2014;510: 84–91.
9. Finck BN. Targeting metabolism, insulin resistance, and diabetes to treat nonalcoholic steatohepatitis. Diabetes 2018;67:2485–93.
10. Simões ICM, Fontes A, Pinton P, Zischka H, Wieckowski MR. Mitochondria in non-alcoholic fatty liver disease. Int J Biochem Cell Biol 2018;95:93–9.
11. Sim KG, Hammond J, Wilcken B. Strategies for the diagnosis of mitochondrial fatty acid β-oxidation disorders. Clin Chim Acta 2002;323:37–58.
12. Rinaldo P, Cowan TM, Matern D. Acylcarnitine profile analysis. Genet Med 2008;10:151–6.
13. Bouchouirab FZ, Fortin M, Noll C, Dubé J, Carpentier AC. Plasma palmitoyl-carnitine (AC16:0) is a marker of increased postprandial nonesterified incomplete fatty acid oxidation rate in adults with type 2 diabetes. Can J Diabetes 2018;42:382–8.
14. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, Herder C, Carstensen M, Krausch M, Knoefel WT, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab 2015;21:739–46.
15. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
16. Strand E, Bjorndal B, Nygard O, Burri L, Berge C, Bohov P, Christensen BJ, Berge K, Wergedahl H, Viste A, et al. Long-term treatment with the pan-PPAR agonist tetradecylthioacetic acid or fish oil is associated with increased cardiac content of n-3 fatty acids in rat. Lipids Health Dis 2012;11:82.
17. Vernez L, Wenk M, Krähenbühl S. Determination of carnitine and acylcarnitines in plasma by high-performance liquid chromatography/electrospray ionization ion trap tandem mass spectrometry. Rapid Commun Mass Spectrom 2004;18:1233–8.
18. Vigerust NF, Bohov P, Bjorndal B, Seifert R, Nygård O, Svardal A, Glintborg D, Berge RK, Gaster M. Free carnitine and acylcarnitines in obese patients with polycystic ovary syndrome and effects of pioglitazone treatment. Fertil Steril 2012;98: 1620–6,
19. Fürst W, Hallström S. Simultaneous determination of myocardial nucleotides, nucleosides, purine bases and creatine phosphate by ion-pair high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1992;578:39–44.
20. Lindquist C, Bjørndal B, Rossmann CR, Tusubira D, Svardal A, Røsland GV, Tronstad KJ, Hallström S, Berge RK. Increased hepatic mitochondrial FA oxidation reduces plasma and liver TG levels and is associated with regulation of UCPs and APOC-III in rats. J Lipid Res 2017;58:1362–73.
21. Lindquist C, Bjørndal B, Lund A, Slettom G, Skorve J, Nygård O, Svardal A, Berge RK. Increased fatty acid oxidation and mitochondrial proliferation in liver are associated with increased plasma kynurenine metabolites and nicotinamide levels in normolipidemic and carnitine-depleted rats. Biochim Biophys Acta Mol Cell Biol Lipids 2020:1865;158543 .
22. Roncari DAK. Fatty acid synthase from human liver. Methods Enzymol 1981;71:73–9.
23. Bjørndal B, Alterås EK, Lindquist C, Svardal A, Skorve J, Berge RK. Associations between fatty acid oxidation, hepatic mitochondrial function, and plasma acylcarnitine levels in mice. Nutr Metab 2018;15:10.
24. Zaman MQ, Leray V, Le Bloc'h J, Thorin C, Ouguerram K, Nguyen P. Lipid profile and insulin sensitivity in rats fed with high-fat or high-fructose diets. Br J Nutr 2011;106:S206–10.
25. Ferramosca A, Conte A, Damiano F, Siculella L, Zara V. Differential effects of high-carbohydrate and high-fat diets on hepatic lipogenesis in rats. Eur J Nutr 2014;53:1103–14.
26. Parks EJ. Effect of dietary carbohydrate on triglyceride metabolism in humans. J Nutr 2001;131:2772S–2774S.
27. Lindquist C, Bjørndal B, Rossmann CR, Svardal A, Hallström S, Berge RK. A fatty acid analogue targeting mitochondria exerts a plasma triacylglycerol lowering effect in rats with impaired carnitine biosynthesis. PLoS One 2018;13:e0194978.
28. Softic S, Meyer JG, Wang GX, Gupta MK, Batista TM, Lauritzen HPMM, Fujisaka S, Serra D, Herrero L, Willoughby J, et al. Dietary sugars alter hepatic fatty acid oxidation via transcriptional and post-translational modifications of mitochondrial proteins. Cell Metab 2019;30:735–53, e4.
29. Czech MP, Tencerova M, Pedersen DJ, Aouadi M. Insulin signalling mechanisms for triacylglycerol storage. Diabetologia 2013;56:949–64.
30. Assimacopoulos-Jeannet F, Brichard S, Rencurel F, Cusin I, Jeanrenaud B. In vivo effects of hyperinsulinemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissues. Metabolism 1995;44:228–33.
31. Jia Y, Yee JK, Wang C, Nikolaenko L, Diaz-Arjonilla M, Cohen JN, French SW, Liu PY, Lue Y, Lee WNP, et al. Testosterone protects high-fat/low-carbohydrate diet-induced nonalcoholic fatty liver disease in castrated male rats mainly via modulating endoplasmic reticulum stress. Am J Physiol Endocrinol Metab 2018;314:E366–76.
32. Nassir F, Rector RS, Hammoud GM, Ibdah JA. Pathogenesis and prevention of hepatic steatosis. Gastroenterol Hepatol 2015;11:167–75.
33. Kucera O, Cervinkova Z. Experimental models of non-alcoholic fatty liver disease in rats. World J Gastroenterol 2014;20:8364–76.
