Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein

Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein[1–3]

Lucas Lindeboom, Christine I Nabuurs, Matthijs KC Hesselink, Joachim E Wildberger, Patrick Schrauwen, and Vera B Schrauwen-Hinderling

ABSTRACT

Background: Fat accumulation in nonadipose tissue is linked to insulin resistance and metabolic diseases. Earlier studies have shown that hepatic lipid accumulation can occur after 4 d of a high-fat diet in humans, and this fat accumulation can be blunted by the ingestion of additional proteins.

Objectives: In this study, we explored whether a single high-fat meal increased the lipid content in liver and skeletal muscle as measured by using in vivo proton magnetic resonance spectroscopy (^{1}H-MRS) and whether the addition of protein can modulate the postprandial ectopic lipid storage.

Design: Intrahepatic lipid (IHL) and intramyocellular lipid (IMCL) concentrations were determined by using ^{1}H-MRS before and 3 and 5 h after a high-fat with added protein meal (61.5% of energy from fat) or a high-fat without added protein meal (mean ± SEM: 51.1 ± 7.9 g of protein; 191.9 ± 9.9 kcal added) in a randomized crossover study. IHL and IMCL concentrations were converted to absolute concentrations (g/kg wet weight) by using water as an internal reference.

Results: Nine lean, healthy subjects [6 men and 3 women; mean (±SD) age: 22.7 ± 3.0 y; mean body mass index (in kg/m^2): 21.8 ± 1.8] were included in this study. IHL concentrations increased ∼20% ($P < 0.01$) at 3 h after the meal and did not further increase after 5 h. In contrast, IMCL concentrations were not altered during the postprandial period ($P = 0.74$). The addition of protein to a single high-fat meal did not change the postprandial accumulation of fat in the liver ($P = 0.93$) or skeletal muscle ($P = 0.84$).

Conclusions: In this study, we showed that a single energy-dense, high-fat meal induced net lipid accumulation in the liver, which was detected by using in vivo ^{1}H-MRS. This noninvasive approach might bring new opportunities to study postprandial hepatic lipid dynamics. The addition of protein did not change the ectopic lipid retention after a single high-fat meal. This trial was registered at clinicaltrials.gov as NCT01709643. *Am J Clin Nutr* 2015;101:65–71.

Keywords ^{1}H-MRS, IHL, IMCL, postprandial, protein

INTRODUCTION

Increased ectopic fat accumulation, which is the storage of triglycerides in nonadipose tissue such as liver and muscle, is associated with reduced insulin sensitivity and other metabolic disorders (1–3). Nutritional studies have revealed that the ectopic fat content can change rapidly and is affected by diet composition. The consumption of a high-fat diet ≥3 d increases intrahepatic lipid (IHL)[4] (4, 5) and intramyocellular lipid (IMCL) (6, 7) concentrations, and evidence is emerging that a high-fructose diet for 1 wk also augments IHL concentrations (8, 9).

It was shown that adding protein to a high-fat (HF) diet blunts the accumulation of hepatic lipids (4). Similarly, a mixture of essential amino acids reduced the high-fructose–induced lipid accumulation in liver (9). The exact mechanism that underlies these protective effects of proteins on ectopic fat storage remains unclear; it is not known whether the addition of protein to the diet has an acute effect on ectopic lipid storage or whether effects are only visible in the longer term.

Studies that investigated the effect of protein intake on lipid deposition in the liver have used proton magnetic resonance spectroscopy (^{1}H-MRS) to measure IHL concentrations. ^{1}H-MRS is a well-established tool for the noninvasive quantification of IHL as well as IMCL (10–12). As indicated, pronounced effects on IHL and IMCL concentrations have been reported after relatively short-term dietary interventions of only a few days; however, studies reporting changes in IHL and IMCL concentrations after a single meal are lacking. One previous study reported no change after a single meal when the hepatic lipid content was investigated at a field strength of 1.5 Tesla (T) (13). We anticipated that the detection of differences after a single meal with ^{1}H-MRS would be facilitated by improving the detection accuracy. Therefore, in the current study, the accuracy of IHL and IMCL measurements

was improved by acquiring spectra with a higher signal-to-noise ratio (SNR) by increasing the number of averaged spectra, choosing a larger volume of interest, and applying the technique on a 3-T magnetic resonance (MR) scanner. Furthermore, we improved the postprocessing of acquired data from the liver by automatic phasing and alignment routines of individual spectra to reduce (breathing) motion artifacts.

In this study, we explored whether postprandial changes in hepatic and skeletal muscle lipid contents are detectable after a single HF meal by using in vivo ^{1}H-MRS. In addition, we investigated whether additional protein blunts postprandial lipid deposition after a single meal.

SUBJECTS AND METHODS

Subjects

Nine healthy, young, lean subjects (6 men and 3 women) participated in the study. Subjects had a mean ($\pm$SD) age of 22.7 $\pm$ 3.0 y, mean BMI (in kg/m^2) of 21.8 $\pm$ 1.8, and mean body fat percentage of 15.1% $\pm$ 8.4% (10.7% $\pm$ 1.9% for men and 23.8% $\pm$ 4.3% for women). All subjects were nonsmokers. All measurements were performed at the Maastricht University Medical Center and were approved by the institutional medical ethics committee. Written informed consent was obtained from all subjects before inclusion.

