Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein1–3

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 (1H-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 1H-MRS before and 3 and 5 h after a high-fat 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 age: 22.7 ± 3.0 y; mean body mass index [kg/m²]: 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 proton magnetic resonance spectroscopy (1H-MRS) to measure IHL concentrations. 1H-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 1H-MRS would be facilitated by improving the detection accuracy. Therefore, in the current study, the accuracy of IHL and IMCL measurements 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 1H-MRS would be facilitated by improving the detection accuracy. Therefore, in the current study, the accuracy of IHL and IMCL measurements by improving the detection accuracy. Therefore, in the current study, the accuracy of IHL and IMCL measurements

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 (1H-MRS) to measure IHL concentrations. 1H-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 1H-MRS would be facilitated by improving the detection accuracy. Therefore, in the current study, the accuracy of IHL and IMCL measurements

1 From the Departments of Radiology (LL, CIN, JEW, and VBS-H), Human Biology (LL, PS, and VBS-H), and Human Movement Sciences (CIN and MKCH), NUTRIM, Maastricht University Medical Center, Maastricht, The Netherlands, and Top Institute Food and Nutrition (LL, CIN, PS, and VBS-H), Wageningen, The Netherlands.

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3 Address correspondence to P Schrauwen, Department of Human Biology, Maastricht University Medical Center, PO Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail: p.schrauwen@maastrichtuniversity.nl.

4 Abbreviations used: iAUC, incremental AUC; DNL, de novo lipogenesis; EMCL, extramyocellular lipid; FFA, free fatty acid; HF, high fat; HFP, high fat with added protein; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; kgww, kg wet weight; MR, magnetic resonance; SNR, signal-to-noise ratio; T, Tesla; TR, repetition time; 1H-MRS, proton magnetic resonance spectroscopy.

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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 (±SD) age of 22.7 ± 3.0 y, mean BMI (in kg/m$^2$) of 21.8 ± 1.8, and mean body fat percentage of 15.1 ± 8.4% (10.7% ± 1.9% for men and 23.8% ± 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 weighing.

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 6% 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 HF 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 of 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.
[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 triglyceride concentrations. All analyses were performed in duplicate. For all metabolites, the incremental AUC (iAUC) was calculated separately.

1H-MRS acquisition

1H-MRS spectra were acquired on a 3.0-T whole-body MR system (Achieva 3T; 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). 1H-MR spectra were obtained from a 40 × 40 × 40 mm³ 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 T2-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 ∼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 main magnetic field and the foot constrained by 2 sandbags. A 30-mm³ voxel was used to eliminate the contamination of the signal from subcutaneous adipose tissue. Shimming was again 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 ∼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).

1H-MRS spectral analysis

1H-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 CH₂ 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, T₂ 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). T₁ relaxation correction was only applied for the water measurement of the IMCL determination, and T₁ 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 ± SDs, whereas all other results are expressed as means ± 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 ± 180 kcal. Because of the addition of protein, the total energy content of the HFP meal was higher (1361 ± 210 kcal; P < 0.01). On average, subjects consumed 80.0 ± 12.3 g fat, 92.3 ± 14.2 g carbohydrates, and 20.0 ± 3.1 g protein in the HF meal. For the HFP meal, 51.1 ± 7.9 g protein was added.
Postprandial IHL and IMCL (1H-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 ± 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 4A) 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 × 40 × 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.
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 the 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 $\gamma$, 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 $^{19}$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

**DISCUSSION**

![FIGURE 3](Image 322x142 to 523x467)

**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 $14 \times 14 \times 40$ 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.

![FIGURE 4](Image 90x620 to 235x728)

**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, intra-hepatic lipid; IMCL, intramyocellular lipid; kgww, kg wet weight.
window, to our knowledge, on investigating effects on postprandial 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 $^1$H-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 combination with hyperinsulinemia (6, 30). In our study setup, we performed $^1$H-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 separation 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 concentrations than elevated triacylglycerol concentrations with respect to lipid deposition.

In conclusion, we observed a net in vivo hepatic lipid accumulation after a single HF meal by using $^1$H-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.

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: solid 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.

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