Increasing Protein Intake Modulates Lipid Metabolism in Healthy Young Men and Women Consuming a High-Fat Hypercaloric Diet1–3

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Abstract

The objective of this study was to evaluate the effect of increasing protein intake, at the expense of carbohydrates, on intrahepatic lipids (IHLs), circulating triglycerides (TGs), and body composition in healthy humans consuming a high-fat, hypercaloric diet. A crossover randomized trial with a parallel control group was performed. After a 2-wk run-in period, participants were assigned to either the control diet [n = 10; 27.8 energy percent (en%) fat, 16.9 en% protein, 55.3 en% carbohydrates] for 4 wk or a high-fat, hypercaloric diet (n = 17; >2 MJ/d) crossover trial with 2 periods of 2 wk, with either high-protein (HP) (37.7 en% fat, 25.7 en% protein, 36.6 en% carbohydrates) or normal-protein (NP) (39.4 en% fat, 15.4 en% protein, 45.2 en% carbohydrates) content. Measurements were performed after 2 wk of run-in (baseline), 2 wk of intervention (period 1), and 4 wk of intervention (period 2). A trend toward lower IHL and plasma TG concentrations during the HP condition compared with the NP condition was observed (IHL: 0.35 ± 0.04% vs. 0.51 ± 0.08%, P = 0.08; TG: 0.65 ± 0.03 vs. 0.77 ± 0.05 mmol/L, P = 0.07, for HP and NP, respectively). Fat mass was significantly lower (10.6 ± 1.72 vs. 10.9 ± 1.73 kg; P = 0.02) with the HP diet than with the NP diet, whereas fat-free mass was higher (55.7 ± 2.79 vs. 55.2 ± 2.80 kg; P = 0.003). This study indicated that an HP, high-fat, hypercaloric diet affects lipid metabolism. It tends to lower the IHL and circulating TG concentrations and significantly lowers fat mass and increases fat-free mass compared with an NP, high-fat, hypercaloric diet. This trail was registered at www.clinicaltrails.gov as NCT01354626. J. Nutr. 144: 1174–1180, 2014.

Introduction

In recent years, high-protein (HP)4 diets have become increasingly popular as a way to reduce obesity and improve metabolic risk factors in the general population (1). Rodent data revealed that the metabolic adaptation to an HP diet included a down-regulation of lipogenesis and increased gluconeogenesis and glycogen synthesis in the liver (2,3). Increasing dietary protein intake also has the potential to reduce intrahepatic lipids (IHLs) (4). Indeed, our group recently showed that mice fed an HP, high-fat diet [50 energy percent (en%) protein, 35 en% fat], had a lower liver lipid content compared with mice given a normal-protein (NP), high-fat diet (5). IHL accumulation can be regarded as the hepatic manifestation of metabolic syndrome and is correlated with hypertriglyceridemia and a reduced suppression of insulin on hepatic glucose production, contributing to hyperglycemia.

Hepatic steatosis, the first stage of so-called non-alcoholic fatty liver disease, is quite prevalent in Western societies, particularly in societies with increased obesity and/or type 2 diabetes mellitus prevalence (6).

Dietary manipulation has the potential to change lipid storage in the liver (4). In humans, a high-fat diet increased IHLs after only a few days (7). This was associated with an increase in markers of insulin resistance (7). A low-fat diet reduced liver fat accumulation and fasting blood insulin concentrations (7). Studies evaluating the effect of increasing protein intake on IHLs in humans are limited, although initial
results appear promising (8–10). Increasing protein intake to 23 en% in a whole-diet approach blunted the effect of a 4-d high-fat diet on IHLs in healthy human volunteers (8). Whey protein supplementation (60 g/d) for 4 wk, without changing the habitual diet, substantially lowered IHLs in obese women (9). Moreover, supplementation with a mixture of the amino acids leucine, isoleucine, valine, lysine, and threonine at 6.77 g, 3 times per day, for 6 d blunted the effect of a fructose-induced increment in IHLs (10).