Study design

All subjects were invited to Maastricht University for a screening visit and filled in a questionnaire regarding their medical history, general health, eating habits, and physical activity. Body composition was determined by using hydrostatic weighting.

Two days before test days, subjects were asked to refrain from strenuous physical activity and alcohol consumption. On evenings before both test days, subjects consumed a standardized meal (total energy content: 505 kcal; 33% of energy from fat, 53% of energy from carbohydrates, and 14% of energy from protein) that was provided. Subjects were asked to remain fasted overnight (from 2200) until they reported at the university at 0700.

In a randomized crossover study, subjects consumed a high-energy, HF meal or a high-energy, high-fat with added protein (HFP) meal at 0830. ^{1}H-MRS was used to determine IHL and IMCL concentrations before and 3 and 5h after the meal. The

2 different measurement days were separated by a washout period $\geq$1 wk. Random assignment was applied by using an online research random assignment tool.

After baseline MRS measurements, a catheter was inserted in the median cubital vein, which was used for blood collections throughout the testing day. A first 10-mL blood sample was taken before the meal. Additional blood samples were taken 30, 60, 120, 180, 240, and 300 min after the meal. A schematic overview of the test day is depicted in **Figure 1**.

Body composition

Hydrostatic weighing and the simultaneous measurement of lung volume were performed to determine body composition in the morning in the fasted state. The equation of Siri (14) was used to calculate fat and fat-free mass.

HF test meal

The HF meal consisted of sausage rolls (61.5% energy from fat, 31.6% energy from carbohydrates, and 6.9% energy from proteins). The total energy content of the meal equaled 50% of the subjects' daily recommended caloric intakes. Daily recommended caloric intake was calculated on the basis of lean body mass as measured during the screening visit by using the Cunningham (15) formula and a correction factor of 1.3 for physical activity during the test day.

For the HFP meal, protein powder (Protifar; Nutricia) was dissolved in water, and subjects drank this in addition to the HF meal. The amount of protein added was calculated to ensure that the total energy content from the protein in the meal equaled 20%, which was in accordance with previously published work (4). Subjects drank water ad libitum during both test days.

Blood analysis

Before and after meals, blood was collected in tubes containing 140 μL 0.2 M EDTA. Samples were immediately centrifuged at high speed, and the plasma was frozen in liquid nitrogen and stored at $-80°C$ for later analyses. Plasma concentrations of insulin, glucose, free fatty acids (FFAs), and total and free glycerol were determined. The plasma insulin concentration was determined by using an RIA (Linco Research). Glucose, FFAs and free and total glycerol were measured by using enzymatic assays automated on a Cobas Fara/Mira analyzer
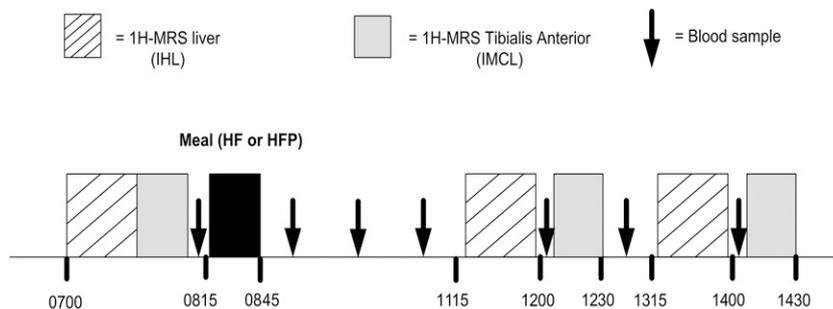
FIGURE 1 Flowchart of study design. Subjects were asked to consume a standardized evening meal on the evening before MR measurements. Subjects reported to the university fasted at 0700, after which first baseline MR measurements were started. Between 0815 and 0845, subjects consumed either an HF meal alone or an HFP meal. Three and 5 h postprandial, MR measurements were repeated. Seven blood samples of 10 mL were taken. HF, high fat; HFP, high fat with added protein; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; MR, magnetic resonance; ^{1}H-MRS, proton magnetic resonance spectroscopy.

[glucose: hexokinase method (Roche); FFAs: Wako Nefa C test kit (Wako Chemicals); glycerol: Enzytec glycerol kit (R-Biopharm)]. Total glycerol and free glycerol were used to calculate triacylglyceride concentrations. All analyses were performed in duplicate. For all metabolites, the incremental AUC (iAUC) was calculated separately.

^{1}H-MRS acquisition

^{1}H-MRS spectra were acquired on a 3.0-T whole-body MR system (Achieva 3Tx; Philips Healthcare). Liver spectra were obtained with the subject in a supine position, head first. Images and MR spectra were obtained by using a 32-element sense cardiac/torso coil (Philips Healthcare). [1]H-MR spectra were obtained from a $40 \times 40 \times 40mm^3$ voxel placed centrally in the right lobe of the liver by using point-resolved spectroscopy volume selection (16) with a repetition time (TR) of 4000 ms, echo time of 32.5 ms, and 2048 sample points. The total measurement was divided into 3 blocks of equal duration. In these blocks, 32 spectra, with the number of spectral averages (NSA) equal to 2, were acquired, which eventually led to 192 individual acquisitions and a total of 96 spectra. Multislice T_2-weighted MR images were acquired before the spectroscopy protocol to facilitate the accurate placement of the voxel inside the liver. Special care was taken to ensure that the voxel was placed at the same location during repeated measurements. The water resonance was suppressed by using a chemical shift selective water-suppression technique (17).

