High protein intake reduces intrahepatocellular lipid deposition in humans[1–3]

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ABSTRACT

Background: High sugar and fat intakes are known to increase intrahepatocellular lipids (IHCLs) and to cause insulin resistance. High protein intake may facilitate weight loss and improve glucose homeostasis in insulin-resistant patients, but its effects on IHCLs remain unknown.

Objective: The aim was to assess the effect of high protein intake on high-fat diet–induced IHCL accumulation and insulin sensitivity in healthy young men.

Design: Ten volunteers were studied in a crossover design after 4 d of either a hypercaloric high-fat (HF) diet; a hypercaloric high-fat, high-protein (HFHP) diet; or a control, isocaloric (control) diet. IHCLs were measured by ^{1}H-magnetic resonance spectroscopy, fasting metabolism was measured by indirect calorimetry, insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp, and plasma concentrations were measured by enzyme-linked immunosorbent assay and gas chromatography–mass spectrometry; expression of key lipogenic genes was assessed in subcutaneous adipose tissue biopsy specimens.

Results: The HF diet increased IHCLs by 90 ± 26% and plasma tissue-type plasminogen activator inhibitor-1 (tPAI-1) by 54 ± 11% ($P < 0.02$ for both) and inhibited plasma free fatty acids by 26 ± 11% and β-hydroxybutyrate by 61 ± 27% ($P < 0.05$ for both). The HFHP diet blunted the increase in IHCLs and normalized plasma β-hydroxybutyrate and tPAI-1 concentrations. Insulin sensitivity was not altered, whereas the expression of sterol regulatory element-binding protein-1c and key lipogenic genes increased with the HF and HFHP diets ($P < 0.02$). Bile acid concentrations remained unchanged after the HF diet but increased by 50 ± 24% after the HFHP diet ($P = 0.14$).

Conclusions: Protein intake significantly blunts the effects of an HF diet on IHCLs and tPAI-1 through effects presumably exerted at the level of the liver. Protein-induced increases in bile acid concentrations may be involved. This trial was registered at www.clinicaltrials.gov as NCT00523562. *Am J Clin Nutr* 2009;90:1002–10.

INTRODUCTION

In animals (1) and healthy humans (2), high-fat or high-fructose diets lead to the development of several features of the metabolic syndrome, such as increased plasma triglycerides, hepatic and extrahepatic insulin resistance, and liver steatosis. In contrast, increasing protein intakes have been suggested to exert beneficial metabolic effects by promoting weight loss and improving glucose homeostasis in insulin-resistant patients (3, 4). The effects of dietary protein on hepatic lipids, however, remain unknown. The aim of this study, therefore, was to investigate the potential beneficial effects of a high-protein diet on hepatic steatosis induced by a high-fat diet. For this purpose we monitored liver fat (intrahepatocellular lipid; IHCL) concentrations in healthy subjects consuming a control, balanced isocaloric (control) diet or hypercaloric diets with a high-fat (HF) or a high-fat and high-protein (HFHP) content. Because adipose tissue metabolism may secondarily affect the hepatic lipid content, the effects of these diets on subcutaneous adipose tissue gene expression were also studied. In addition, bile acids (BAs) have been recently recognized as important regulators of hepatic lipid metabolism and possibly of total energy expenditure (5–8). Therefore, circulating BA concentrations were also monitored.

SUBJECTS AND METHODS

Participants

Two groups of healthy male volunteers were included in this study. Ten volunteers aged 24 ± 1 y with a mean body mass index (in kg/m^2) of 22.4 ± 0.6 took part in the main protocol and were studied in a crossover design after an isocaloric diet, a hypercaloric high-fat (HF) diet, or a hypercaloric high-fat, high-protein (HFHP) diet. Another group of 6 volunteers aged 25 ± 1 y were studied in a complementary crossover study after consuming a high-fat, high-cholesterol (HF-high chol) and

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[2] Supported by the Swiss National Science Foundation (grant 310000-109737 to LT and CB).

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Received November 28, 2008. Accepted for publication July 24, 2009.
First published online August 26, 2009; doi: 10.3945/ajcn.2008.27296.

a high-fat, low-cholesterol diet (HF-low chol). All subjects were sedentary, were nonsmokers, and had no family history of diabetes. The experimental protocol was approved by the Ethical Committee of Lausanne University School of Medicine. All participants provided written informed consent at inclusion.

Study design

For the main study (effects of HF and HFHP diets), each of the 10 subjects was studied on 3 occasions in a crossover design. They received, in randomized order, over 4 d either a standard isocaloric control diet or an HF or HFHP diet. All 3 diets were designed to provide the same total carbohydrate intake, and the HF and HFHP diets were designed to provide the same fat intake (**Table 1**). The extra fat content in the HF and HFHP diets was provided mainly by dairy products (eg, butter, milk, and cheese), and the extra protein content was provided as eggs, ham, salami, and tuna. The study was performed on an outpatient basis, and the subjects received all of their food as prepackaged food items with instruction on how and when to prepare and consume them. The subjects were instructed not to consume any other food or drinks. The experimental periods were separated by a washout period of $\geq$2 wk.