34. Romestaing C, Piquet MA, Bedu E, Rouleau V, Dautresme M, Hourmand-Ollivier I, Filippi C, Duchamp C, Sibille B. Long term highly saturated fat diet does not induce NASH in Wistar rats. Nutr Metab 2007;4:4.
35. Mei S, Yang X, Guo H, Gu H, Zha L, Cai J, Li X, Liu Z, Bennett BJ, He L, et al. A small amount of dietary carbohydrate can promote the HFD-induced insulin resistance to a maximal level. PLoS One 2014;9:e100875.
36. Pichon L, Huneau J-F, Fromentin G, Tomé D. A high-protein, high-fat, carbohydrate-free diet reduces energy intake, hepatic lipogenesis, and adiposity in rats. J Nutr 2006;136:1256–60.
37. Mardinoglu A, Wu H, Bjornson E, Zhang C, Hakkarainen A, Räsänen SM, Lee S, Mancina RM, Bergentall M, Pietiläinen KH, et al. An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. Cell Metab 2018;27:559–71, e5.
38. Softic S, Gupta MK, Wang GX, Fujisaka S, O'Neill BT, Rao TN, Willoughby J, Harbison C, Fitzgerald K, Ilkayeva O, et al. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. J Clin Invest 2017;127:4059–74.
39. Poudyal H, Panchal SK, Ward LC, Waanders J, Brown L. Chronic high-carbohydrate, high-fat feeding in rats induces reversible metabolic, cardiovascular, and liver changes. Am J Physiol Metab 2012;302:E1472–82.
40. Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr 1997;65: 1628S–44S.
41. Zock PL, De Vries JHM, Katan MB. Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arterioscler Thromb Vasc Biol 1994;14: 567–75.
42. Jenkins BJ, Seyssel K, Chiu S, Pan PH, Lin SY, Stanley E, Ament Z, West JA, Summerhill K, Griffin JL, et al. Odd chain fatty acids; new insights of the relationship between the gut microbiota, dietary intake, biosynthesis and glucose intolerance. Sci Rep 2017;7:44845.
43. MacIejewska D, Palma J, Dec K, Skonieczna-Zydecka K, Gutowska I, Szczuko M, Jakubczyk K, Stachowska E. Is the fatty acids profile in blood a good predictor of liver changes? Correlation of fatty acids profile with fatty acids content in the liver. Diagnostics 2019;9:197.
44. Volk BM, Kunces LJ, Freidenreich DJ, Kupchak BR, Saenz C, Artistizabal JC, Fernandez ML, Bruno RS, Maresh CM, Kraemer WJ, et al. Effects of step-wise increases in dietary carbohydrate on circulating saturated fatty acids and palmitoleic acid in adults with metabolic syndrome. PLoS One 2014;9:e113605.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022

45. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 2008;134:933–44.
46. Weir NL, Johnson L, Guan W, Steffen B, Djousse L, Mukamal KJ, Tsai MY. cis-Vaccenic acid is associated with lower HOMA-IR and incident T2D in participants from the MESA cohort. Diabetes 2018;67:1552.
47. Safaei A, Oskouie AA, Mohebbi SR, Rezaei-Tavirani M, Mahboubi M, Peyvandi M, Okhovatian F, Zamanian-Azodi M. Metabolomic analysis of human cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis diseases. Gastroenterol Hepatol from Bed to Bench 2016;9:158–73.
48. Iguchi K, Okumura N, Usui S, Sajiki H, Hirota K, Hirano K. Myristoleic acid, a cytotoxic component in the extract from Serenoa repens, induces apoptosis and necrosis in human prostatic LNCaP cells. Prostate 2001;47:59–65.
49. Volk BM, Kunces LJ, Freidenreich DJ, Kupchak BR, Saenz C, Artistizabal JC, Fernandez ML, Bruno RS, Maresh CM, Kraemer WJ, et al. Effects of step-wise increases in dietary carbohydrate on circulating saturated fatty acids and palmitoleic acid in adults with metabolic syndrome. PLoS One 2014;9:e113605.
50. Yasuda S, Okahashi N, Tsugawa H, Ogata Y, Ikeda K, Suda W, Arai H, Hattori M, Arita M. Elucidation of gut microbiota-associated lipids using LC-MS/MS and 16S rRNA sequence analyses. iScience 2020;23:101841.
51. Kindt A, Liebisch G, Clavel T, Haller D, Hörmannsperger G, Yoon H, Kolmeder D, Sigruener A, Krautbauer S, Seeliger C, et al. The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. Nat Commun 2018;9:1–15.
52. McCoin CS, Von Schulze A, Allen J, Fuller KNZ, Xia Q, Koestler DC, Houchen CJ, Maurer A, Dorn GW, Shankar K, et al. Sex modulates hepatic mitochondrial adaptations to high-fat diet and physical activity. Am J Physiol Endocrinol Metab 2019;317: E298–311.
53. Pramfalk C, Pavlides M, Banerjee R, McNeil CA, Neubauer S, Karpe F, Hodson L. Sex-specific differences in hepatic fat oxidation and synthesis may explain the higher propensity for NAFLD in men. J Clin Endocrinol Metab 2015;100: 4425–33.
54. Childs CE, Romeu-Nadal M, Burdge GC, Calder PC. The polyunsaturated fatty acid composition of hepatic and plasma lipids differ by both sex and dietary fat intake in rats. J Nutr 2010;140:245–50.
55. Ostwald R, Okey R, Shannon A, Tinoco J. Changes in tissue lipids in response to diet. I. Fatty acids of subcutaneous, mesenteric and interscapular fat. J Nutr 1962;76:341–52.

Downloaded from https://academic.oup.com/jn/article/151/9/2610/6300464 by Universitetsbiblioteket i Bergen user on 15 March 2022