To keep the TR constant, no gating was applied, but the subject was instructed to breathe exactly at the 4s-rhythm of the sequence (expired state at gradient switching). The breathing pattern was carefully monitored with the use of a pressure-sensitive sensor on the belly of the subject, and online monitoring of the breathing sensor signal assured that the acquisition was indeed always during the expired phase. If necessary, subjects were further coached via the intercom. Shimming was performed by using second-order FASTMAP-based shimming (18). The total duration of the protocol was $\sim$30 min.

For IMCL measurements, subjects were positioned supine and feet first in the magnet bore with the left leg parallel to the main magnetic field and the foot constrained by 2 sandbags. A 2-element flexible-surface coil was placed over the lower leg muscle (centered 15 cm below the patella). Also, for muscle measurements, T_2-weighted images were acquired for the accurate positioning of the voxel. [1]H-MR spectra were obtained from a $14 \times 14 \times 40$-mm^3 volume (point-resolved spectroscopy sequence localization) within the tibialis anterior by using the following acquisition variables: TR: 2000 ms; echo time: 38 ms; NSA: 128 and 2048 sample points. Water suppression was performed by using a selective excitation pulse followed by dephasing gradients. An additional outer volume suppression was used to eliminate the contamination of the signal from subcutaneous adipose tissue. Shimming was again performed by using second-order FASTMAP-based shimming. The IMCL measurement, including imaging and spectroscopy, was performed in $\sim$15 min.

For both liver and muscle, a second nonwater-suppressed spectrum was acquired subsequently from the same volume, which enabled us to use the water signal as an internal reference (liver: NSA, 2×8; muscle: NSAs, 8).

^{1}H-MRS spectral analysis

[1]H-MR spectra obtained from the liver were analyzed by using a home-built MATLAB (version 7, August 2007; The Mathworks Inc.) script. This script allowed for the reduction of motion artifacts by automatic phasing and alignment of all spectra individually. Furthermore, the script automatically removed spectra with a full width at half maximum or peak height that was 1.5 SD above or below the mean of all acquired spectra. Eventually, fitting of the averaged CH_2 or water resonance in the spectral domain was performed by using a combination of a Gaussian and Lorentzian line shape (50% Gaussian:50% Lorentzian).

For the analysis of muscle measurements, all spectra were analyzed and fitted in the time domain by using the nonlinear least-squares AMARES algorithm in the jMRUI software package (www.mrui.uab.es) (19). Spectra were apodized with a 3-Hz Lorentzian line shape. Previous knowledge was set such that the extramyocellular lipid (EMCL) methylene and methyl peaks were fitted with a combination of a Lorentzian and Gaussian line (equal amplitude). Methylene and methyl IMCL peaks were fitted with a single Lorentzian line shape. The water peak was fitted separately while applying the same apodization and again with a Lorentzian line shape.

Both IHL and IMCL concentrations were expressed in mmol/kg wet weight (kgww) by using water as an internal reference and based on the assumptions as published before for liver and muscle, respectively (11, 12, 20, 21). For the liver measurement, T_2 relaxation times of 60 ms for the methylene peak and 26 ms for water were used (20, 21). For muscle measurements, these values were 91 and 29 ms, respectively (22). T_1 relaxation correction was only applied for the water measurement of the IMCL determination, and T_1 was assumed to be 1387 ms (22).

Statistics

The statistical analysis was performed with SPSS Statistics 20 software (released 2011; IBM Corp.). Subject characteristics are expressed as means $\pm$ SDs, whereas all other results are expressed as means $\pm$ SEMs.

To test for differences in blood plasma metabolite concentrations, IHL, and IMCL, a repeated-measures ANOVA was performed. A post hoc analysis was performed by using Bonferroni post hoc tests. A paired-samples Student's t test was performed to test for differences between the iAUC of blood plasma metabolites on HF and HFP meals. Pearson's correlation coefficients were calculated to test for associations between postprandial IHL differences and the iAUC of blood plasma metabolites. $P < 0.05$ was considered statistically significant in all tests.

RESULTS

Subjects and meal

Subject characteristics are summarized in **Table 1**. The total energy content in the HF meal was 1169 $\pm$ 180 kcal. Because of the addition of protein, the total energy content of the HFP meal was higher (1361 $\pm$ 210 kcal; $P < 0.01$). On average, subjects consumed 80.0 $\pm$ 12.3 g fat, 92.3 $\pm$ 14.2 g carbohydrates, and 20.0 $\pm$ 3.1 g protein in the HF meal. For the HFP meal, 51.1 $\pm$ 7.9 g protein was added.