At the end of the fourth day of each period, IHCL concentrations were measured by proton magnetic resonance spectroscopy (^{1}H-MRS). On the morning of the fifth day, a periumbilical adipose tissue biopsy specimen was obtained by needle aspiration under local anesthesia. Thereafter, an infusion of 6,6 $^2H_2$-glucose (bolus: 2 mg/kg; continuous: 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Cambridge Isotope Laboratories, Cambridge, MA) was started. Blood samples were collected at baseline and after 90, 105, and 120 min to measure concentrations of basal hormones, substrates, and 6,6 $^2H_2$-glucose enrichment and at time 60 min to measure plasma lipids, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). After this 120-min baseline measurement period, a 2-step hyperinsulinemic-euglycemic clamp (0.3 mU $\cdot$ kg^{-1} $\cdot$ min^{-1} from 120 to 210 min, then 1.0 mU $\cdot$ kg^{-1} $\cdot$ min^{-1} in from 210 to 300 min) with variable 6,6 $^2H_2$ glucose infusion was performed, and plasma hormone and substrate concentrations were measured at 30-min intervals. Substrate oxidation and energy expenditure were continuously measured throughout the test by open-circuit indirect calorimetry (Deltatrac II; Datex Instrument, Helsinki, Finland) by using the equations of Livesey and Elia (9). Net protein oxidation was calculated from the urinary urea nitrogen excretion rate, assuming that urea excretion accounted for 90% of total urinary nitrogen excretion (10). Urine collected overnight was used for basal protein oxidation, whereas urine collected at the end of the clamp was used for protein oxidation during hyperinsulinemia.

Because the HFHP diet was considerably enriched in cholesterol compared with the other diets, because it included eggs and meat-based products, a second complementary crossover study was performed in 6 volunteers. In this study, subjects received over 4 d either an HF-low chol diet or an HF-high chol diet. The high-fat supplementation (+30% of total energy as fat) was similar to that used in the previous study, and a high cholesterol intake was ensured by adding 30 g egg yolk/d ($\approx$300 mg cholesterol/d). At the end of this period, variations in body weight were assessed, IHCLs were measured by ^{1}H-MRS, and a fasting blood sample was obtained for measurement of plasma glucose, nonesterified fatty acids (NEFAs), triglycerides, cholesterol, HDL-cholesterol BAs, AST, and ALT.

TABLE 1
Nutrient content of the experimental diets (n = 10)[1]

	C diet	HF diet	HFHP diet
Energy (kcal)	2248	2923	3370
Carbohydrate (kcal)	1237	1237	1237
Total fat (kcal)	674	1349	1349
Protein (kcal)	337	337	784
Saturated fat (%)	33.5	51.6	52.6
Monounsaturated fat (%)	37.0	27.6	26.5
Polyunsaturated fat (%)	16.5	8.8	7.9
Cholesterol (mg)	226	376	653

[1] C, control; HF, high-fat; HFHP, high-fat, high-protein.

Analytic procedures

Plasma metabolites (glucose, NEFAs, triglycerides, and β-hydroxybutyrate) and insulin were determined by using classic enzymatic methods and radioimmunoassay, respectively, as previously reported (2). Adiponectin, tissue-type plasminogen activator inhibitor-1 (tPAI-1), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6 were analyzed by immunoassay using a multiplex assay (Linco Research, St Charles, MO). Total plasma BA concentrations were measured with an enzyme-linked immunosorbent assay kit (Randox Laboratories, Crumlin, United Kingdom). Individual BA concentrations were measured by gas chromatography–mass spectroscopy (GC-MS) (11). Plasma isotopic enrichment of 6,6 $^2H_2$-glucose was calculated by GC-MS, as previously described (12). Total glucose turnover was calculated by using Steele's equations for steady state conditions (13). Endogenous glucose production was calculated as total glucose turnover minus the exogenous glucose infusion rate.

^{1}H Magnetic resonance spectroscopy

For the main study (effects of the HF and HFHP diets), ^{1}H-MRS examinations were performed with a clinical 1.5 T magnetic resonance (MR) scanner (Signa; General Electric Medical Systems, Waukesha WI) using a flexible receive radiofrequency coil in combination with the body transmit coil. Data acquisition and processing were similar to those in a protocol described previously (2). In brief, single-voxel ^{1}H-MR spectra were acquired with an optimized point-resolved spectroscopy (PRESS) sequence (20-ms echo time; 2-kHz bandwidth, 1024 points). On the basis of coronal (spoiled gradient recalled echo sequence, 60° flip angle, 1.5-ms echo time, 0.11-s repetition time, 8-mm slice thickness, 2.5-mm gap between slices, 48-cm field of view, 512 $\times$ 192 matrix size) and axial (fast-spin echo sequence, 9-ms echo time, 4-s repetition time, 4-mm slice thickness, 1-mm gap between slices, 40-cm field of view, 512 $\times$ 128 matrix size) MR images obtained under breath hold in expiration, a volume of interest of 55 cm^3 was placed in a lateral area of the liver and repositioned at the same location in follow-up examinations. Positioning of the receive coil made use of external anatomic landmarks. Thirty-two acquisitions with water presaturation were recorded and stored individually for each spectrum. The repetition time (5–6 s) was adjusted such that the subjects could breathe normally, and acquisitions could occur during brief breathing arrests in expiration (14). Effects of residual motion were found to be evidenced by small shifts in resonance frequency. They were accounted for by aligning all individual

