

Small amounts of dietary medium-chain fatty acids protect against insulin resistance during caloric excess in humans

Anne-Marie Lundsgaard¹, Andreas M. Fritzen¹, Kim. A. Sjøberg¹, Maximilian Kleinert^{1,2}, Erik A. Richter¹, and Bente Kiens¹

¹Section of Molecular Physiology, Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark.

²Institute for Diabetes and Obesity, Helmholtz Diabetes Center at Helmholtz Zentrum München, German Research Center for Environmental Health, Germany.

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Correspondence:

Bente Kiens, PhD & Dr.Sci.

Section of Molecular Physiology

Department of Nutrition, Exercise and Sports

University of Copenhagen,

Universitetsparken 13,

DK-2100 Copenhagen

Denmark

Telephone: + 45 28751619

E-mail: bkiens@nexs.ku.dk

Abstract

Medium-chain fatty acids (MCFAs) have in rodents been shown to have protective effects on glucose homeostasis during high-fat overfeeding. In this study, we investigated whether dietary MCFAs protect against insulin resistance induced by a hypercaloric high-fat diet in humans. Healthy, lean men ingested a eucaloric control diet and a three-day hypercaloric high-fat diet (+75% energy, 81-83% fat) in randomized order. For one group (n=8), the high-fat diet was enriched with saturated long-chain FAs (LCSFA-HFD), while the other group (n=9) ingested a matched diet, but with ~30 g (5%) saturated MCFAs (MCSFA-HFD) in substitution for a corresponding fraction of the saturated LCFAs. A hyperinsulinemic-euglycemic clamp with femoral arteriovenous balance and glucose tracer was applied after the control and hypercaloric diets. In LCSFA-HFD, whole body insulin sensitivity and peripheral insulin-stimulated glucose disposal were reduced. These impairments were prevented in MCSFA-HFD, accompanied by increased basal FA oxidation, maintained glucose metabolic flexibility, increased non-oxidative glucose disposal related to lower starting glycogen content and increased glycogen synthase activity, together with increased muscle lactate production. In conclusion, substitution of a small amount of dietary LCFAs with MCFAs rescues insulin action in conditions of lipid-induced energy excess.

Introduction

For decades, saturated fatty acids (FAs) have been considered detrimental to insulin sensitivity and cardiovascular health, as reflected by dietary guidelines. However, this consensus has recently been challenged (1;2), and it has been suggested to be of importance to consider the specific type of saturated FA (1). Saturated FAs are specified by their number of carbon atoms, and those with medium chain length (MCFAs), like hexanoic (C6:0), octanoic (C8:0), capric (C10:0) and lauric acid (C12:0), have received particular attention in metabolic research (3). Besides their presence in coconut oil and synthetic medium-chain triacylglycerol (MCT) oils, MCFAs make up 15-28% of FAs in bovine and human milk (4;5) and are enriched in palm kernel oil. Saturated long-chain FAs (LCFAs), like palmitic acid (C16:0) and stearic acid (C18:0), are typically enriched in animal fat, but are also present in dairy products. Dairy products are thus a source of both saturated MCFAs and LCFAs.

Due to their lower lipophilicity, MCFAs primarily enter the portal vein in their non-esterified form after intestinal absorption (6;7). This is in contrast to the chylomicron incorporation and lymphatic absorption route of LCFAs. Therefore, intake of TGs containing MCFAs leads to a blunted elevation in plasma TG concentration compared with intake LCFA-rich TGs in humans (8). In rats, ingested MCFAs are predominately oxidized in the liver (9), suggesting that most MCFAs are metabolized there. Still, following intake of a diet that is very enriched in MCFAs (40E%), up to 17% of ingested MCFAs were found to reach the circulation in humans (10).

MCFAs are thought to enter mitochondria independently of the carnitine transport system, which facilitates the mitochondrial import and hence oxidation of LCFAs. Hence, when CPT was inhibited in isolated rat muscle (11) and in vivo in mice (12) oxidation of LCFA was inhibited whereas oxidation of MCFAs was not. Moreover, carnitine independency by MCFAs has been demonstrated in isolated liver mitochondria (13;14).

In humans, MCFAs are shown to be oxidized to a greater extent than LCFAs (35-58% of ingested dose versus 15-25%) following intake, as shown in both lean and obese individuals by use of FA tracers (15-17). Feeding mice or rats diets with 42E%-60E% fat comprised of coconut oil or MCT oil for 4-12 weeks maintained insulin and glucose tolerance at the level of chow diet, in contrast to diets with saturated or unsaturated LCFAs (12, 18-20), which induced insulin resistance. These studies in rodents imply that MCFAs have protective effects on glucose homeostasis during conditions of high energy and FA availability. Of note, although MCFAs are suggested to mainly be taken up and metabolized in the liver, increased skeletal muscle mitochondrial oxidative capacity has been reported with MCFA feeding in mice (21).

