# Long- and medium-chain Fatty acids induce insulin resistance to a similar extent in humans despite marked differences in muscle fat accumulation.

# Long- and Medium-Chain Fatty Acids Induce Insulin Resistance to a Similar Extent in Humans Despite Marked Differences in Muscle Fat Accumulation

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**Context:** Animal studies revealed that medium-chain fatty acids (MCFA), due to their metabolic characteristics, are not stored in skeletal muscle and may therefore not give rise to potentially hazardous lipid species impeding insulin signaling.

**Objective:** We here hypothesized that infusion of medium-chain triacylglycerols (MCT) in healthy lean subjects does not lead to ectopic fat accumulation and hence does not result in lipid-induced insulin resistance.

**Design and Methods:** Nine healthy lean male subjects underwent a 6-h hyperinsulinemic-euglycemic clamp with simultaneous infusion of 1) a 100% long-chain triacylglycerols (LCT) emulsion, 2) a 50/50% MCT/LCT emulsion, or 3) glycerol in a randomized crossover design. Muscle biopsies were taken before and after each clamp.

**Results:** MCT/LCT infusion raised plasma free fatty acid levels to a similar level compared with LCT infusion alone. Despite elevated free fatty acid levels, intramyocellular triacylglycerol (IMTG) levels were not affected by the MCT/LCT emulsion, whereas LCT infusion resulted in an approximately 1.6-fold increase in IMTG. These differences in muscle fat accumulation did not result in significant differences in lipid-induced insulin resistance between LCT ($-28\%$, $P = 0.003$) and MCT/LCT ($-20\%$, $P < 0.001$). Total skeletal muscle ceramide content as well as lactosyl- and glucosylceramide levels were not affected by any of the interventions. In addition, the distribution pattern of all ceramide species remained unaltered.

**Conclusions:** Although we confirm that MCFA do not lead to ceramide and IMTG accumulation in skeletal muscle tissue in humans, they do induce insulin resistance. These results indicate that, in humans, MCFA may not be beneficial in preventing peripheral insulin resistance. **(*J Clin Endocrinol Metab* 97: 208–216, 2012)**

Prolonged consumption of a high-fat diet or acute elevation of plasma free fatty acid (FFA) levels via infusion of a lipid emulsion induces insulin resistance in both animals and humans (1–3). Ectopic accumulation of lipid intermediates such as ceramides and diacylglycerols (DAG) in nonadipose tissues is generally believed to be the mechanistic link between the elevated supply of fatty acids and the resulting insulin resistance (2, 4). It is therefore anticipated that (dietary) manipulation of ectopic fat depots is a promising approach to accomplish beneficial changes in insulin sensitivity. In this context, medium-chain fatty acids (MCFA), fatty acids consisting of six to

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Abbreviations: DAG, Diacylglycerol; FFA, free fatty acids; GIR, glucose infusion rate; iAUC, incremental area under the curve; IMTG, intramyocellular triacylglycerols; LCFA, long-chain fatty acids; LCT, long-chain triacylglycerols; MCFA, medium-chain fatty acids; MCT, medium-chain triacylglycerols; NS, not significant.

12 carbon atoms, are of interest. Due to their shorter chain length, MCFA are taken up by mitochondria in a carnitine palmitoyltransferase-independent manner (5), are rapidly oxidized as shown by studies with stable isotopes (6–8), and hence are diverted away from incorporation into ectopic fat. This may result in blunted lipid-induced insulin resistance upon MCFA administration.

Indeed, we have recently shown that consumption of a high-fat diet rich in medium-chain triacylglycerols (MCT), as opposed to long-chain triacylglycerols (LCT), does not lead to ectopic fat accumulation in skeletal muscle and liver of both rats and mice (9, 10). However, we were unable to show beneficial effects of the reduction in intramyocellular lipid levels upon high-fat MCT feeding in mice with respect to insulin-stimulated glucose uptake in the peripheral organs (primarily accounted for by skeletal muscle) (10).

In line with our findings, a recent study showed that a 4- to 5-wk dietary intervention with a high-fat MCFA diet in mice was associated with a lack of intramyocellular fat accumulation compared with a high-fat diet based on long-chain fatty acids (LCFA) (11). In contrast to our study (10), however, this study also showed that mice fed the high-fat MCFA diet, in contrast to the high-fat LCFA diet, maintained a normal whole-body glucose tolerance as well as normal insulin-stimulated muscle glucose uptake, as assessed by a hyperinsulinemic-euglycemic clamp (11). Furthermore, these findings associated with increased markers of mitochondrial metabolism in skeletal muscle tissue (11).

To date, very little is known about the effects of MCFA on insulin sensitivity in humans. Therefore, we here employed the model of acute lipid-induced insulin resistance in healthy human subjects and compared the induction of insulin resistance upon the iv infusion of an LCT- *vs.* an MCT-containing lipid emulsion. We anticipated that administration of the MCT-containing lipid emulsion does not lead to ectopic fat accumulation and hence does not result in lipid-induced insulin resistance.

## Subjects and Methods

### Subjects

Nine healthy lean male volunteers participated in the present study. Subject characteristics are displayed in Table 1. All subjects were in good health, and none had a family history of diabetes mellitus or any other endocrine disorder or was involved in endurance training or engaged in sports activities for more than 2 h/wk.