The main objective of the present study was to evaluate the effects of increasing protein intake on markers of lipid metabolism in healthy humans. Protein intake was increased at the expense of carbohydrates in a high-fat, hypercaloric diet (HD) with unsaturated and saturated fats and dietary fiber comparable across diets. Markers of lipid metabolism considered were circulating TGs, body composition, and especially IHLs. Last, we evaluated adipose tissue gene expression that might reflect the uptake and use of circulating TGs. A group of healthy participants given a balanced control diet (CD) was included as a reference.

Participants and Methods

Participants. Twenty-nine healthy, young, lean Caucasian men and women participated in this strictly controlled dietary intervention study. Participants had no family history of type 2 diabetes and were not taking any medication. The experimental protocol was approved by the Medical Ethical Committee of Wageningen University. The sample size calculation was based on the following: in young healthy men, the addition of protein to the diet attenuated the increase in IHLs by 22 ± 32% (P < 0.02) (8). In addition, we assumed an SD of 0.7% in our healthy young population without overweight based on data from literature (11), and we aimed to include 10 participants per condition.

Study design. The total dietary intervention lasted for 6 wk. All participants started with a 2-wk run-in period on a weight-maintaining CD (27.8 en% fat, 16.9 en% protein, 55.3 en% carbohydrates) to get familiarized with the dietary regimen and to adapt to the same diet. Thereafter, participants were randomly assigned to either the HD group (n = 19) or the CD reference group (n = 10). Stratified randomization was performed by an independent research assistant using a computer-generated table of random numbers. Participants were unaware of their assigned diets until the end of the study, although participants could have guessed their study status based on the diets. After run-in, participants in the HD group were overfed with 2 MJ/d for the following 4 wk of intervention. Within the HD group, a randomized crossover design consisting of 2 periods of 2 wk was applied: 1) a 2-wk HP intervention; and 2) a 2-wk NP intervention (Fig. 1). Participants started randomly with either the NP condition or the HP condition and crossed to the other condition after 2 wk. Parallel to the HD group, the reference group continued on the weight-maintaining CD for another 4 wk.

Measurements of IHL content and fasting blood glucose, insulin, and TG concentrations were performed after 2 wk of run-in (baseline), 2 wk of intervention (period 1), and 4 wk of intervention (period 2). Body composition, adipose tissue gene expression, and resting energy expenditure (REE) were measured after each intervention period (periods 1 and 2) (Fig. 1). All measurements were performed after an overnight fast at the research facilities of Wageningen University.

Dietary intervention. Diets were strictly controlled. During the entire 6-wk study period, participants consumed foods covering 90% of their designated needs. The remaining 10% had to be selected from a predefined free-choice list. All food items chosen from the free-choice list were recorded. Participants came to the research facility every working day during lunchtime. They consumed a hot meal, which was weighed to the nearest gram by the research dietitians. Breakfast, evening bread meals, snacks, beverages, and all meals for the weekends were provided in take-home packages. Participants were carefully instructed how to prepare the take-home meals. All the foods were precalculated for macronutrient composition and energy content for each individual participant by the research dietitians. Participants were instructed to eat all the provided food and not to change their physical activity pattern for the duration of the study.

Body weight was measured two times per week on a calibrated scale. During the run-in period, energy intake was adjusted in case of weight change. Duplicate portions of a mean daily energy amount of 11 MJ of each intervention diet were collected each day, pooled per intervention group, and analyzed for energy, measured by bomb-calorimetry, macronutrients, FAs, and dietary fiber composition (Table 1). Protein contribution was 16.9 en%, 15.4 en%, and 25.7 en% for the CD, NP diet, and HP diet, respectively. Protein was of mixed origin: dairy, animal, and plant sources, with the contribution of dairy protein more prominent in the HP condition (~55% compared with 30% for both other diets). The contribution of SFAs was kept at ~10 en%, as dietary guidelines recommend, and accounted for 9.5 en% in the CD and 11.5 en% in the HD in both the HP and NP conditions. Dietary compliance was assessed by completion of a diary by the participants, return of emptied food packages, and measurement of 24-h urine urea concentration by kinetic UV assay (Roche Diagnostics). Physical activity was controlled through a regular check by the dietitians, who informed participants about the allowed physical activity. Moreover, a diary on