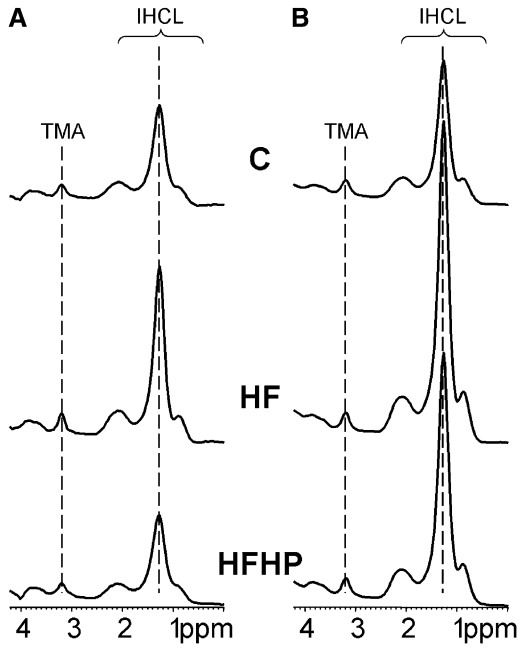

FIGURE 1. Localized proton magnetic resonance spectroscopy spectra of liver showing data quality (A) and overall results (B) for intrahepatocellular lipids (IHCLs). The 3 rows show the spectra for the 3 dietary regimens (C, control; HF, high-fat; HFHP, high-fat, high-protein), Panel A shows data from a single subject, and panel B shows the averaged spectra from all subjects (n = 10). Trimethylammonium (TMA) compounds were unaffected by diet. The dashed line indicates the peak of methyl protons used for quantitation.

scans that fell into a frequency window of 12 Hz and by discarding those acquisitions with a lipid peak that was shifted by >6 Hz. Spectra were processed, fitted, and quantitated similarly to earlier descriptions (2) by using prior knowledge fitting (**Figure 1**). Quantitation to obtain IHCL in units of mmol/kg was based on the median water signal from 8 separate acquisitions obtained without water suppression, a transverse relaxation time (T_2) of 50 ms for this water signal (as determined earlier), and a liver water content that was experimentally determined for each investigation, using proton density weighted MR images acquired with the body coil in breath hold and with a water reference standard placed on the subject's breast (spoiled gradient recalled echo sequence, single slice of 10 mm, 12° flip angle, 1.8-ms echo time, 200-ms repetition time, 21-s total scan time).

For the complementary study of the influence of cholesterol intake, all MRS measurements were performed on a 3-T MR scanner (Trio; Siemens, Erlangen, Germany) with slightly adapted measurement conditions: localization with a stimulated echo sequence (20-ms echo time, 5-s repetition time) without water presaturation. Three spectra were recorded in each session. Data processing and model fitting were performed with jMRUI (15), including realignment of spectra, elimination of the water peak (Henkel Lancosz singular value decomposition using 3 components) for the determination of the lipid peak areas, and peak area fitting using prior knowledge parameter relations. T_2 relaxation was corrected with values taken from the literature. IHCL values obtained at 3 T with a somewhat modified method are probably systematically somewhat different from those obtained in the main study at 1.5 T. However, this influence is expected to be small and does not affect the conclusions of the study because both parts of the MR investigations were self-contained.

Gene expression in adipose tissue

The real-time quantitative polymerase chain reaction assay for mRNA was previously described and validated (16). Hypoxanthine phosphoribosyltransferase mRNA was measured by real-time quantitative polymerase chain reaction as a reference gene, and the mRNA level of the genes of interest was expressed as a percentage ratio relative to the expression of hypoxanthine

TABLE 2
Gene expression in adipose tissue from healthy subjects after 4 d of each dietary condition (n = 8)[1]

				P for pairwise comparison		
Lipid metabolism	C diet (n = 7)	HF diet (n = 8)	HFHP diet (n = 8)	C vs HF	C vs HFHP	HF vs HFHP
Lipolysis						
Hormone-sensitive lipase	1856 ± 185	1616 ± 168	1487 ± 211	>0.2	0.028	>0.2
Adipose triglyceride lipase	2296 ± 241	2267 ± 226	2358 ± 219	>0.2	>0.2	>0.2
Perilipin	8699 ± 986	6539 ± 716	8212 ± 1713	0.018	>0.2	>0.2
Phosphodiesterase 3b	44,563 ± 6017	47,592 ± 5674	44,679 ± 5393	>0.2	>0.2	>0.2
Lipogenesis						
Sterol regulatory element-binding protein-1c	636 ± 54	1085 ± 159	1349 ± 208	0.018	0.018	0.036
Fatty acid synthase	144 ± 29	336 ± 69	276 ± 41	0.043	0.063	0.161
Hexokinase II	377 ± 37	496 ± 54	520 ± 69	0.018	0.018	>0.2
Lipid oxidation						
Fatty acid translocase	6743 ± 891	7511 ± 619	8407 ± 854	>0.2	0.018	>0.2
Carnitine palmitoyltransferase	67 ± 8	58 ± 9	52 ± 8	0.063	0.018	>0.2
Cholesterol-related						
Liver X receptor-α	234 ± 18	275 ± 18	331 ± 30	0.028	0.018	>0.2
ATP-binding cassette A	80 ± 13	83 ± 15	81 ± 12	>0.2	>0.2	>0.2
LDL receptor	37 ± 5	34 ± 5	48 ± 7	>0.2	0.028	0.012

[1] All values are means ± SEMs. Data are expressed as a percentage ratio referring to the expression of hypoxanthine phosphoribosyltransferase. C, control; HF, high-fat; HFHP, high-fat, high-protein. Wilcoxon's signed-rank test with Bonferroni's correction was used for pairwise comparisons.

phosphoribosyltransferase. Expression of selected genes involved in lipid, carbohydrate, and energy metabolism were measured. The complete list is shown in **Table 2**.

Statistical analysis

All data were expressed as means ± SEs. The nonparametric Wilcoxon's signed-paired rank test with Bonferroni's correction was used to test the null hypothesis between all 3 paired dietary conditions (control, HF, and HFHP diets). A nonparametric one-factor analysis of variance was performed to assess the difference between the HF-high chol and HF-low chol diets). The SEM of the percentage changes from referred values was calculated as the mean of the relative SEM to the mean value for both variables. The software used was STATA version 9.1 (Stata Corp, College Station, TX).