The study protocol was reviewed and approved by the Medical Ethical Committee of Maastricht University, and all subjects gave their written informed consent before participating in the study. Some other aspects of the current study have been previously published (1).

**TABLE 1.** Subject characteristics

| Parameter | Mean ± SD |
|---|---|
| Age (yr) | 20.3 ± 1.5 |
| Height (m) | 1.82 ± 0.07 |
| Body weight (kg) | 72.7 ± 8.0 |
| Body mass index (kg/m$^2$) | 21.8 ± 1.9 |
| Fat-free mass (kg) | 61.1 ± 8.0 |
| Body fat (%) | 16.1 ± 4.2 |

### Body composition

Body density was determined by hydrostatic weighing in the morning in the fasted state, as described previously (12). Body composition was calculated according to the equation of Siri (13). Fat free mass was calculated by subtracting fat mass from total body weight.

### Experimental design

Subjects arrived at the laboratory at 0800 h after an overnight fast. Subjects were asked to avoid strenuous physical activity at least 24 h before each test and came to the laboratory by car or by public transportation. On three separate days, subjects underwent a 6-h euglycemic-hyperinsulinemic clamp (for details see Ref. 1) with simultaneous infusion of two distinct lipid emulsions or glycerol, in a randomized crossover design. Between conditions, a washout period of at least one week was maintained.

Before and after every clamp, a percutaneous muscle biopsy was taken from the vastus lateralis muscle, according to the technique by Bergström *et al.* (14). The muscle biopsy was frozen immediately in melting isopentane cooled with liquid nitrogen and stored at −80 C until assayed.

### Lipid emulsions

Simultaneously to the hyperinsulinemic-euglycemic clamp, a lipid emulsion was infused through a cannula in the contralateral arm. In the LCT condition, a 20% triglyceride emulsion (Intralipid; Fresenius Kabi, Bad Homburg, Germany) was infused at a rate of 1.3 ml/min for 6 h. This emulsion is based on soybean oil and consists of fatty acids longer than 16 carbon atoms or more (LCFA). Together with the lipid emulsion, heparin was coadministered (0.2 U/kg · min) to stimulate hydrolysis of the infused triglycerides, and subjects received a bolus injection of heparin (200 IU) upon the start of the test. In the MCT/LCT condition, a 20% triglyceride emulsion (Lipofundin; Braun Medical, Melsungen, Germany) was infused, together with a primed (200 IU) continuous (0.2 U/kg · min) infusion of heparin. In contrast to the LCT emulsion, 50% of the fatty acids present in the MCT/LCT emulsion were of medium chain length (≤12 carbon atoms), whereas the other 50% consisted of LCFA (≥16 carbon atoms), again derived from soybean oil. An infusion rate of 0.82 ml/min was used to match the plasma FFA levels obtained during the LCT clamp. This was established by pilot experiments. In the control condition, glycerol was infused to match the mean glycerol content of the lipid emulsions in the experimental conditions.

### Indirect calorimetry

Before and during the clamp, whole-body energy expenditure and substrate oxidation were measured by indirect calorimetry, as described previously (12). Energy expenditure was calculated

according to the Weir formula (15). Total carbohydrate and fat oxidation were calculated using stoichiometric equations (16).

### Blood analysis

Blood was collected in tubes containing 30 μl 0.2 M EGTA per 5 ml blood (to prevent clotting and *in vitro* lipolysis of triacylglycerols) and immediately centrifuged at high speed. Plasma was rapidly frozen in liquid nitrogen and stored at −80 C until further analysis.

Plasma FFA concentrations were measured using the Wako NEFA C kit (Wako Chemicals, Neuss, Germany), with adjustments made to allow valid measurement of FFA levels exceeding 1000 μmol/liter. Plasma glucose was measured using the hexokinase method (Glucose HK 125 kit; ABX Diagnostics, Montpellier, France). Glycerol and triacylglycerols were determined by the glycerol kinase-lipase method (Roche Molecular Biochemicals GmbH, Mannheim, Germany). Plasma insulin levels were measured using a human insulin RIA kit (Linco Research, St. Charles, MO).

### Oil Red O staining

Fresh cryosections (5 μm) were stained for intramyocellular triacylglycerols (IMTG) by Oil Red O staining combined with immunolabeling of the basal membrane marker laminin, to allow quantification of IMTG, as described previously (17, 18).

### Lipidomics analysis

#### *Sample homogenization*

Freeze-dried muscle fibers were weighed before addition of 500 μl cold methanol and transferred into 2 ml Safe-Lock Eppendorf tubes. The muscle fibers were homogenized with zirconium beads using a Mixer Mill 301 system (RETSCH, Haan, Germany) for 5 min at 20 Hz and 4 C. The homogenates were stored at −80 C before protein determination and lipid extraction.

#### *Protein determination*

An aliquot of the homogenate was used for protein determination. The protein amounts were determined using the Micro BSA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA) and performed in triplicate. The absorbance of the samples was measured at 560 nm using a Multiskan EX (Thermo Fisher Scientific).