![Figure 1](https://academic.oup.com/jn/article-abstract/144/8/1174/4571765?redirectedfrom=fulltext)

**FIGURE 1** Study design and composition of the diets. Measurements are indicated as follows: 1) after 2 wk of run-in (Baseline); 2) after 2 wk of intervention (Period 1); and 3) after 4 wk of intervention (Period 2). BW, body weight; CD, control diet; HD, high-fat, hypercaloric diet; HP, high-protein condition (within the high-fat, hypercaloric diet); IHL, intrahepatic lipid; NP, normal-protein condition (within the high-fat, hypercaloric diet); REE, resting energy expenditure.
physical activity had to be filled out every day. Physical activity amount was calculated as total energy expenditure/basal metabolic rate. Total energy expenditure was estimated from energy intake when fed in energy balance during the 2-wk run-in, and basal metabolic rate was estimated by the Schofield equation (12).

**IHLs.** IHL content was measured by image-guided single-voxel spectroscopy, a quantitative version of 1H-magnetic resonance spectroscopy. Measurements were performed on a 3.0 tesla magnetic resonance scanner (Syngo MR B17; Siemens) with a flexible receiver and transmitter body coil. In brief, the voxel (30 × 30 × 20 mm) was placed in the right hepatic lobe, avoiding big structures and the proximity of subcutaneous adipose tissue, using scout images in all 3 planes. Shimming of the magnetic field to optimize magnetic field homogeneity was performed manually. Spectra were acquired using a point-resolved spectroscopy sequence (bandwidth, 1200 Hz; echo time, 30 ms; repetition time, 4000 ms; 1024 points; 32 averages).

Participants were asked to breathe to the rhythm of the measurement and to be at end-expiration when they heard the sound of gradient switching. An H2O signal was suppressed using preps (60 Hz; flip angle, 90°). In addition, a reference spectrum was acquired without H2O suppression to determine H2O signal intensity (16 averages). At visits 2 and 3, the voxel was placed visually at the same location in the liver using the images of the first visit. Post-processing of the spectral data were done by magnetic resonance user interface software (jMRUI version 4.0, build 162) (13). After manual phasing of the spectra, the observed lipid peak [methylen (CH2)] and H2O peak were fitted by a Gaussian line shape. For the lipid spectra, the residual H2O peak was removed using a Hankel Lanczos singular values decomposition filter.

The lipid and H2O signals were analyzed using the AMARES (advanced method for accurate, robust, and efficient spectral fitting) algorithm, which enables the inclusion of prior knowledge (14). The lipid area and of adipokines was investigated. The mRNA expression of all genes was normalized to ribosomal protein, large, P0 (RPLP0) expression.

**Statistical analyses.** All data are expressed as means ± SEMs. Data were judged on normality; if data were not normally distributed, a log transformation was done before analyzing the data. We analyzed the data using a random intercept model (SAS PROC MIXED), including diet and period as independent variables. Additionally, the effect of protein intake within the HD group was assessed using a paired-samples t test to test the equality of the means between the HP and NP conditions (HP vs. NP). All P values were considered significant if P < 0.05. Statistical analyses were performed with SAS 9.2 (2002–2008; SAS Institute).

**Results**

**Participants.** Two participants dropped out for personal reasons after the run-in period, before starting the intervention diets. Only the data from the remaining 27 participants (17 participants in the HD group and 10 participants in the CD group) were analyzed. Baseline participant characteristics can be found in Table 2. Participant recruitment and screening were done from August 2011 until November 2011. The intervention had phased inflow of participants and was performed from October 2011 until March 2012. No differences in any of the baseline variables were observed between the HD and CD groups. The diets were well received by the participants and compliance was good, as measured by urrea intake.