TABLE 1
Subject characteristics

	Values
Age, y	22.7 ± 3.0[1]
Sex, M:F	6:3
Body mass, kg	69.2 ± 10.8
BMI, kg/m^2	21.8 ± 1.8
Fat percentage	15.1 ± 8.4
M	10.7 ± 1.9
F	23.8 ± 4.3

[1]Mean $\pm$ SD (all such values).

Postprandial IHL and IMCL (^{1}H-MRS)

A typical example of liver spectra collected from one subject at baseline and 3 and 5 h after the HF meal is shown in **Figure 2**. In all hepatic spectra, we were able to identify methyl and methylene peaks of IHL. The CV between the 3 separate recorded blocks at one time point was $3.6 \pm 0.4\%$ (i.e., without repositioning of the subject).

In **Figure 3**, a muscle spectrum of one of the subjects is shown, which shows well-separated methylene peaks of EMCL and IMCL. One spectrum (baseline IMCL of HFP) was excluded because of an insufficient spectral quality.

IHL concentrations (**Figure 4**A) increased from baseline to 3 h postprandially (from 3.13 ± 0.68 to 3.72 ± 0.71 g/kgww after the HF meal and from 2.88 ± 0.30 to 3.66 ± 0.38 g/kgww after the HFP meal, $P < 0.01$). There were no significant differences in IHL between 3 and 5 h ($P = 0.99$), but IHL concentrations were still elevated with respect to baseline (5-h value: 3.68 ± 0.71 g/kg with the HF meal and 3.77 ± 0.44 g/kgww with the HFP meal; $P < 0.01$). The IHL concentration and postprandial increase in IHL was similar for both meals ($P = 0.93$ and 0.43, respectively).

IMCL concentrations (Figure 4B) at baseline were 2.57 ± 0.38 g/kgww for the HF group and 2.80 ± 0.35 g/kgww for the HFP group and were not significantly different after the meals ($P = 0.74$; 3-h value of 2.70 ± 0.42 g/kgww and 5-h value of 2.74 ± 0.48 g/kgww for the HF group and 3-h value of 2.79 ± 0.38 g/kgww and 5-h value of 2.81 ± 0.32 g/kgww for the HFP group). The addition of protein to the diet did not have a significant effect on IMCL concentrations in skeletal muscle ($P = 0.84$), and we did not show an effect on the postprandial IMCL time course ($P = 0.51$).

There were no significant differences in IHL ($P = 0.89$) and IMCL ($P = 0.34$) concentrations between male and female subjects. Furthermore, sex did not influence the postprandial

increase in IHL ($P = 0.90$) and IMCL ($P = 0.80$), and the response to the addition of protein was similar ($P = 0.60$ for IHL and $P = 0.14$ for IMCL).

Blood plasma concentrations

In **Figure 5**, plasma concentrations of insulin (Figure 5A), FFAs (Figure 5B), glucose (Figure 5C), and triacylglycerol (Figure 5D) in time are depicted.

Insulin showed the expected meal-induced increase ($P < 0.01$) and returned to baseline within the time course of the experiment for both conditions. There was no significant interaction effect ($P = 0.64$) and no main effect for the meal ($P = 0.07$). Insulin concentrations were significantly higher at $t = 30$, 60, 120, 180, and 240 min compared with at baseline ($P < 0.05$). The iAUC of insulin on HFP was significantly higher than on HF ($P < 0.01$).

Also, for FFA concentrations, there was a significant time effect ($P < 0.01$) but no interaction effect ($P = 0.28$) or main effect for the meal ($P = 0.09$). FFA concentrations were significantly reduced at $t = 60$ and 120 min after the meal than at baseline ($P < 0.05$). The iAUC of FFAs was not different between HF and HFP meals ($P = 0.12$).

For glucose concentrations, there was again no significant interaction effect ($P = 0.27$) or meal effect ($P = 0.41$), whereas a significant time effect ($P = 0.03$) was observed. However, the post hoc analysis revealed that none of the individual time points were significantly different compared with at baseline. The iAUC of glucose on HF and HFP was not different ($P = 0.47$).

Triacylglycerol concentrations were significantly increased at $t = 60$, 120, 180, and 240 min ($P < 0.05$) after the meals than at baseline (P-overall time effect < 0.01). No interaction effect was observed ($P = 0.36$), and a main effect of the meal was not observed ($P = 0.61$). However, the iAUC of triacylglycerol was significantly higher for the HFP meal than HF meal ($P = 0.01$).