#### *Lipid extraction*

A 200-μl volume of the homogenate was used for sphingolipid extraction. The homogenates were extracted using a modified Folch lipid extraction method (19) performed on a custom-made Hamilton Microlab Star robotic system. Before the sphingolipid extraction, a known concentration of the ceramide d18:1/17:0 internal standard was added. The lipid extracts were reconstituted in chloroform/methanol (1:2, vol/vol) and stored at −80 C before analysis.

#### *Sphingolipid analysis*

The sphingolipid extracts were diluted in chloroform/methanol (1:2, vol/vol) containing 5 mM ammonium acetate and analyzed on a 4000 QTRAP mass spectrometer equipped with an ultra-performance liquid chromatography system using multiple reaction monitoring. The ceramide species were separated on an Acquity BEH C18, 2.1- × 50-mm column with a particle size of 1.7 μm (Waters, Milford, MA). A 25-min gradient using 10 mM ammonium acetate in water with 0.1% formic acid (mobile phase A) and 10 mM ammonium acetate in acetonitrile/2-propanol (4:3, vol/vol) containing 0.1% formic acid (mobile phase B) was applied. A collision energy of 40–45 eV was used. The mass spectrometry data files were processed using MultiQuant. Ceramide and cerebroside species were normalized to ceramide d18:1/17:0 and total protein amount. Ceramides are presented as picomoles per milligram protein and cerebrosides as relative amounts.

### Statistical analysis

All values are expressed as mean ± SD. Changes in energy expenditure, fat oxidation, glucose oxidation, and blood and muscle metabolites were calculated as incremental area under the curve (iAUC) or as pre- *vs.* postclamp difference.

Differences within conditions were analyzed by paired Student's *t* tests, whereas differences between conditions were evaluated by ANOVA (one-way repeated-measures ANOVA). When significant differences were found, a Bonferroni adjusted *post hoc* test was used to determine the exact location of the difference. Outcomes were regarded as statistically significant if $P < 0.05$.

## Results

### Energy expenditure and substrate oxidation

Baseline energy expenditure was similar in all three conditions [5.59 ± 0.59, 5.57 ± 0.32, and 5.58 ± 0.38 kJ/min in glycerol, LCT, and MCT/LCT, respectively, not significant (NS)] and increased during the clamp by 0.32 ± 0.24 ($P = 0.004$), 0.68 ± 0.23 ($P < 0.001$), and 0.79 ± 0.19 ($P < 0.001$) kJ/min upon glycerol LCT and MCT/LCT, respectively (Fig. 1A). The increase in energy expenditure was significantly higher in both lipid conditions ($P = 0.006$ for LCT and $P = 0.003$ for MCT/LCT) compared with glycerol, but no difference was detected between LCT and MCT/LCT (Fig. 1B).

Baseline fat oxidation was not different between the conditions (glycerol, 88.5 ± 30.3; LCT, 80.1 ± 20.2; MCT/LCT, 88.5 ± 24.0 mg/min) as well as baseline glucose oxidation (glycerol, 135.6 ± 52.2; LCT, 156.2 ± 42.7; MCT/LCT, 135.6 ± 44.5 mg/min). Insulin infusion suppressed fat oxidation by 48.4 ± 24.8 mg/min ($P < 0.001$) in the glycerol trial and by 13.5 ± 14.8 mg/min in the MCT/LCT trial ($P = 0.04$), but insulin-induced suppression of fat oxidation was absent upon infusion of LCT emulsion (Fig. 1D).

Insulin infusion increased glucose oxidation (Fig. 1E) in all conditions (glycerol, 142.6 ± 57.1, $P < 0.001$; LCT, 30.8 ± 44.7, $P = 0.07$; MCT/LCT, 84.7 ± 39.8 mg/min, $P = 0.001$). However, insulin-induced increase in glucose oxidation was significantly blunted upon LCT ($P = 0.009$)