**TABLE 1** Nutrient composition of the diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>CD</th>
<th>NP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ/d</td>
<td>12.1</td>
<td>14.5</td>
<td>14.4</td>
</tr>
<tr>
<td>Protein, en%</td>
<td>16.9</td>
<td>15.4</td>
<td>25.7</td>
</tr>
<tr>
<td>Fat, en%</td>
<td>27.8</td>
<td>39.4</td>
<td>37.7</td>
</tr>
<tr>
<td>SFA, en%</td>
<td>9.6</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>MUFA, en%</td>
<td>9.1</td>
<td>13.8</td>
<td>11.9</td>
</tr>
<tr>
<td>PUFA, en%</td>
<td>7.7</td>
<td>11.9</td>
<td>12.1</td>
</tr>
<tr>
<td>Cholesterol, mg/MJ</td>
<td>26.9</td>
<td>23.9</td>
<td>25.1</td>
</tr>
<tr>
<td>Carbohydrate, en%</td>
<td>55.3</td>
<td>45.2</td>
<td>36.6</td>
</tr>
<tr>
<td>Fiber, g/MJ</td>
<td>3.6</td>
<td>3.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

1 Values are means of duplicate portions. CD, control diet; en%, energy percent; HP, high-protein condition (within the high-fat, hypercaloric diet); NP, normal-protein condition (within the high-fat, hypercaloric diet).

2 Carbohydrate content includes dietary fiber.

**Body composition, REE, plasma analyses, and insulin sensitivity.** Body composition was measured by DXA (Lunar Prodigy Advance, 70 Kev, enCORE version 13.40; GE Healthcare) to assess fat mass and fat-free mass.

REE was measured by indirect calorimetry with a ventilated hood using a canopy (Deltatrac II; Datex-Ohmeda, GE Healthcare). Participants lay down on a bed for ≥20 min before measurements were performed. A canopy was placed over their head, and the measurement started. After 10 min, oxygen consumption (VO2) was recorded for 5 min, and these values were averaged to calculate REE according to the formula 3.9 (VO2) + 1.1 (0.85 × VO2)/1000 (17), and a respiration quotient of 0.85 was assumed because carbon dioxide was not recorded.

Fasting blood samples were collected from an antecubital vein in EDTA-containing tubes. Glucose was analyzed by the hexokinase method (Roche Diagnostics), TGs were determined photometrically (Roche Diagnostics), and insulin was measured by ELISA (Merodia). Insulin and glucose concentrations were used to calculate insulin sensitivity using the HOMA-IR: [glucose (mmol/L) × insulin (μU/L)]/22.5 (18).

**Adipose tissue gene expression.** Subcutaneous adipose tissue samples were obtained caudally from the umbilicus under local anesthetic (1% lidocaine) with a small liposuction cannula after an overnight fast. The samples were rinsed with PBS to eliminate blood, immediately frozen in liquid nitrogen, and stored at −80°C. Total RNA was isolated with TRIzol reagent (Invitrogen), purified (RNeasy Micro kit; Qiagen), and quantified (Nanodrop ND 1000; Nanodrop Technologies). Quality was determined by Bioanalyzer (Agilent 2100 Bioanalyzer; Agilent Technologies). Total RNA with a RNA integrity number of 7.2 ± 0.12 was then reverse transcribed (cDNA synthesis kit; Promega) and analyzed by standard qPCR (SensiMix SYBR; Bioline) on a CFX384 Real-Time System (Bio-Rad). Primers were designed using Beacon design version 6.7 (Premier Biosoftware). Primer sequences are available on request. Expression of key genes involved in FA uptake, de novo lipogenesis (DNL), lipid storage, lipolysis, and β-oxidation and of adipokines was investigated. The mRNA expression of all genes was normalized to ribosomal protein, large, P0 (RPLP0) expression.