Correlations

With the HFP meal, the increase in IHL was positively correlated with the AUC of insulin and triacylglycerol after 3 h (for insulin: Pearson's $r = 0.74$, $P = 0.02$; for triacylglycerol: $r = 0.83$, $P < 0.01$) and 5 h (for insulin: $r = 0.85$, $P < 0.01$; for triacylglycerol: $r = 0.80$, $P < 0.01$). We did not show these same significant correlations for plasma metabolites with the IHL increase with the HF meal alone.
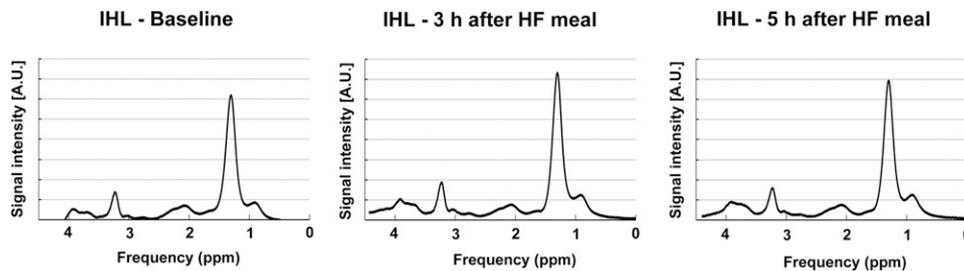
FIGURE 2 Typical example of spectra collected from one subject in the liver (IHL) before and after the HF meal. A total of 192 individual acquisitions were collected from the liver in a voxel of $40 \times 40 \times 40$ mm. Spectra were aligned and phased automatically, and spectra with movement artifacts were excluded. To show the meal-induced response, spectra in this figure were scaled to the water peak area of the corresponding reference scan. A.U., arbitrary units; HF, high fat; IHL, intrahepatic lipid.

FIGURE 3 Typical example of a spectrum acquired in one subject from the muscle (IMCL). A total of 128 spectra were collected in a voxel of 14 × 14 × 40 mm and averaged without additional postprocessing. The EMCL-CH_2 (around 1.5 ppm) and IMCL-CH_2 resonance (at 1.28 ppm) are well separated. EMCL, extramyocellular lipid; IMCL, intramyocellular lipid.

DISCUSSION

In this study, we showed that a single HF meal led to an increase in IHL as measured by using in vivo ^{1}H-MRS at 3 T. The observed increase in liver fat occurred during t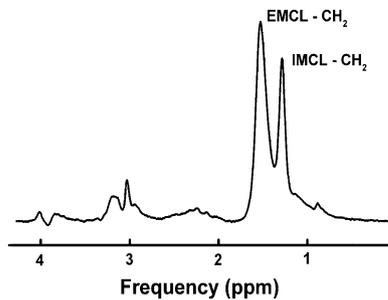he first 3 h after the meal, and the IHL concentration remained elevated after 5 h. In contrast to the increase in liver fat, we did not show any significant differences on IMCL concentrations after the same HF meal. The addition of protein to the HF meal did not affect postprandial IHL and IMCL dynamics and concentrations.

A previous study that used H-MRS at 1.5 T failed to detect differences in IHL concentrations at 4 h after a single HF meal that contained 50 g fat (13). In the current study, we showed that it was possible to detect the net postprandial lipid increase in the liver with ^{1}H-MRS by performing measurements at a higher field (3 T) and using an extensive postprocessing of acquired spectra, thereby reducing (breathing) motion (frequency and phase) artifacts. Subjects were asked to breathe in a rhythm of 4 s (equal to the TR). To ensure subjects were able to comply with this rhythm for a longer period, the measurement was divided in 3 equal blocks. Between these blocks, subjects were free to breathe in their own rhythm. This setup enabled us to average a total of 192 spectra, which ensured a high SNR. Because measurements were performed in 3 blocks, the frequency and phase alignment of individual spectra were of crucial importance. Because we chose a relatively large voxel (64 cm^3), we focused on acquiring spectra with a high SNR. The large voxel inherently led to minimization of the influence of inhomogeneity in liver-fat distribution, and it also minimized the intra-measurement variability during the day (repositioning of the voxel). Notably, the HF meal in the current study contained a higher amount of fat (80 g on average) than the 50 g fat in the previous study mentioned.

Because it has been suggested that the addition of protein to the diet blunts lipid deposition in the liver as caused by either HF overfeeding for 4 d (4) or fructose overfeeding for 6 d (9), we were interested to see if protein would diminish lipid retention in the liver already after a single meal. Our results did not show different IHL concentrations when protein was added to a single HF meal. This result implicates that the net result of storage and oxidation of lipids in the liver in the postprandial state is not different after the addition of protein to a single HF meal. The mechanism that underlies the protective effect of protein on the longer term remains to be elucidated but most likely does not involve direct postprandial effects. Protein may interfere with de novo lipogenesis (DNL), lead to increased VLDL secretion, or enhance whole-body or organ-specific lipid oxidation. It has also been speculated that dietary protein induces the expression of peroxisome proliferator–activated receptor γ, which has a role in adipose tissue differentiation, possibly funneling fatty acids to storage in adipose tissue rather than to ectopic sites.