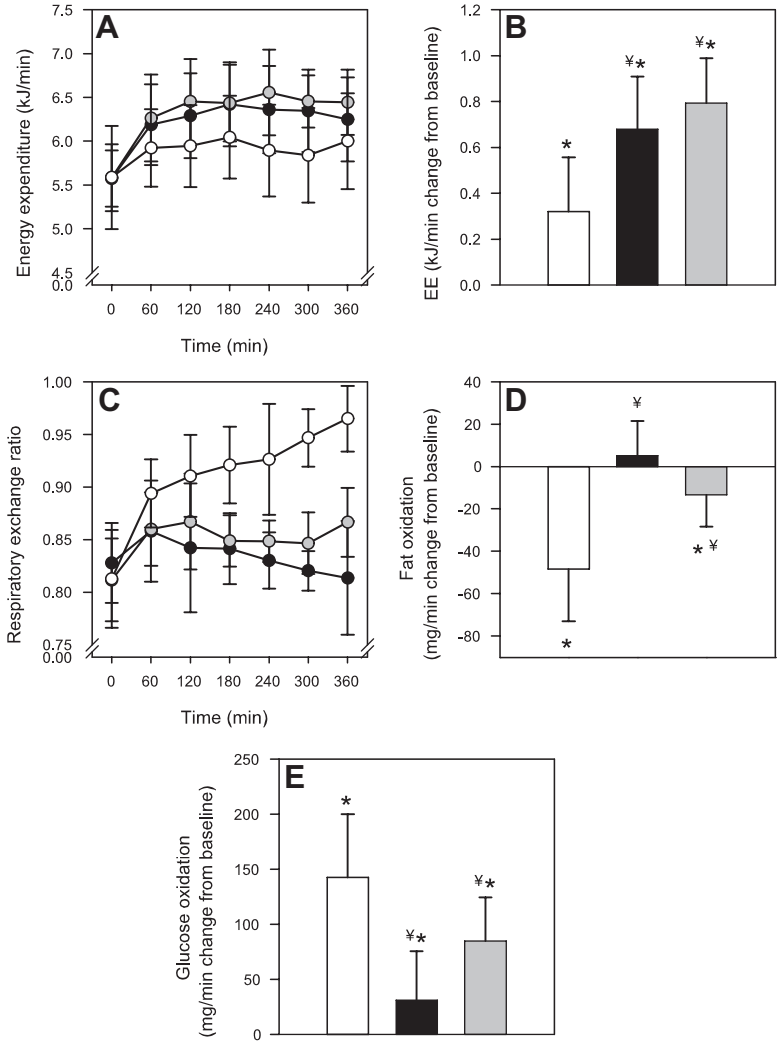


**FIG. 1.** A, Changes in energy expenditure over time during the hyperinsulinemic-euglycemic clamp with simultaneous glycerol (*white circles*), LCT (*black circles*), and MCT/LCT (*gray circles*) infusion. B, Average changes in energy expenditure (iAUC) upon the glucose clamp with simultaneous infusion of glycerol (*white bars*), LCT (*black bars*), or MCT/LCT (*gray bars*). C, Similar to A, but for the respiratory exchange ratio. D and E, From the respiratory exchange ratio, changes (iAUC) in fat (D) and carbohydrate (E) oxidation upon the glucose clamp with simultaneous infusion of glycerol (*white bars*), LCT (*black bars*), or MCT/LCT (*gray bars*) were calculated. Values are mean ± SD. *, $P < 0.05$ compared with baseline; ¥, $P < 0.05$ compared with glycerol.

and MCT/LCT infusion ($P = 0.029$) compared with glycerol infusion. The difference in insulin-induced increase in glucose oxidation between LCT and MCT/LCT did not reach statistical significance ($P = 0.25$).

## Blood metabolites

Baseline plasma FFA were similar in all experimental conditions (467 ± 144, 551 ± 84, and 559 ± 66 μmol/liter in glycerol, LCT and MCT/LCT, respectively, NS). Plasma FFA levels were elevated 3-fold upon both LCT and MCT/LCT infusion during the hyperinsulinemic clamp ($P < 0.001$, Fig. 2A). Insulin plus glycerol suppressed plasma FFA levels down to approximately 15% of basal values ($P < 0.001$). The changes in plasma FFA levels were similar between the LCT and MCT/LCT condition, whereas glycerol infusion during hyperinsulinemia resulted in significantly lower plasma FFA levels compared with both LCT and MCT/LCT (both $P < 0.001$, Fig. 2B).

Baseline plasma triacylglycerol (TAG) levels did not differ between the three conditions and averaged 614 ± 155, 642 ± 371, and 838 ± 575 μmol/liter in glycerol, LCT, and MCT/LCT, respectively (NS). Insulin plus glycerol administration decreased plasma TAG levels significantly ($P = 0.001$), whereas both LCT ($P < 0.001$) and MCT/LCT ($P = 0.009$) infusion during the clamp increased plasma TAG concentrations (Fig. 2C). The changes in plasma TAG levels over time (Fig. 2D) upon glycerol were statistically different from both LCT ($P < 0.001$) and MCT/LCT ($P = 0.01$). Furthermore, the increase in plasma TAG levels was more pronounced upon LCT infusion in comparison with the MCT/LCT infusion ($P = 0.008$).

Plasma insulin levels were similar at baseline (glycerol, 14.3 ± 6.5; LCT, 13.2 ± 5.0; MCT/LCT, 13.1 ± 3.1 μU/ml) and increased in all conditions upon starting the clamp procedure by on average approximately 7.5-fold in all conditions ($P < 0.001$). The changes in plasma insulin levels did not differ between experimental conditions, resulting in similar insulin levels at the end of all clamps.

Glucose levels were clamped on approximately 5 mmol/liter and measured directly by online determination of whole-blood glucose concentrations. Baseline whole-blood glucose levels were similar between glycerol, LCT, and MCT/LCT (4.71 ± 0.28, 4.66 ± 0.43, and 4.73 ± 0.36 mmol/liter, respectively, NS) and increased slightly, although significantly, during the euglycemic-hyperinsulinemic clamp procedure in all conditions ($P = 0.002$). However, no differences were detected in the change in whole-blood glucose levels over time between the experimental conditions and glucose levels were similar at the end of all clamps.