**TABLE 2** Participant characteristics

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (males), n</td>
<td>27 (19)</td>
</tr>
<tr>
<td>Age, y</td>
<td>22.8 ± 0.74</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.5 ± 0.29</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>68.8 ± 1.58</td>
</tr>
<tr>
<td>IHL, % of H2O peak</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>Fasting plasma TGs, mmol/L</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.20 ± 0.11</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/L</td>
<td>5.15 ± 0.48</td>
</tr>
<tr>
<td>Physical activity, TEE/BMR</td>
<td>1.74 ± 0.04</td>
</tr>
</tbody>
</table>

1 Data are means ± SEMs unless otherwise indicated. BMR, basal metabolic rate; IHL, intrahepatic lipid; TEE, total energy expenditure.
concentration in the 24-h urine samples after periods 1 and 2. After the CD, the urine urea concentration was 222 ± 26.7 mmol/L (period 1) and 234 ± 25.7 mmol/L (period 2) (P = 0.7). Within the HD group, the urine urea concentration was substantially higher after the HP condition compared with the NP condition (440 ± 29.9 vs. 272 ± 25.7 mmol/L, respectively; P < 0.0001).

**IHLs and fasting TGs.** IHL measurements were analyzed in 8 participants of the CD group and 15 participants of the HD group, because not all spectra could be considered as a result of poor quality of the spectra and/or very low IHL content. Mean IHL concentrations were low at baseline (0.40 ± 0.05% of the H2O peak).

In general, IHLs tended to increase over time (P = 0.08) (Fig. 2A), particularly for those consuming the CD. When comparing all 3 groups, no clear effect of diet was seen (P = 0.18) (Fig. 2A). However, a trend toward a lower IHL content on the HP condition compared with the NP condition was observed (P = 0.08) (Fig. 2B).

Overall, fasting TG concentration was significantly different between the diets (P = 0.003), with no effect of time (P = 0.79) (Fig. 3A). After the HP HD, participants had a significantly lower TG concentration compared with those on the CD (P = 0.0007). Furthermore, the fasting TG concentration tended to be lower with the HP condition compared with the NP condition (P = 0.07) (Fig. 3B).

**Body weight, body composition, insulin sensitivity, and energy expenditure.** Body weight did not differ significantly between dietary groups (P = 0.30), but a trend toward an increase in time was observed (P = 0.09) due to a slight expected increase in the HD groups (Supplemental Table 1). Body weight was not significantly different between the HP and NP conditions (P = 0.22) (Table 3). However, fat-free mass was significantly higher after 2 wk of the HP condition with the NP condition (P = 0.003) (Table 3), whereas fat mass was significantly lower with the HP condition compared with the NP condition (P = 0.02) (Table 3). No differences were observed between all 3 dietary groups or both protein conditions in REE, fasting glucose, fasting insulin, or insulin sensitivity as measured by HOMA-IR (Supplemental Table 1, Table 3).

**Adipose tissue gene expression.** The response to the HP and NP conditions of a selection of key genes from different lipid metabolic pathways was assessed in subcutaneous adipose tissue of 16 participants; in 1 participant, not enough adipose tissue was obtained (Supplemental Table 2). No differences were observed in the expression of genes involved in FA uptake [lipoprotein lipase (LPL), angioptin-like 4 (ANGPTL4), FA binding protein 4 (FABP4), cluster of differentiation 36 (CD36), and Caveolin 1 (CAV1)], nor for genes involved in DNL [glucose-transporter type 4 (GLUT4), FA synthase (FASN), insulin receptor substrate 1 (IRS1), and sterol regulatory element binding transcription factor 1, transcript variants 1 and 2 (SREBF1a and SREBF1c)] or lipid storage [diacylglycerol O-acyltransferase 2 (DGAT2) and peroxisome proliferator-activated receptor-γ (PPARγ)]. Also, gene expression of hormone-sensitive lipase (HSL) and carnitine palmitoyltransferase 1B (CPT1B), leptin, and adiponectin were not different between protein conditions.

**Discussion**

The main objective of the present study was to investigate the effect of increasing protein intake, at the expense of carbohydrates, on markers of lipid metabolism in healthy adults. The results indicated that, after 2 wk, an HP diet compared with an NP diet substantially affected body composition, i.e., lowered fat mass, increased fat-free mass, tended to lower circulating TGs and reduced IHLs. Accordingly, it appeared that, in the HD group, the surplus of energy derived from fat did not result in increased IHLs, plasma TGs, or adipose tissue, nor did it result in changes in glucose metabolism.