In a 14 year prospective study, intake of C6:0-C12:0 MCFAs from full-fat dairy products was associated with lower risk of developing type 2 diabetes (22). Dietary intervention studies investigating the effect of MCFAs on glucose homeostasis in humans are scarce and have not investigated the underpinning physiological and molecular mechanisms (23-25). The purpose of the present human study was thus to delineate whether inclusion of food sources with MCFAs have protective effects on whole body, hepatic, and skeletal muscle insulin action and glucose metabolism in skeletal muscle during overfeeding. For this purpose, volunteers consumed a diet with caloric excess high in FAs. Healthy, lean men ingested, in a randomized order, a eucaloric control diet and a three-day hypercaloric high-fat diet with 81-83E% fat, corresponding to ~450 g fat per day. For one group, the high-fat diet was enriched in saturated LCFAs, while the other group ingested a similar diet with a small fraction of the saturated LCFAs substituted with ~30 g (5E%) MCFAs, derived from coconut oil, palm kernel oil and dairy products.

Research design and methods

Nine and eight young (23 ± 3 and 23 ± 3 years) (mean \pm SD) healthy men were randomized to each intervention group in a parallel design (fig. 2A). Subjects were normal weight (BMI 23.9 ± 2.1 and 23.8 ± 1.9 kg·m⁻²) and moderately exercise trained (maximal oxygen uptake 52.0 ± 2.7 and 52.2 ± 3.7 ml·kg LBM⁻¹·min⁻¹). The study was approved by the Copenhagen Ethics Committee (KF 01 261127). A few data from the LCSFA-HFD intervention have been published (26), as specified in the legend of table 1, figure 2 and 3.

Diets: Habitual energy and macronutrient intake were registered by weighing all ingested foods and drinks for 3 days and subsequently quantified by Dankost 2000, DK. Subjects consumed in randomized order a eucaloric control diet (CON) (63E% carbohydrate, 14E% protein, 24E% fat) and a hypercaloric (+75% energy) high-fat diet, enriched in either saturated LCFAs (LCSFA-HFD) or saturated LCFAs of which ~30 g (5E%) were substituted by MCFAs (MCSFA-HFD) of C8:0, C10:0 and C12:0 origin (coconut oil, palm kernel oil and dairy products). Both high-fat diets were matched in total fat content (~82E%), equivalent to ~450 g (table S1, figure 1). The FA composition was calculated from a food product data base (Dankost 2000, DK). Control and high-fat diets were separated by three weeks. The hypercaloric diets were ingested for three days, preceded by five days of CON diet. In the CON trial, subjects ingested the eucaloric CON diet for eight days (fig. 2A). All food items were delivered and subjects were supervised to ensure compliance.

Protocol: Each subject completed two identical experimental days. 72 hours before, subjects abstained from strenuous physical activity and on the day of the experiment arrived by passive transport under post-absorptive conditions, following a small meal (1.6 MJ) ingested at 5 A.M (26), i.e. 7 h before the clamp. After 30 min rest, a venous catheter was inserted into an antecubital vein and teflon catheters inserted in one femoral artery and one femoral vein under local anesthesia. After

basal blood sampling, a bolus of 6,6-²H₂ glucose (3.203 mg·kg⁻¹) was administered in the cubital vein, followed by constant infusion (0.055 mg·kg⁻¹·min⁻¹) for 120 min during basal conditions. Subjects then underwent a 120 min hyperinsulinemic-euglycemic clamp (1.4 mU insulin·kg⁻¹·min⁻¹), initiated with an insulin bolus (9.0 mU·kg⁻¹) (Actrapid, Novo Nordisk, DK). During the clamp, 6,6-²H₂ glucose tracer was added to the non-labeled glucose infusate, so tracer infusion followed the variable glucose infusion rate which was continuously adjusted to maintain euglycemia. Femoral arterial blood flow was determined by ultrasound Doppler (Philips Ultrasound, US) every 20 min, concomitant with femoral arteriovenous blood sampling. Before and at the end of the clamp, indirect calorimetry was performed and vastus lateralis muscle biopsies were obtained under local anesthesia with 2-3 ml lidocaine.

Plasma parameters

Plasma glucose and blood lactate concentrations were determined with an ABL615 analyzer, Radiometer Medical, DK). Plasma insulin was measured by ELISA (ALPCO, US). The concentrations of FA (NEFA C kit, Wako Chemicals GmbH, DE), TG (GPO-PAP kit, Roche Diagnostics, DE), HDL, LDL, and total cholesterol (HORIBA Medicals, JA) were measured by colorimetric kits according to the manufacturers' instructions. Plasma epinephrine and norepinephrine concentrations were determined by radioimmunoassay (2-CAT 125I RIA kit, Labor Diagnostika, GE). Plasma tumor necrosis factor α (TNF α) and interleukin 6 (IL-6) were measured on an AutoDELFIA (Perkin Elmer, US) analyser. Plasma enrichment of ²H glucose was measured using liquid chromatography mass spectrometry (ThermoQuest Finnegan AQA, US).