## Insulin sensitivity

Insulin sensitivity, as assessed by determination of the glucose infusion rate (GIR) needed to maintain euglyce-

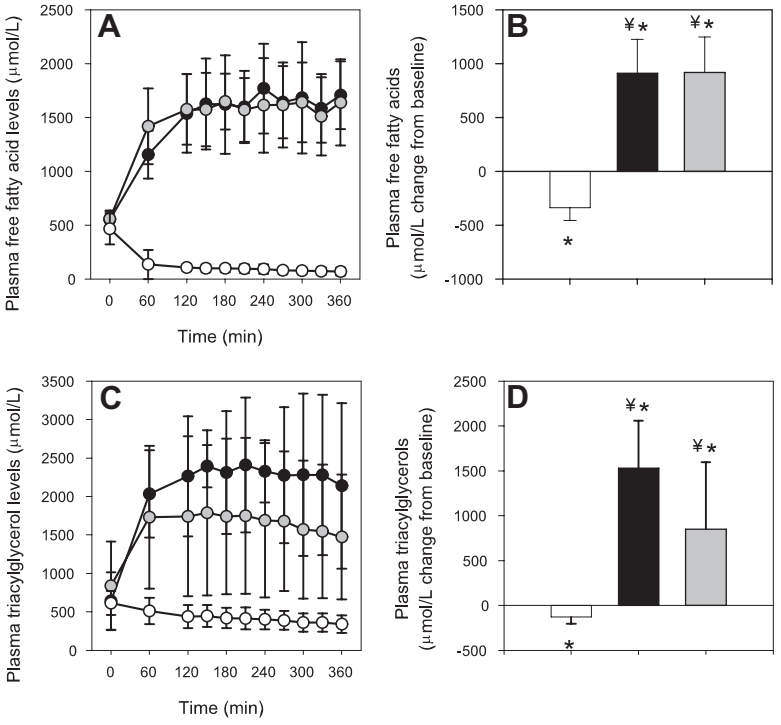


**FIG. 2.** A, Changes in plasma FFA levels over time during hyperinsulinemic-euglycemic clamp with simultaneous glycerol (*white circles*), LCT (*black circles*), and MCT/LCT (*gray circles*) infusion. B, Average changes in plasma FFA (iAUC) upon the glucose clamp with simultaneous infusion of glycerol (*white bars*), LCT (*black bars*), or MCT/LCT (*gray bars*). C, Similar to A, but for plasma triacylglycerol levels. D, Similar to B, but for plasma triacylglycerol. Values are mean ± SD. *, $P < 0.05$ compared with baseline; ¥, $P < 0.05$ compared with glycerol.

mia, is depicted in Fig. 3. GIR and thus insulin sensitivity gradually increased (Fig. 3A, $P < 0.001$) in the control (glycerol) condition up to 61.4 ± 13.3 μmol/kg · min in the last 30 min of the test (330–360 min). Upon LCT infusion, GIR reached a plateau (46.8 ± 12.9 μmol/kg · min at 120–150 min) and subsequently decreased by approximately 28% to a value of 32.6 ± 8.6 μmol/kg · min at 330–360 min ($P = 0.003$). GIR upon MCT/LCT infusion followed a similar pattern and decreased ($P < 0.001$) by approximately 20% from 51.5 ± 13.9 μmol/kg · min at 120–150 min to 41.0 ± 10.8 μmol/kg · min at 330–360 min (Fig. 3B).

During the last half-hour of the clamp, the GIR was significantly higher in the glycerol condition compared with both the LCT ($P < 0.001$) and the MCT/LCT ($P < 0.001$) condition. More importantly, however, we also detected a significant difference between the two lipid emulsions, *i.e.* the GIR was significantly lower in the LCT condition compared with the MCT/LCT condition ($P = 0.004$). However, there was no statistical difference between the two lipid conditions with respect to the reduction in GIR from 120–150 to 330–360 min.

### Intramyocellular triacylglycerols

Baseline IMTG levels were similar in all experimental conditions (2.97 ± 2.32, 2.84 ± 2.04, and 2.87 ± 2.39% in glycerol, LCT, and MCT/LCT, respectively, NS). In the glycerol condition, IMTG levels tended to decrease to 1.56 ± 1.71% ($P = 0.065$), whereas LCT infusion significantly increased IMTG levels (Fig. 4A) by approximately 1.6-fold ($P = 0.02$). In accordance with our hypothesis, MCT/LCT infusion did not lead to muscular fat accumulation (from 2.87 ± 2.39 to 3.13 ± 2.70%, NS). The change in IMTG levels was significantly different between glycerol and LCT ($P = 0.02$), whereas the difference in IMTG change between glycerol and MCT/LCT ($P = 0.17$) and between LCT and MCT/LCT ($P = 0.28$) did not reach statistical significance. Finally, we observed a significant correlation ($r = 0.52$; $P = 0.006$) between the change in IMTG levels and the GIR (*i.e.* insulin sensitivity) but only at the end (time = 360 min) of the clamp (Fig. 4B).

### Skeletal muscle ceramides and cerebrosides

Total skeletal muscle ceramide content was similar at baseline in the glycerol (561.2 ± 144.5 pmol/mg protein), LCT (468.1 ± 135.4 pmol/mg protein), and MCT/LCT (661.3 ± 200.3 pmol/mg protein) condition (NS). Total ceramide levels in skeletal muscle did not significantly change in any of the experimental conditions. Accordingly, the changes in total skeletal muscle ceramides were similar in all conditions (Fig. 5A). Glucosylceramides were also similar at baseline and averaged 13.8 ± 4.4, 13.6 ± 5.0, and 21.3 ± 19.0 pmol/mg protein in glycerol, LCT, and MCT/LCT, respectively. After the clamp, skeletal muscle glucosylceramide levels were significantly increased (Fig. 5B) upon glycerol ($P = 0.01$), LCT ($P = 0.03$), and MCT/LCT ($P = 0.05$) infusion. In other words, we did not observe any difference in the change in glucosylceramide levels across the three conditions.