RESULTS

Effects of high-fat and high-fat, high-protein diets

All 3 diets were well tolerated, and no problems of compliance with the diets or side effects were reported. Compliance was verified by interview, and a 49 ± 8% increase in net protein oxidation corroborated that compliance with the HFHP diet was good.

Substrates, intrahepatocelullar lipids, and liver enzymes

Compared with the control diet, the HF diet did not change substrate concentrations, except for a 26 ± 11% reduction in NEFAs ($P < 0.05$), a 61 ± 27% reduction in β-hydroxybutyrate ($P < 0.05$), and a 22 ± 14% reduction in plasma VLDL-triglycerides ($P < 0.05$) (**Figure 2**). The HFHP diet suppressed NEFAs to the same extent as did the HF diet ($P < 0.02$) but normalized plasma VLDL-triglyceride and β-hydroxybutyrate concentrations. The HF diet nearly doubled IHCL concentrations ($P < 0.02$). Compared with the HF diet, the HFHP diet led to a significant reduction in IHCLs by 22 ± 32% ($P < 0.02$) (Figure 2). AST and ALT were not altered by either diet, except for a 28 ± 15% increase in ALT with the HFHP diet ($P < 0.02$) (**Table 3**).

Insulin sensitivity

Fasting endogenous glucose production was not affected by the HF and HFHP diets. The HFHP diet increased fasting glucagon concentrations by 14 ± 9% compared with the HF diet ($P < 0.05$) but did not alter insulin concentrations. Total glucose turnover, endogenous glucose production, and the percentage inhibition of glucose production were similar under all conditions at both low and high insulin infusion rates, which indicated that the HF and HFHP diets did not significantly alter hepatic or extrahepatic insulin sensitivity (Table 3).

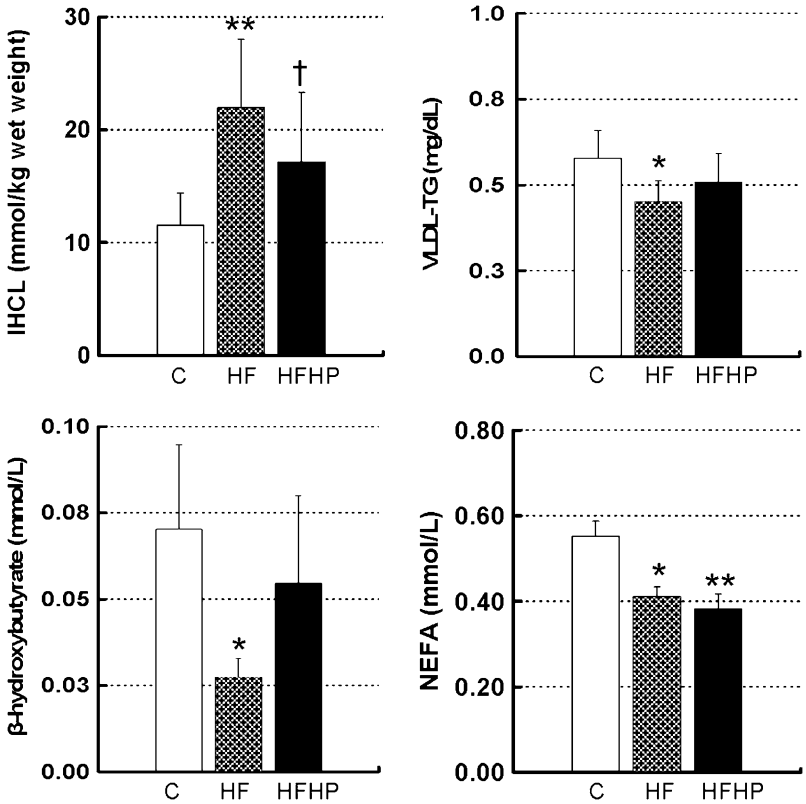

FIGURE 2. Mean (±SEM) effects of a balanced isocaloric diet (control, C), a hypercaloric high-fat (HF) diet, or a hypercaloric high-fat, high-protein (HFHP) diet on intrahepatocellular lipid (IHCL), VLDL-triglyceride (TG), plasma β-hydroxybutyrate, and nonesterified fatty acid (NEFA) concentrations in healthy men (n = 10). Wilcoxon's signed-rank test with Bonferroni's correction was used for pairwise comparisons. *·**Significantly different from control: $^{*}P < 0.05$, $^{**}P < 0.02$. †Significantly different from HF, $P < 0.02$.

TABLE 3
Body weight, glucoregulatory hormones, and insulin sensitivity ($n = 10$)[1]

				P for pairwise comparison		
	C diet	HF diet	HFHP diet	C vs HF	C vs HFHP	HF vs HFHP
Body weight (kg)	73.8 ± 2.3	73.9 ± 2.6	74.9 ± 2.3	>0.2	0.005	0.034
Glucose (mg/dL)[2]	93.8 ± 1.3	96.5 ± 2.2	93.5 ± 1.4	0.092	>0.2	>0.2
Insulin (μU/mL)[3]	8 ± 1	9 ± 1	10 ± 1	>0.2	>0.2	0.097
Glucagon (pg/mL)	56 ± 4	56 ± 5	64 ± 6	>0.2	0.169	0.022
AST (U/L)	26 ± 3	24 ± 2	26 ± 3	>0.2	>0.2	>0.2
ALT (U/L)	22 ± 3	23 ± 3	28 ± 6	0.172	0.018	0.120
Insulin sensitivity						
EGP ($mg \cdot kg^{-1} \cdot min^{-1}$)						
Basal	2.13 ± 0.12	2.17 ± 0.11	2.17 ± 0.11	>0.2	>0.2	>0.2
First step	0.82 ± 0.16	0.84 ± 0.22	0.68 ± 0.15	>0.2	>0.2	>0.2
Second step	0.04 ± 0.03	0.00 ± 0.00	0.13 ± 0.06	>0.2	>0.2	>0.2
TO ($mg \cdot kg^{-1} \cdot min^{-1}$)						
First step	2.87 ± 0.13	3.05 ± 0.18	3.19 ± 0.27	>0.2	>0.2	>0.2
Second step	5.77 ± 0.54	5.84 ± 0.59	6.25 ± 0.45	>0.2	>0.2	>0.2
IGP (%)	62 ± 8	62 ± 9	69 ± 6	>0.2	>0.2	>0.2