One limitation of the applied ^{1}H-MRS methodology was that only the total lipid content was measured (i.e., only the net effect of fat influx, DNL, and oxidation could be determined). It was not possible to distinguish the direct contribution from dietary fatty acids by using ^{1}H-MRS. We chose to measure IHL concentrations at 3 and 5 h after the meal on the basis of 2 considerations. First, previous research has indicated that the first appearance of dietary fatty acids in VLDL was shown at 180 min after meal intake (23), indicating that at least part of the lipids from the meal were taken up by the liver at that time point. Furthermore, studies that used labeled dietary lipids, either in (in)direct carbon (^{13}C)-magnetic resonance spectroscopy (24, 25) or fluorine (^{18}F) positron emission tomography (26), showed that the maximum lipid retention in the liver was reached ~5 h after the meal. Nevertheless, the accuracy of ^{1}H-MRS measurements and the protocol of this study opens a new
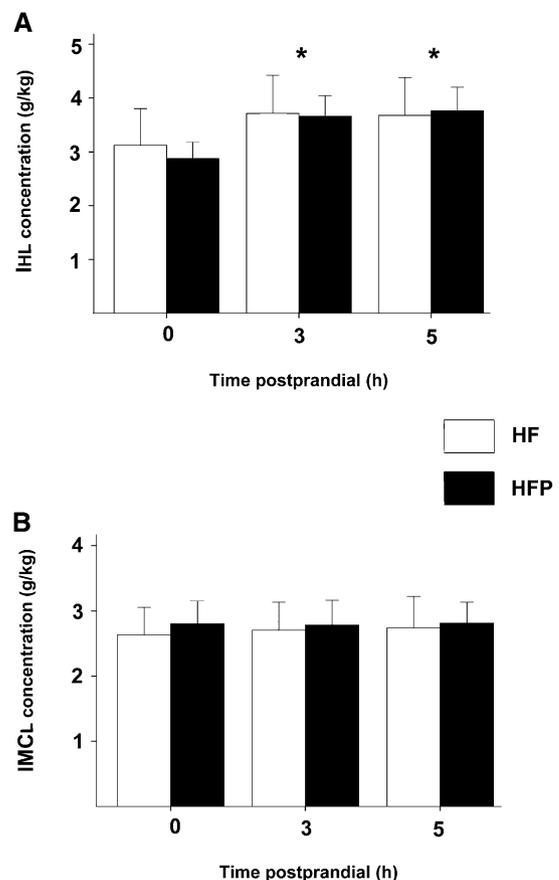
FIGURE 4 Mean (±SE) IHL (A) and IMCL (B) concentrations measured at baseline and 3 and 5 h postprandially either after an HF or HFP meal. All concentrations are expressed in g/kgww tissue. IHL concentrations increased significantly after the meal (P-time < 0.01), but no differences were shown between HF and HFP groups (P-interaction = 0.43). No significant differences were shown in IMCL concentrations after meals (P-time = 0.84, P-interaction = 0.51). *Significant differences (post hoc of main effect of time, P < 0.05). HF, high fat; HFP, high fat with added protein; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; kgww, kg wet weight.

FIGURE 5 Mean (±SE) blood plasma metabolite concentrations in time. Insulin (A), free fatty acid (B), glucose (C), and triacylglycerol (D) concentrations are shown for both meals (HF: so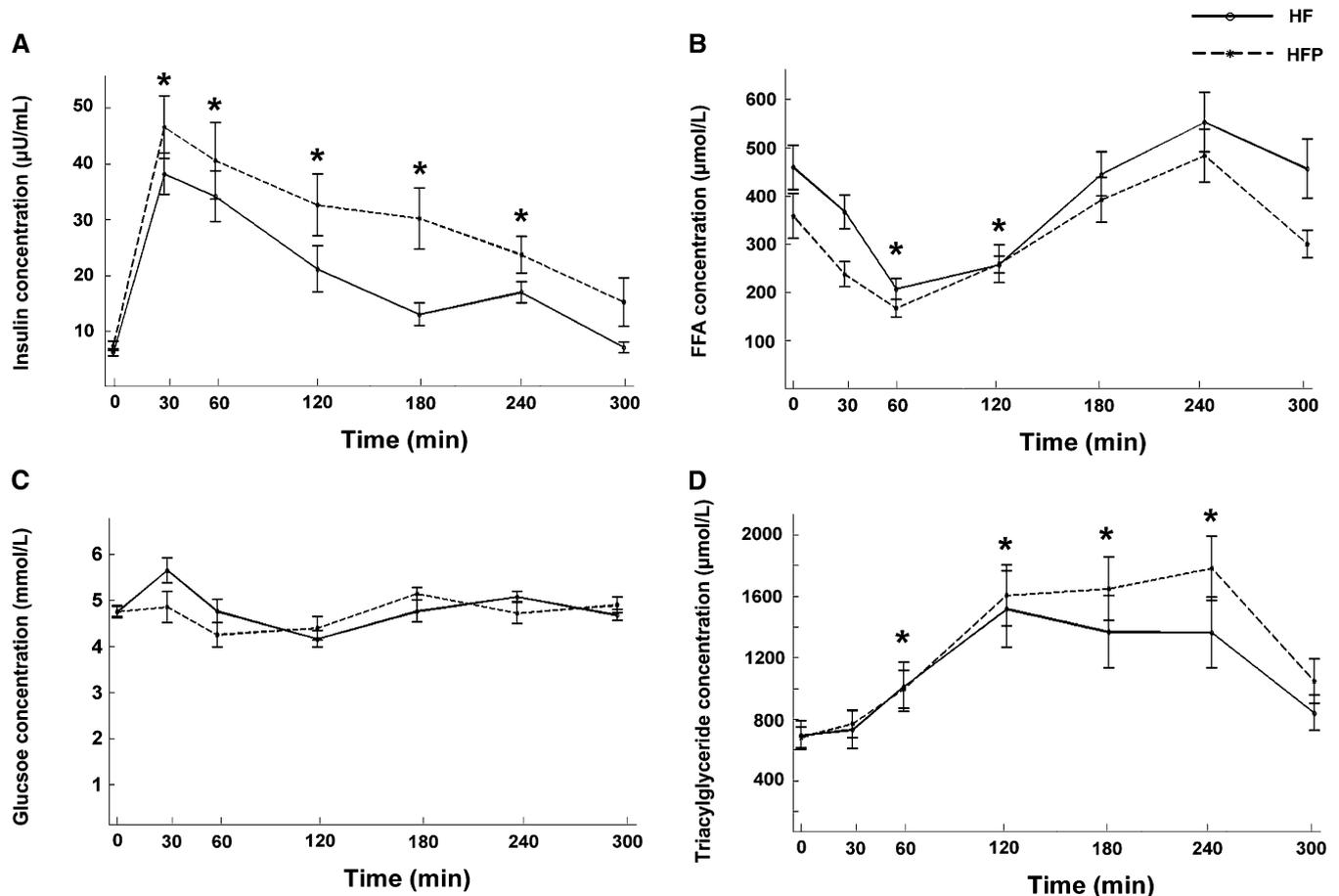lid lines and HFP: dashed lines). A significant time effect was shown for all metabolites (P-time < 0.01 for insulin, FFA, and triacylglycerol; P-time = 0.03 for glucose). None of the metabolite curves were significantly different after the HF meal compared with the HFP meal (insulin: P-interaction = 0.51; FFA: P-interaction = 0.64; glucose: P-interaction = 0.27; triacylglycerol: P-interaction = 0.36). *Concentrations significantly different from those at baseline (post hoc of main effect of time, P < 0.05). FFA, free fatty acid; HF, high fat; HFP, high fat with added protein.