The results showed a considerable effect of diet on fasting blood TG concentrations, with lower TGs after the HD compared with the CD. A trend toward a lower TG concentration was seen in the HP condition compared with the NP condition in the context of the HD. Furthermore, the lower TG concentration was not the only change during the HP condition in the present study. Remarkably, a substantial reduction of adipose tissue mass was seen also when comparing the HP condition with the NP condition. This occurred without any changes in adipose tissue gene expression of genes involved in lipid uptake or storage. Besides this reduction of fat mass, fat-free mass turned out to be 0.5 kg higher during the HP condition compared with the NP condition, despite a comparable total body mass. Although lifestyle was not completely controlled, participants did not report any extra physical activity. An increase of fat-free mass was observed previously during an HP
HD (>40% energy, 25 en% protein) (19). It was stated that the surplus of energy in that diet was used for diet-induced thermogenesis due to the high protein turnover and protein storage for increasing lean body mass. In the present study, other metabolic markers, including insulin, glucose, and insulin sensitivity, did not differ between the dietary conditions, although HP diets are known to have an insulinitropic effect and modulate glucose metabolism (20).

It is known that IHLs can be influenced rapidly by dietary changes. Previous studies showed that a high-fat diet increased IHLs by 35%, and similarly, a low-fat diet decreased IHLs by 20% in only 2 wk (7). Additionally, a higher protein intake was able to attenuate the effect of a high-fat diet in 4 d (8). Similarly, we also observed that increasing the protein content of the diet to 26 en% was associated with an ~30% lower IHL content, although the difference was not significant (P = 0.08). However, it should be noted that at the start of the dietary intervention, all our participants had a very low IHL content (~0.5%). Additionally, to generate more statistical power, we did not separately analyze male and female participants. Some studies suggested that females have a lower IHL content compared with males (21,22), although others had no sex-related differences in IHLs (23). The suggestion of lower IHLs when increasing protein intake is consistent with previous observations in both humans (8–10) and rodents (3,5), and different mechanisms can be proposed.

One mechanism that could explain the observed effects on lipid metabolism is an increase of energy needs in the liver due to HP feeding. However, in the present study, whole-body REE was measured, and no differences in energy expenditure between dietary conditions were detected. Conversely, in other studies, REE did increase after 1.5 d of adaptation to an HP diet consisting of 30 en% protein (24) and after 8 wk of an HP diet consisting of 25 en% of protein (19). It is likely that postprandial and 24-h energy expenditure may have been increased with the HP diet, but this was not assessed in the present study. Another mechanism could be a reduced DNL rate after an HP diet. An increased flux of amino acids reaching the hepato-portal area in HP diet conditions might induce an increased amino acid deamination and production of amino acid–derived carbon skeletons. From mouse data it was suggested that the carbon skeletons derived from amino acids are poorly transferred to glucose and not converted to FAs. Therefore, DNL rates might be lower or even absent after an HP diet (5). A decreased rate of hepatic lipogenesis after an HP diet was also observed in rats; this was in parallel with a lower hepatic expression of Fasn, which is the rate-limiting enzyme of hepatic lipogenesis (3). Moreover, expression of sterol regulatory element binding protein-1c (Srebplc) was found to be lower; this modulated insulin and thereby also prevented hepatic lipid accumulation (3,25). In humans, it was also concluded previously that hepatic DNL is highly sensitive to dietary changes (26). The observed lower blood TGs in the present study might be a result of lower DNL due to the lower carbohydrate content of the HP diet compared with both the NP diet and CD. Finally, an increase in the release of bile acids in response to the high-fat diet could play a role. Bile acids are formed in the liver out of cholesterol and can affect lipid, glucose, and energy metabolism via the bile acid receptors farnesoid X receptor and G protein-coupled bile acid receptor 1. Activation of the farnesoid X receptor and G protein-coupled bile acid receptor 1 receptors by the secreted bile acids were shown to reduce liver lipid concentrations by increasing liver lipid oxidation (27). However, no increase in bile acid concentrations was observed previously after an HP diet (8). In the present study, bile acids were not measured.