Similar results were observed for skeletal muscle lactosylceramides (Fig. 5C). Thus, preclamp levels were identical in the glycerol (1.09 ± 0.42 pmol/mg protein), LCT (1.00 ± 0.37 pmol/mg of protein), and MCT/LCT (1.30 ± 0.52 pmol/mg protein) condition, whereas postclamp lactosylceramide levels were elevated in all conditions, although this did not reach statistical significance for the glycerol arm ($P = 0.10$). Again, the changes in lactosylceramides were similar between all conditions.

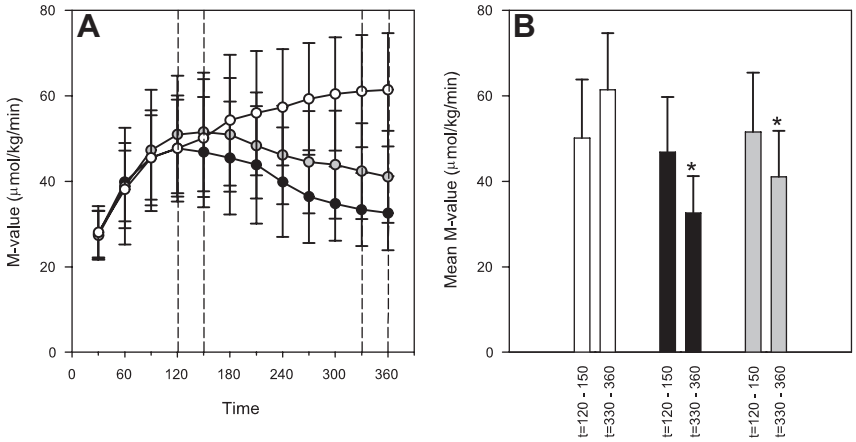


**FIG. 3.** A, GIR during the hyperinsulinemic-euglycemic clamp with simultaneous glycerol (*white circles*), LCT (*black circles*), and MCT/LCT (*gray circles*) infusion. B, Changes in insulin sensitivity, analyzed by comparing the mean GIR at 120–150 min with the GIR at 330–360 min, upon the coinfusion of glycerol (*white bars*), LCT (*black bars*), and MCT/LCT (*gray bars*). Values are mean ± SD. *, $P < 0.05$ compared with time (t) = 120–150 min.

## Discussion

Intramuscular fat accumulation is negatively related to muscle insulin sensitivity (20–22) and reducing IMTG content has been shown to beneficially affect muscle insulin sensitivity (23, 24). We and others have previously shown in animal studies that consumption of a high-fat diet based on MCFA, in contrast to LCFA, does not induce im fat accumulation (9–11) and might therefore maintain a healthy muscle insulin sensitivity (11). Human data on the effects of MCFA with respect to im fat accumulation and insulin resistance were, however, largely lacking. In line with the animal data, we show here that infusion of an MCT-containing emulsion (*i.e.* 50% MCT/50% LCT) in humans does not lead to im fat accumulation. Surprisingly, however, the lack of im fat accumulation upon the MCT-containing emulsion did not ameliorate lipid-induced insulin resistance; *i.e.* both the MCT/LCT and the LCT emulsion induced insulin resistance to the same extent.

In agreement with previous studies (25–27), short-term infusion with a lipid emulsion based on LCT during a glucose clamp induced insulin resistance in healthy young individuals in the present study. This lipid-induced insulin resistance typically occurred after 2–3 h of infusion, indicating that the insulin-desensitizing signal is not directly caused by increased fatty acid levels but most likely arises alongside the process of triacylglycerol synthesis. Indeed, the infusion of the LCT emulsion was also accompanied by a significant increase in muscle triacylglycerol content (Fig. 4). We then tested the hypothesis that the infusion of a MCT/LCT emulsion would not lead to im fat accumulation and would therefore ameliorate lipid-induced insulin resistance. Indeed, the infusion of the MCT/LCT emulsion, resulting in similar plasma FFA levels (Fig. 2, A and B), failed to induce muscle triacylglycerol accumulation. Although we did observe a slight delay in the induction of insulin resistance upon MCT/LCT infusion, resulting in a higher plateau in GIR after 2–3 h of the clamp and therefore also a slightly higher GIR at the end of the clamp (Fig. 3A), the relative reduction of insulin sensitivity (percent reduction from 120–150 to 330–360 min) upon MCT/LCT infusion was similar when compared with LCT infusion. We therefore conclude that MCT/LCT infusion induced insulin resistance to the same extent compared with infusion of the LCT emulsion, despite pronounced differences in skeletal muscle triacylglycerol accumulation. This finding underscores that muscle insulin sensitivity does not simply depend on the total amount of IMTG, which is in line with several reports showing that IMTG accumulation does not in itself induce insulin resistance but may instead be a marker of lipotoxicity (28, 29). In this context, it is also well known that endurance-trained athletes are highly insulin sensitive despite the accumulation of IMTG up to levels similar or even greater than in the insulin-resistant or diabetic state (the so-called athletes' paradox) (30, 31). Thus, it is the balance between the fatty acid supply to the muscle and the skeletal muscle oxidative capacity that seems to deter-