[1] All values are means ± SEMs. C, control; HF, high-fat; HFHP, high-fat, high-protein; TO, total glucose turnover; EGP, endogenous glucose production; IGP, inhibition of glucose production, AST, aspartate aminotransferase; ALT, alanine aminotransferase. Wilcoxon's signed-rank test with Bonferroni's correction was used for pairwise comparisons.
[2] To convert mg/dL to mmol/L, multiply by 0.0555.
[3] To convert μU/mL to pmol/L, multiply by 6.945.

Adipokines, energy metabolism, and lipid profile

The HF and HFHP diets significantly increased plasma leptin concentrations (HF: 2.7 ± 0.4 ng/mL; HFHP: 3.1 ± 0.6 ng/mL; both $P < 0.02$; control: 2.3 ± 0.4 ng/mL). Adiponectin concentrations were not altered by any of the diets (HF: 15.8 ± 2.3 μg/mL; HFHP: 15.9 ± 2.4 μg/mL; control: 14.2 ± 1.7 μg/mL; NS) (not reported in tables or figures). Energy expenditure as net carbohydrate, lipid, and protein oxidation rates were not altered by the HF diet relative to the control diet. The HFHP diet increased net protein oxidation by 49 ± 8% ($P < 0.02$) and decreased net lipid oxidation by 28 ± 13% ($P < 0.05$) but did not significantly alter net carbohydrate oxidation or energy expenditure (**Table 4**). Both the HF and HFHP diets increased total, LDL-, and HDL-cholesterol concentrations relative to the control condition ($P < 0.02$ for all). Total ($P < 0.05$) and VLDL-triglycerides ($P < 0.02$) were slightly decreased with the HF diet relative to the control diet (Table 4).

Inflammatory markers

tPAI-1 concentrations increased by 54 ± 11% with the HF diet ($P < 0.02$) but were completely normalized with the HFHP diet. In contrast, TNF-α, IL-1β, and IL-6 were not significantly altered by either diet (**Figure 3**).

TABLE 4
Energy metabolism and plasma lipid profile ($n = 10$)[1]

				P for pairwise comparison		
	C diet	HF diet	HFHP diet	C vs HF	C vs HFHP	HF vs HFHP
Energy metabolism						
Energy expenditure ($kcal \cdot kg^{-1} \cdot h^{-1}$)	1.05 ± 0.02	1.06 ± 0.03	1.06 ± 0.03	>0.2	>0.2	>0.2
Carbohydrate oxidation ($mg \cdot kg^{-1} \cdot min^{-1}$)	1.36 ± 0.16	1.49 ± 0.10	1.41 ± 0.11	>0.2	>0.2	>0.2
Lipid oxidation ($mg \cdot kg^{-1} \cdot min^{-1}$)	0.66 ± 0.09	0.64 ± 0.04	0.48 ± 0.06	>0.2	0.028	0.028
Protein oxidation ($mg \cdot kg^{-1} \cdot min^{-1}$)	0.65 ± 0.05	0.54 ± 0.03	0.97 ± 0.07	0.079	0.015	0.008
Plasma lipid profile (mg/dL)						
Total triglycerides[2]	73 ± 8	61 ± 6	68 ± 9	0.032	>0.2	0.053
VLDL triglycerides[2]	51 ± 7	40 ± 5	45 ± 7	0.017	>0.2	0.169
Total cholesterol[3]	139 ± 9	154 ± 10	160 ± 9	0.059	0.007	0.139
LDL cholesterol[3]	80 ± 7	92 ± 7	96 ± 6	0.013	0.007	>0.2
HDL cholesterol[3]	36 ± 2	42 ± 3	41 ± 2	0.006	0.005	>0.2

[1] All values are means ± SEMs. C, control; HF, high-fat; HFHP, high-fat, high-protein. Wilcoxon's signed-rank test with Bonferroni's correction was used for pairwise comparisons.
[2] To convert mg/dL to mmol/L, multiply by 0.01129.
[3] To convert mg/dL to mmol/L, multiply by 0.02586.