This study was conducted using a realistic dietary approach during a strictly controlled dietary intervention instead of supplementing protein. Compliance was ensured by means of diaries and urinary urea excretion. This approach gave the opportunity to study the effect of nutrition in a real-life setting under controlled conditions. The crossover design used did not have a washout period, because we considered 2 wk a long enough period to adapt to a diet. Adding a control group gave

TABLE 3 Effect of increasing dietary protein content on metabolic markers in the HD group

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>HP</th>
</tr>
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<tbody>
<tr>
<td>Body weight, kg</td>
<td>69.5 ± 1.80</td>
<td>69.8 ± 1.85</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>55.1 ± 2.79</td>
<td>55.7 ± 2.78*</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>10.9 ± 1.73</td>
<td>10.6 ± 1.72*</td>
</tr>
<tr>
<td>REE, kcal/min</td>
<td>1.11 ± 0.04</td>
<td>1.11 ± 0.04</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.04 ± 0.08</td>
<td>5.05 ± 0.09</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/L</td>
<td>4.21 ± 0.62</td>
<td>3.95 ± 0.63</td>
</tr>
<tr>
<td>HOMA-IR, mmol/L × μU/L/ml</td>
<td>0.95 ± 0.14</td>
<td>0.90 ± 0.15</td>
</tr>
</tbody>
</table>

1 Data are means ± SEMs and are pooled from the NP and HP conditions, n = 17. *Significantly different compared with the NP condition, P < 0.05. HD, high-fat, hypercaloric diet; HP, high-protein condition (within the high-fat, hypercaloric diet); NP, normal-protein condition (within the high-fat, hypercaloric diet); REE, resting energy expenditure.
insight into IHL responses to a normal, weight-maintaining diet. This was of interest because both IHL and circulating TG concentrations were higher on this presumably healthy diet compared with both HD conditions. Therefore, it suggests not only a positive effect of increasing protein but also a more negative effect of high carbohydrate intake on these metabolic markers. In the experimental design, participants were young, lean, and healthy. Thus, they are expected to have a flexible metabolism that is able to adapt easily, possibly within 14 d—the duration per intervention period—to dietary changes. This might explain the relatively small changes observed in our study compared with the somewhat larger changes in IHLs observed previously in an intervention of 4 d only (8). Those results might be a consequence of participants not being fully adapted to the diet yet. Furthermore, the fat content in the HD conditions was just <40 en%, which is considered a high-fat content, because dietary guidelines advise a fat intake <30 en%. The contribution of SFAs was at a normal level (<10–12 en%) and comparable between CD and HD. However, national food surveys revealed that 46% of the Dutch population do have a fat intake >35 en% (28). The habitual dietary fat intake of our participants resembled this (data not shown). Therefore, we cannot exclude that this relatively high habitual fat intake could have attenuated the consequences of our experimental HD, although the contrast with the low-fat CD, which contained 27 en%, was considered to be big enough, and, in addition, the participants were overfed by 2 MJ/d. For future research, it might be important to study participants who are already suffering to some degree from metabolic syndrome or have a fatty liver and consequently decreased metabolic flexibility. This could give information on the effect of HP diets on reversing increased IHL content and possibly the positive effects on other metabolic markers, such as insulin sensitivity and body composition.

Hence, it might be concluded that, by increasing protein content, some of the additional energy provided by the HD was expended through protein and amino acid processing in the liver and in other peripheral tissues. In addition, the results of this study may suggest that the effect on lipid metabolism observed in the HP HD condition most likely originates from decreased liver lipogenesis and not from a higher release of TGs from the liver to the circulating blood, or from a direct effect of HP on adipose tissue.

In conclusion, our study indicated that the HP HD condition resulted in changes in lipid metabolism, namely a trend toward lower IHL content and circulating TGs, substantially lower fat mass, and higher fat-free mass compared with the NP HD condition. These effects may possibly be attributed to lower DNL and to increased energy needs by the liver when consuming HP diets.

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References