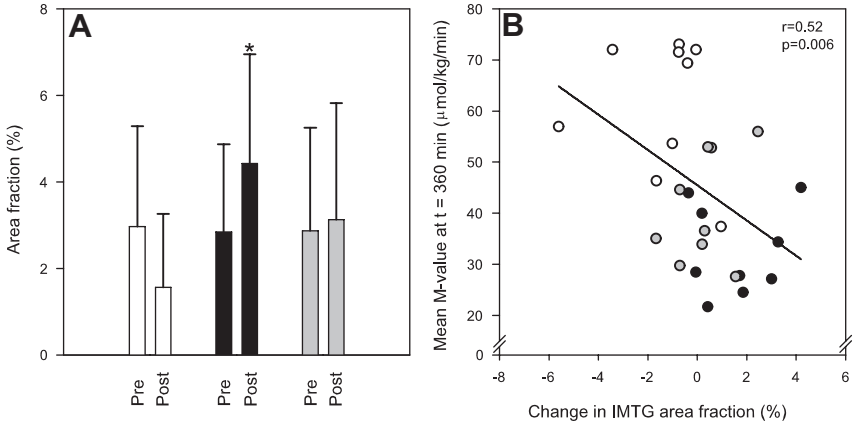


**FIG. 4.** A, IMTG levels before (pre) and after (post) the glucose clamp with coinfusion of glycerol (*white bars*), LCT (*black bars*), and MCT/LCT (*gray bars*). B, The changes in intramyocellular lipid levels (post- minus preclamp values) correlated with the GIR at the end of the clamp (330–360 min). Values are mean ± SD. *, $P < 0.05$ compared with preclamp levels.

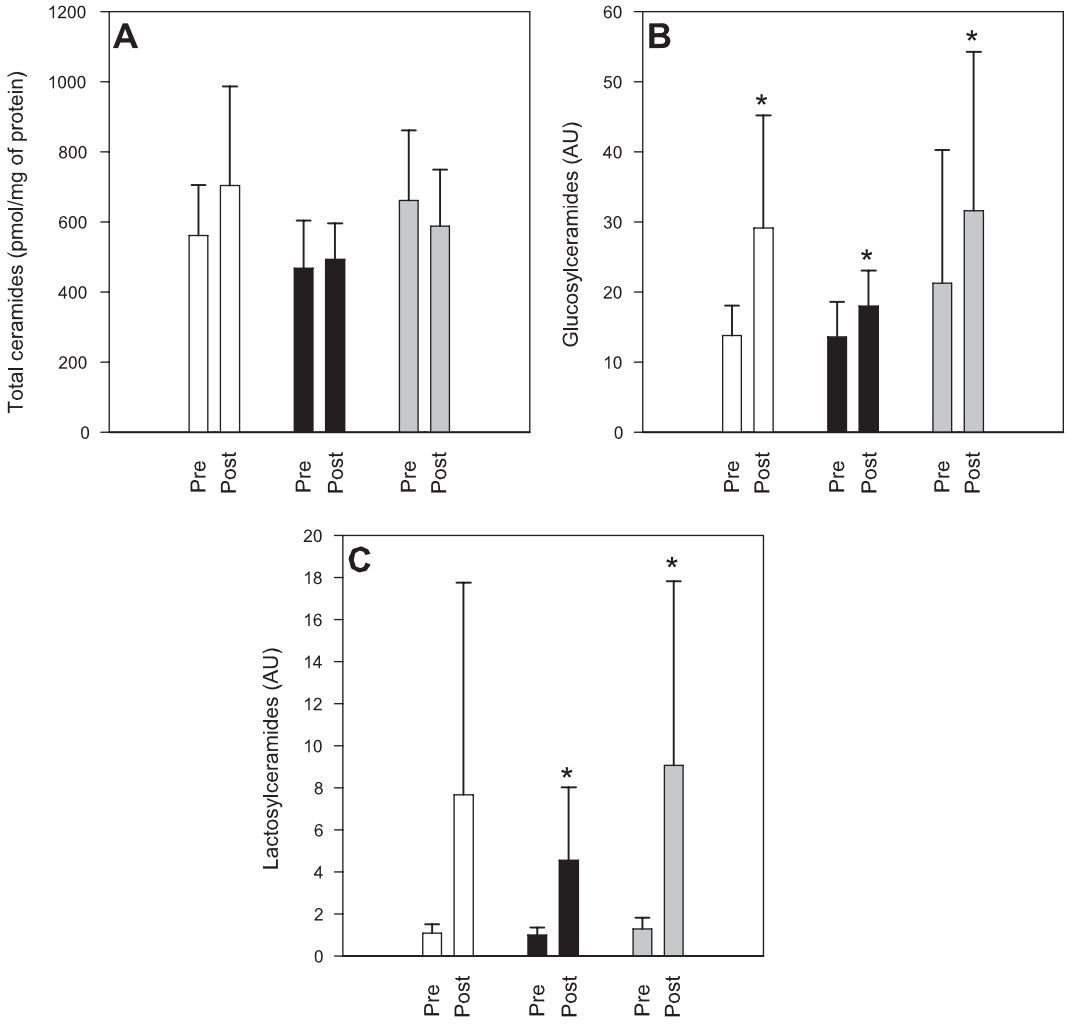


**FIG. 5.** A–C, Total ceramides (A), glucosylceramides (B), and lactosylceramides (C) in skeletal muscle tissue before (pre) and after (post) the hyperinsulinemic-euglycemic clamp with simultaneous infusion of glycerol (*white bars*), LCT (*black bars*), and MCT/LCT (*gray bars*). Values are mean ± SD. *, $P < 0.05$ compared with preclamp levels.

mine skeletal muscle insulin sensitivity rather than the magnitude of lipid intake and/or IMTG accumulation alone.