window, to our knowledge, on investigating effects on post-prandial lipid retention in vivo within the same subject.

The addition of protein to the HF meal induced a higher iAUC of insulin that with the HF meal alone. This increased insulin response on the addition of protein is in line with previous reports that showed that protein can stimulate insulin secretion (27–29). In addition to the increased iAUC of insulin, we also showed an elevated iAUC of triacylglycerol when protein was added, which was correlated with the postprandial increase in IHL. The main reason for the higher iAUC of triacylglycerol after the HFP meal was shown in elevated triacylglycerol concentrations in the late-postprandial phase, whereas triacylglycerol concentrations after the HF meal seemed to return to baseline after 180 min. These results may have indicated that there was increased secretion of VLDL with the HFP meal, whereas high insulin concentrations may potentially increase DNL. As mentioned, such effects could not be determined by using ¹H-MRS, but both effects could have contributed to the IHL concentration in a counterbalanced way.

Although very little is known about postprandial changes in the IMCL content, it has been shown that IMCL concentrations can be influenced rapidly by intravenous lipid challenges in com-bination with hyperinsulinemia (6, 30). In our study setup, we performed ¹H-MRS in the tibialis anterior muscle directly after

the IHL measurement. Differences in IMCL concentrations in the tibialis anterior muscle were reported after a 3-d HF diet, whereas this increase was absent in the soleus muscle (6). The IMCL measurement in the tibialis anterior muscle is furthermore facilitated by the parallel muscle-fiber orientation. The separa-tion of EMCL and IMCL lipid compartments is maximal when the muscle is parallel to the main magnetic field (12). However, IMCL concentrations did not change after the HF or HFP meal. Note that studies that showed a rapid increase in IMCL [e.g., after lipid infusion (6, 30), submaximal exercise (31), or fasting (32)] all reported concomitant increased FFA concentrations. In contrast, after a single HF meal, plasma FFA concentrations were not elevated. Therefore, these results may suggest that skeletal muscle is more sensitive to elevated plasma FFA con-centrations than elevated triacylglycerol concentrations with respect to lipid deposition.

In conclusion, we observed a net in vivo hepatic lipid accu-mulation after a single HF meal by using ¹H-MRS. In contrast, we did not find increased IMCL concentrations after the same HF meal. The addition of protein to the meal did not change the postprandial IHL or IMCL retention, thereby suggesting that it may take longer before additional protein to an HF diet blunts the lipid accumulation in the liver.