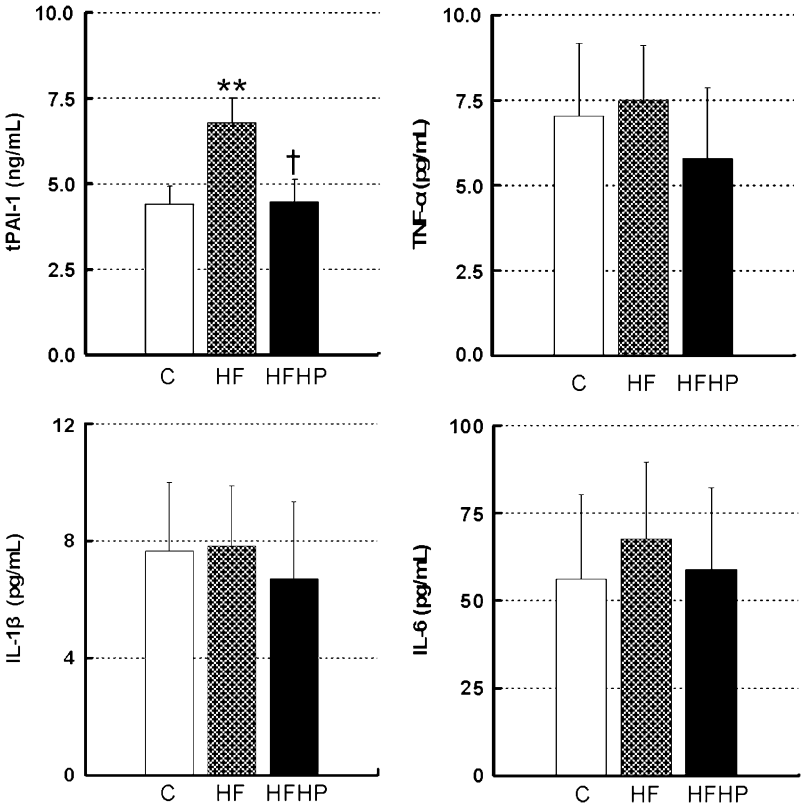

FIGURE 3. Mean (±SEM) effects of a balanced isocaloric diet (control, C), a hypercaloric high-fat (HF) diet, or a hypercaloric high-fat, high-protein (HFHP) diet on plasma concentrations of tissue-type plasminogen activator inhibitor-1 (tPAI-1), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 in healthy men (n = 10). Wilcoxon's signed-rank test with Bonferroni's correction was used for pairwise comparisons. **Significantly different from control, $P < 0.05$. †Significantly different from HF, $P < 0.02$.

Gene expression in adipose tissue

The HF diet increased significantly the expression of key genes related to the lipogenic pathway, such as those coding for the master regulator SREBP-1c (+70 ± 12%), hexokinase II (+32 ± 10%) ($P < 0.02$), and fatty acid synthase (+133 ± 20%) ($P < 0.05$). In contrast, the expression of most genes related to lipolysis and lipid oxidation (*HSL*, *ATGL*, *PDE-3b*, *CD36*, and *CPT-1*) was not affected, whereas the expression of *PLIN*, a protein involved in lipid droplet coating, decreased ($P < 0.02$). There was also no effect of the HF diet on genes related to cholesterol utilization and metabolism (*ABCA-1* and *LDLR*), except for *LXR*α (+17 ± 7%). The expression of adiponectin and peroxisome proliferator–activated receptor γ (PPARG) was also not modified after the HF diet. The HFHP diet tended to further increase the expression of lipogenic genes, particularly sterol regulatory element-binding protein-1c, increased importantly the expression of *CD36*, reduced the expression of *CPT1*—a gene related to fatty acid oxidation ($P < 0.02$ for both). The HFHP diet increased *PPARG* mRNA concentrations (+45 ± 15%; $P < 0.02$) and significantly increased the expression of *LXR*α ($P < 0.02$) and *LDLR* ($P < 0.05$) (Table 2).

Plasma bile acid concentrations

Total BA concentrations were 64 ± 22% and 50 ± 24% higher after the HFHP and HF diets, respectively, than after the control diets. Although not significant, this difference prompted us to proceed with the measurement of individual BAs by GC-MS (**Figure 4**). Compared with the control and HF diets, the HFHP diet specifically increased plasma cholic acid concentrations by 269 ± 27% ($P < 0.02$) and 248 ± 27% ($P < 0.05$), respectively. A similar, albeit less pronounced profile was observed for chenodeoxycholic acid (+125 ± 19% HFHP compared with control, $P < 0.02$; +108 ± 23% HFHP compared with HF, $P < 0.05$) and deoxycholic acid (+63 ± 19% HFHP compared with control, $P < 0.05$; +46 ± 21% HFHP compared with HF, $P < 0.05$). The degree of conjugation was also assessed under all 3 dietary conditions. Conjugated cholic acid concentrations decreased by 40 ± 21% with the HFHP diet compared with the HF diet (HFHP: 28.6 ± 5.9%; HF: 47.8 ± 9.2%; $P < 0.05$). Circulating BA concentrations were not correlated with IHCL concentrations under any of the dietary conditions tested.

Effects of high-fat, high-cholesterol compared with high-fat, low-cholesterol diets

Concentrations of the substrates AST, ALT, and IHCL after the HF-high chol and HF-low chol diets were similar to those observed in the first study after the HF diet, except for glucose and BA concentrations ($P < 0.02$) (**Table 5**). Body weight did not change between pre– and post–high fat supplementation (ΔHF-low chol: −0.02 ± 0.01 kg; ΔHF-high chol: +0.4 ± 0.1 kg). Also, there was no difference in any of the variables tested between the HF-high chol and HF-low chol diets (Table 5).