In line with the current study, it was previously reported that whole-body insulin sensitivity (*i.e.* GIR during a hyperinsulinemic-euglycemic clamp) was reduced after consumption of both a high-fat MCT and high-fat LCT diet for 4–5 wk in mice, although the effect was more pronounced with the latter diet (11). Despite this reduction in whole-body insulin sensitivity, however, Turner *et al.* (11) observed beneficial effects of the high-fat MCT diet on muscle fat accumulation and muscle insulin sensitivity, whereas liver insulin sensitivity was in fact similarly reduced upon both the MCT and the LCT diet.

However, we have previously shown that 8 wk of high-fat MCT *vs.* high-fat LCT feeding in mice resulted in a similar reduction in whole-body insulin resistance, despite dramatically lower lipid levels in both liver and skeletal muscle after the high-fat MCT diet (9). Intriguingly, and in contrast to the previously mentioned study by Turner *et al.* (11), the reduction in whole-body insulin sensitivity upon the high-fat MCT diet was mainly accounted for by a reduced peripheral glucose uptake (primarily skeletal muscle), whereas liver insulin sensitivity appeared normal (9). The discrepancy in results between these two animal studies might be explained by the specific type of MCFA used in the respective studies; whereas the diets used by Turner *et al.* (11) primarily contained fatty acids of 12 and 14 carbon atoms in chain length, we previously used fatty acids of shorter chain length (C8 and C10). In this context, it is relevant to mention that the MCT portion of the MCT/LCT emulsion used in the present study was mainly composed of fatty acids with eight or 10 carbon atoms in chain length, similar to the diet used in our mouse study. Furthermore, because LCFA and MCFA are processed differently during intestinal absorption, discrepancies seen between the current human study and the earlier published

diet-based animal studies may also be (partially) explained by the difference in administration route.

Next we intended to pinpoint the mechanism responsible for the induction of muscle insulin resistance upon both lipid emulsions. As stated before, the delay in the onset of insulin resistance upon both LCT and MCT/LCT infusion during the clamp indicates that lipid species originating alongside triacylglycerol synthesis may negatively impact insulin signaling (25, 26). One of the candidate events that may interfere with muscle insulin signaling upon the infusion of our lipid emulsions is the accumulation of skeletal muscle ceramides (32, 33). Surprisingly, however, our lipidomics analysis of skeletal muscle biopsies obtained before and after the three experimental procedures showed that total ceramide content was not significantly affected by the infusion of lipids, either upon the LCT or the MCT/LCT emulsion (Fig. 5A). The distribution of the different ceramide species is depicted in Supplemental Figs. (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Glucosyl- and lactosylceramide levels (Fig. 5, B and C) did show an increase in concentration upon the infusion of both LCT and MCT/LCT, but this increase was also observed upon glycerol infusion. The lack of skeletal muscle ceramide accumulation upon lipid infusion are in agreement with findings obtained in rats (34) and humans (25, 27, 35) and do not support a causal role for ceramides in the acute induction of skeletal muscle insulin resistance due to lipid infusion in humans, as opposed to other findings (36). We can, however, not exclude that ceramide accumulation contributes to insulin resistance upon more prolonged exposure to high-fat diets and/or an inactive lifestyle. In this context, it was previously shown that skeletal muscle ceramide content was indeed elevated in obese, insulin-resistant individuals (37, 38), although it should be mentioned that this concept has also been challenged by reports showing that skeletal muscle ceramide levels were similar among individuals that varied widely in their degree of insulin sensitivity (39, 40).

Finally, it should be noted that the emulsion used in the present study is a mixture of MCT (50%) and LCT (50%), because due to the ketogenic properties of MCFA, larger quantities of MCT are not suitable for human use. Although we previously observed that diacylglycerol (DAG) levels followed IMTG levels upon long-term high-fat LCT or MCT diets, and therefore DAG levels could not explain MCT-induced glucose intolerance in our previous rat study (10), we cannot exclude involvement of DAG accumulation in the induction of insulin resistance upon LCT and MCT/LCT infusion in humans.

In summary, a 6-h infusion of a LCT lipid emulsion in healthy young individuals increased skeletal muscle triacylglycerol content whereas intramuscular triacylglycerol levels remained the same upon the infusion of an MCT/LCT emulsion, despite a similar increase in plasma FFA levels. Despite this pronounced difference in im fat accumulation, insulin sensitivity reduced to a similar extent upon both lipid emulsions. The reductions in insulin sensitivity upon LCT and MCT/LCT infusion could not be explained by skeletal muscle ceramide levels.

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