The authors' responsibilities were as follows—LL, CIN, MKCH, JEW, VBS-H, and PS: designed the research and discussed results; LL and CIN: conducted the research and analyzed data; LL, VBS-H, and PS: wrote and had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. The public partners were responsible for the study design, data collection and analysis, decision to publish, and preparation of the manuscript and contributed to the project through regular discussion. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. Lancet 2010;375:2267–77.
2. Schrauwen P, Schrauwen-Hinderling V, Hoeks J, Hesselink MK: Mitochondrial dysfunction and lipotoxicity. Biochim Biophys Acta 2010, 1801:266–71.
3. Gastaldelli A, Basta G. Ectopic fat and cardiovascular disease: what is the link? Nutr Metab Cardiovasc Dis 2010;20:481–90.
4. Bortolotti M, Kreis R, Debard C, Cariou B, Faeh D, Chetiveaux M, Ith M, Vermathen P, Stefanoni N, Le KA, et al. High protein intake reduces intrahepatocellular lipid deposition in humans. Am J Clin Nutr 2009;90:1002–10.
5. van Herpen NA, Schrauwen-Hinderling VB, Schaart G, Mensink RP, Schrauwen P. Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. J Clin Endocrinol Metab 2011;96:E691–5.
6. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, et al. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. Diabetes 2001;50:2579–84.
7. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WH, Nicolay K, Schrauwen P. Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. Obes Res 2005;13:2088–94.
8. Lê KA, Ith M, Kreis R, Faeh D, Bortolotti M, Tran C, Boesch C, Tappy L. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr 2009;89:1760–5.
9. Theytaz F, Noguchi Y, Egli L, Campos V, Buehler T, Hodson L, Patterson BW, Nishikata N, Kreis R, Mittendorfer B, et al. Effects of supplementation with essential amino acids on intrahepatic lipid concentrations during fructose overfeeding in humans. Am J Clin Nutr 2012;96:1008–16.
10. Thomsen C, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O. Quantification of liver fat using magnetic resonance spectroscopy. Magn Reson Imaging 1994;12:487–95.
11. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol 1999;276:E977–89.
12. Boesch C, Machann J, Vermathen P, Schick F. Role of proton MR for the study of muscle lipid metabolism. NMR Biomed 2006;19:968–88.
13. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 2005;288:E462–8.
14. Siri WE. The gross composition of the body. Adv Biol Med Phys 1956;4:239–80.
15. Cunningham JJ. A reanalysis of the factors influencing basal metabolic rate in normal adults. Am J Clin Nutr 1980;33:2372–4.
16. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. Ann N Y Acad Sci 1987;508:333–48.
17. Haase A, Frahm J, Hanicke W, Matthaei D. 1H NMR chemical shift selective (CHESS) imaging. Phys Med Biol 1985;30:341–4.
18. Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn Reson Med 1993;29:804–11.
19. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson 1997;129:35–43.
20. Guiu B, Loffroy R, Petit JM, Aho S, Ben Salem D, Masson D, Hillon P, Cercueil JP, Krause D. Mapping of liver fat with triple-echo gradient echo imaging: validation against 3.0-T proton MR spectroscopy. Eur Radiol 2009;19:1786–93.
21. Guiu B, Petit JM, Loffroy R, Ben Salem D, Aho S, Masson D, Hillon P, Krause D, Cercueil JP. Quantification of liver fat content: comparison of triple-echo chemical shift gradient-echo imaging and in vivo proton MR spectroscopy. Radiology 2009;250:95–102.
22. Krssák M, Mlynarik V, Meyerspeer M, Moser E, Roden M. 1H NMR relaxation times of skeletal muscle metabolites at 3 T. MAGMA 2004;16:155–9.
23. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. J Lipid Res 2003;44:2065–72.
24. Ravikumar B, Carey PE, Snaar JE, Deelchand DK, Cook DB, Neely RD, English PT, Firbank MJ, Morris PG, Taylor R. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. Am J Physiol Endocrinol Metab 2005;288:E789–97.
25. Jonkers RA, Geraedts TR, van Loon LJ, Nicolay K, Prompers JJ. Multitissue assessment of in vivo postprandial intracellular lipid partitioning in rats using localized 1H-[13C] magnetic resonance spectroscopy. Magn Reson Med 2012;68:997–1006.
26. Labbé SM, Grenier-Larouche T, Croteau E, Normand-Lauziere F, Frisch F, Ouellet R, Guerin B, Turcotte EE, Carpentier AC. Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. Am J Physiol Endocrinol Metab 2011;300:E445–53.
27. Westphal S, Kastner S, Taneva E, Leodolter A, Dierkes J, Luley C. Postprandial lipid and carbohydrate responses after the ingestion of a casein-enriched mixed meal. Am J Clin Nutr 2004;80:284–90.
28. Floyd JC Jr, Fajans SS, Conn JW, Knopf RF, Rull J. Insulin secretion in response to protein ingestion. J Clin Invest 1966;45:1479–86.
29. Nuttall FQ, Mooradian AD, Gannon MC, Billington C, Krezowski P. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. Diabetes Care 1984;7:465–70.
30. Brechtel K, Dahl DB, Machann J, Bachmann OP, Wenzel I, Maier T, Claussen CD, Haring HU, Jacob S, Schick F. Fast elevation of the intramyocellular lipid content in the presence of circulating free fatty acids and hyperinsulinemia: a dynamic 1H-MRS study. Magn Reson Med 2001;45:179–83.
31. Schrauwen-Hinderling VB, van Loon LJ, Koopman R, Nicolay K, Saris WH, Kooi ME. Intramyocellular lipid content is increased after exercise in nonexercising human skeletal muscle. J Appl Physiol (1985) 2003;95:2328–32.
32. Stannard SR, Thompson MW, Fairbairn K, Huard B, Sachinwalla T, Thompson CH. Fasting for 72 h increases intramyocellular lipid content in nondiabetic, physically fit men. Am J Physiol Endocrinol Metab 2002;283:E1185–91.