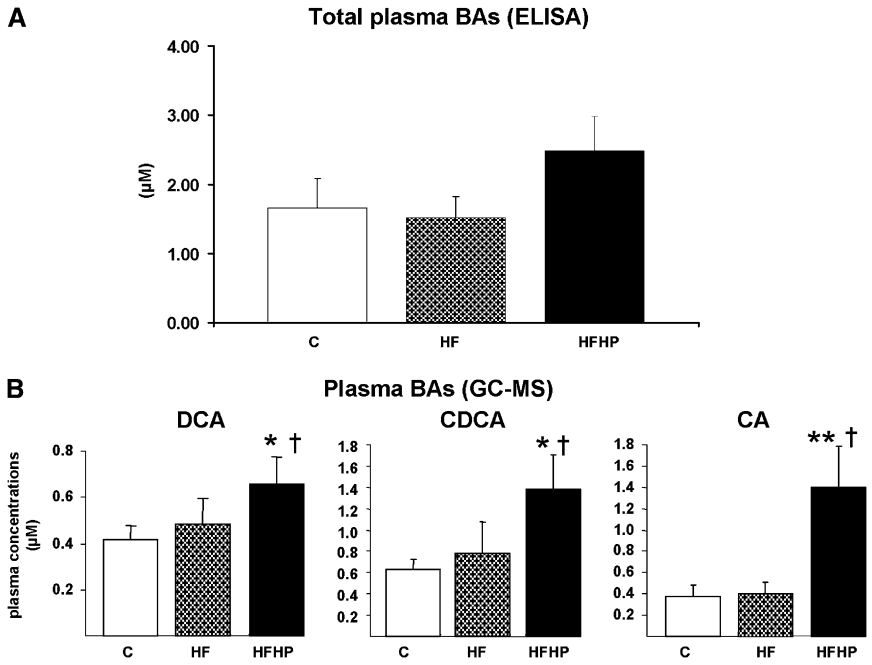

FIGURE 4. A: Mean (±SEM) effects of a balanced isocaloric diet (control, C), a hypercaloric high-fat (HF) diet, or a hypercaloric high-fat, high-protein (HFHP) diet on total bile acids (BAs). B: Mean (±SEM) concentrations of deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and cholic acid (CA) in healthy men (n = 10). ELISA, enzyme-linked immunosorbent assay; GC-MS, gas chromatography–mass spectrometry. Wilcoxon's signed-rank test with Bonferroni's correction was used for pairwise comparisons. *,**Significantly different from control: $^{*}P < 0.05$, $^{**}P < 0.02$. †Significantly different from HF, $P < 0.02$.

DISCUSSION

High fat is known to induce intrahepatic fat deposition in rodents (17). Consistent with these findings, we also observed that high fat feeding produced a significant 90% increase in IHCLs after only 4 d in healthy volunteers. There was, however, no change in glucose production or its suppression by hyperinsulinemia. This may appear surprising considering the association between IHCL concentrations and hepatic insulin sensitivity observed in many studies (17–19) and suggests that IHCLs are not directly related to hepatic insulin resistance (20). Surprisingly, we observed a major 26% reduction in fasting plasma NEFAs. The analysis of adipose tissue documented an increased expression of fatty acid synthase, whereas the expression of genes involved in lipolysis (*HSL*, *ATGL*, *PDE*3, and *perilipin*) and lipid oxidation (*CPT1*) were not significantly affected during the HF diet. Similar observations were also reported by Meugnier et al (20), who found a decrease in NEFAs concomitant with a marked induction of genes related to the lipogenic pathway in skeletal muscle in response to 4 wk of a high-fat diet in healthy subjects. Altogether, these results indicate that, in humans, short-term high-fat feeding shifts the balance between lipogenesis and lipolysis toward lipogenesis in adipose tissue, thus stimulating the deposition of excess dietary lipids. Under such conditions, plasma NEFAs concentrations remains low, and a high flux of plasma NEFAs can therefore not be held responsible for intrahepatic fat accumulation. We observed instead a major 61 ± 27% decrease in fasting plasma β-hydroxybutyrate concentrations, which strongly suggests that a suppression of β-oxidation and ketogenesis contributed to the accumulation of triglycerides in liver cells. Altogether, these results suggest that hypercaloric, high-fat intakes stimulate net fat storage in adipose tissue and in the liver, which results in IHCL deposition and in adipose tissue triglyceride storage.

Insulin sensitivity was not altered during hyperinsulinemia with a high-fat diet, which contrasts with the hepatic insulin resistance observed in rodents very early after they are fed a high-fat diet (17), but agrees with the data observed after 4 wk of high-fat diet in a group of young healthy individuals (20). Because the study was performed on an outpatient basis, it is possible that poor diet compliance would be responsible. However, this appears unlikely because urinary urea excretion increased as expected during protein overfeeding. Changes in body fat were not monitored, but the fat overload provided in this study is not expected to significantly change body fat content over such a short period (21). Hepatic insulin resistance may nonetheless develop with a longer exposure to a hypercaloric high-fat diet and lead to a substantial increase in body fat.

Increasing the dietary protein intake produced a 22% decrease in IHCLs. A high protein intake also increased plasma β-hydroxybutyrate to normal concentrations, but did not reverse the suppression of plasma NEFAs. In adipose tissue, the effects of the HFHP diet were quite similar to those observed after the HF diet alone, with an induction of lipogenic genes, consistent with a switch of adipose tissue metabolism toward net fat storage. This pattern even tended to be enhanced with the HFHP diet. Furthermore, the HFHP diet increased the expression of *PPARG*. Given the role of *PPARG* in adipose tissue differentiation, and the metabolic effects of *PPARG* activation by thiazolidinediones (22), it is possible to speculate that, in the long term, enhanced expression of *PPARG* by dietary protein may prevent HF-induced insulin resistance by promoting fat deposition in adipose rather than ectopic fat depots.

TABLE 5
Effects of a high-fat (HF) diet with a high cholesterol (HF-high chol) or a low cholesterol (HF-low chol) content ($n = 6$)[1]

	HF-low chol	HF-high chol	Pairwise comparison
Body weight (kg)	73.4 ± 2.2	73.2 ± 2.1	>0.2
Glucose (mg/dL)[2]	85.5 ± 1.5	85.8 ± 1.3	>0.2
NEFA (mmol/L)	0.280 ± 0.025	0.378 ± 0.053	0.120
Bile acids (μmol/L)	2.80 ± 0.24	2.62 ± 0.39	>0.2
Hepatic metabolism			
IHCL (μmol/kg wet wt)	27 ± 11	25 ± 10	>0.2
AST (U/L)	22 ± 2	25 ± 3	>0.2
ALT (U/L)	24 ± 2	25 ± 3	>0.2
Plasma lipid profile (mg/dL)			
Total triglyceride[3]	97 ± 30	89 ± 16	>0.2
Total cholesterol[4]	175 ± 12	182 ± 8	>0.2
HDL cholesterol[4]	54 ± 6	55 ± 9	>0.2

[1] All values are means ± SEMs. IHCL, intrahepatocellular lipid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NEFA, nonesterified fatty acid. A nonparametric one-factor ANOVA was used for paired comparisons.
[2] To convert mg/dL to mmol/L, multiply by 0.0555.
[3] To convert mg/dL to mmol/L, multiply by 0.01129.
[4] To convert mg/dL to mmol/L, multiply by 0.02586.

The HF and HFHP diets had concordant effects in adipose tissue (ie, a switch toward lipogenesis and energy storage), but different effects in the liver (an increase in IHCLs with the HF diet and a decrease in IHCLs with the HFHP diet). This suggests that a high protein intake primarily affected liver metabolism through a stimulation of hepatic β-oxidation and ketogenesis. The mechanisms by which protein exerted these effects remain speculative, but several tracks can be proposed for future investigation. Firstly, high-protein intake increases total amino acid degradation at the whole body level. Because the bulk of amino acid catabolism takes place in the liver and is an energy-requiring process, a high protein intake may have merely increased hepatic lipid oxidation through an increase in hepatic energy expenditure (23). Second, protein increased plasma BAs, and BAs may inhibit lipogenesis and favor hepatic lipid oxidation through stimulation of liver X receptor and farnesoid X receptor (7, 24). Because the HFHP diet was also enriched in cholesterol, we initially considered the possibility that this increase was merely secondary to stimulation of BA synthesis by enhanced dietary cholesterol intake. We therefore performed an additional study that compared the effects of diets providing the same overload of saturated fat, one high and one low in cholesterol. The results indicate that the dietary cholesterol content did not significantly affect IHCL and BA concentrations; hence, the increase in BAs was most likely attributed to dietary proteins. Third, a high protein intake causes an increased day-long secretion of glucagon, which may stimulate hepatic ketogenesis (25, 26). Finally, numerous metabolic genes are regulated by amino acids or their metabolites (27), and it is possible that the expression of genes involved in lipid synthesis and oxidation, or in lipoprotein metabolism, were altered in liver cells after a high protein intake (28).

A high-fat diet is known, in rodents, to increase the release of proinflammatory cytokines through activation of the transcription factors IκB kinase β and nuclear transcription factor κB (29). Furthermore, these proinflammatory cytokines are thought to play a role in the development of fat-induced insulin resistance (30, 31). It was therefore a secondary aim of this study to evaluate the effects of HF and HFHP intakes on several markers of inflammation. The HF diet significantly increased tPAI-1 but failed to alter several plasma proinflammatory cytokines, whereas the HFHP diet seemed to restore initial concentrations. tPAI-1, a fibrinolytic inhibitor, is produced by several cell types in the organism, eg, hepatic stellate cells and adipocytes. An increase in tPAI-1 in adipose tissue has been consistently associated with insulin resistance and obesity (32) in several large epidemiologic studies and, hence, may be an early marker of insulin resistance (33). In addition, tPAI-1 is thought to be involved in the development of hepatic fibrosis (34). In patients with nonalcoholic fatty liver disease (NAFLD), elevated plasma tPAI-1 was strongly correlated with liver fat, whereas tPAI-1 expression was observed in liver cells. Furthermore, liver tPAI-1 expression was linked to an increase in TNF-α and TNF-α receptor II, which suggests a link between intrahepatic fat accumulation and inflammation (35). Our observation therefore suggests that dietary protein modulates fat-induced inflammation.

Our study had several limitations that need to be considered. First, the effects of a high protein intake were documented in a group of healthy subjects in whom an increase in IHCLs was produced by short-term hypercaloric high-fat feeding. Although this procedure almost doubled baseline IHCLs, the increase in liver fat was small compared with the fatty liver infiltration observed in obese patients with NAFLD. Furthermore, the mechanisms responsible for fat liver deposition may not be identical in NAFLD patients and in our experimental model (36). Second, an increase in protein intake may possibly improve liver metabolism while exerting unwanted effects on other systems at the same time. In this regard, deleterious effects of protein on glomerular filtration rate and kidney function are of special concern (37, 38).

In summary, our findings indicate that a high protein intake significantly prevents intrahepatic fat deposition induced by a short-term hypercaloric, high-fat diet in humans. It remains to be evaluated whether modulating the dietary protein intake may be included in therapeutic or preventive strategies for NAFLD without adverse events.

The authors' responsibilities were as follows—MB and LT: supervised all the data in the study and responsibility for the integrity of the data; MB, DF, K-AL, PS, and LT: designed and developed the protocol; MB, DF, NS, and PS: recruited subjects, carried out the clinical trial, and conducted the data analysis; RK, MI, PV, and CB: measured IHCL by ^{1}H-MRS, developed the technique, and analyzed the data; CD and HV: analyzed adipose tissue in the biopsy samples and analyzed the data; BC, MC, and MK: analyzed plasma bile acid concentrations and analyzed the data; MB, DF, K-AL, PS, and LT: analyzed and interpreted the data; and MB, PS, and LT: wrote the manuscript, which was reviewed and modified by all authors. None of the authors reported any conflicts of interest.

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