Muscle analyses

Glycogen was measured in basal samples by a fluorometric method that detects glycosyl units after acid hydrolysis of freeze-dried muscle (27).

Glycogen synthase (GS) activity was measured in homogenates of basal and insulin-stimulated samples in the presence of 0.25, and 12 mmol·l⁻¹ glucose-6-phosphate (G6P), using a Unifilter 350 microtiter plate (Whatman, UK) assay (28). GS activity was expressed as % of fractional velocity (FV %), calculated as 100 x activity in presence of 0.25 mmol·l⁻¹ divided by activity in the presence of 12 mM G6P (saturated conditions).

Western blotting. Muscle lysis and western blotting were conducted as described previously (29). The primary antibodies used were anti-pyruvate dehydrogenase-E1 α (PDH-E1 α) Ser³⁰⁰ and anti-PDH-E1 α (Prof. Graham Hardie, University of Dundee, UK), anti-AKT^{Ser473} and anti-AKT2 (#9271 and #3063, Cell signalling, US). After 45 min incubation with horse radish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch, UK), signals were visualized (Bio-Rad ChemiDoc™ MP Imaging System, US) and quantified (Image Lab Version 4.0).

Calculations

Homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as [basal insulin]·[basal glucose]/22.5. Insulin-stimulated leg glucose uptake was calculated as the arteriovenous difference multiplied by blood flow. Endogenous glucose production was calculated from triplicate measures during the last 20 min of the basal conditions, and during the last 20 min of the clamp period using Steele's equation. Oxidative glucose disposal was calculated from VO₂ and VCO₂ values $(4.55 \cdot VCO_2 - 3.21 \cdot VO_2) \cdot 1000$. Non-oxidative glucose disposal during the clamp was calculated by (glucose infusion rate + glucose Ra) - oxidative glucose disposal.

Data and resource availability

The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. No resources were generated.

Statistics

Data in table 1 show means \pm SE, while the subject characteristics described in the methods are means \pm SD. In figure 2 and 3, data are shown as univariate scatter plots where the bars are showing mean \pm SE. Shapiro-Wilkinson test was performed to test for normal distribution and Brown-Forsythe to test for variance homogeneity. Two-way repeated measures ANOVAs were applied to test for effect of diet type (LCSFA-HFD or MCSFA-HFD) and effect of intervention (fat surplus) or effect of insulin and intervention within each group. When ANOVA revealed an interaction, Tukey's was used as a post hoc test. A significance level of $p < 0.05$ was chosen. Statistical analyses were performed in GraphPad PRISM 8, Graphpad, US.

Results

Subjects ingested 12.2 ± 0.3 MJ/day during CON and 21.3 ± 0.3 MJ/day during the hypercaloric high-fat diets. Postabsorptive plasma glucose and insulin concentrations increased following LCSFA-HFD (table 1), but were not altered following MCSFA-HFD (table 1). HOMA-IR index increased by 90% following LCSFA-HFD (figure 2B). The basal endogenous glucose production (EGP), measured during basal conditions, was not affected by either type of hypercaloric high-fat diet (figure 2C). Postabsorptive plasma TG levels decreased by 26-31% with both high-fat interventions (table 1). Plasma LDL and total cholesterol concentrations remained unchanged, while HDL cholesterol concentration increased 9-24% with both high-fat interventions (table 1). The plasma level of the cytokine, $\text{TNF}\alpha$, was increased 17% in LCSFA-HFD, but remained unchanged in MCSFA-HFD (table 1).

Glucose infusion rate was 17% lower following three days of LCSFA-HFD, while a small amount of MCSFA substitution prevented this reduction in insulin sensitivity (figure 2D+E). The endogenous glucose production, measured during the clamp, was not altered by either type of hypercaloric high-fat diet (figure 2F). In agreement, insulin-mediated suppression of endogenous glucose production relative to basal values (suppression of ~60%) was not affected by either high fat diet intervention (figure 2C+F). Whole body glucose disposal rate (Rd) was decreased by 21% in LCSFA-HFD, with no change in MCSFA-HFD (figure 2G). Insulin-stimulated leg glucose uptake was decreased by 26% in LCSFA-HFD, but remained unchanged in MCSFA-HFD (figure 2H+I). In LCSFA-HFD, RER was lower during the clamp compared with CON (0.82 ± 0.02 versus 0.90 ± 0.01) (figure 2J). In MCSFA-HFD, RER was lower during both basal and insulin-stimulated conditions compared with CON (figure 2K). The increase in RER in response to insulin was reduced in LCSFA-HFD, but not in MCSFA-HFD (figure 2L). The oxidative glucose disposal during the clamp was lower following both high-fat interventions compared with CON (figure 2M). Non-oxidative glucose disposal tended to be decreased in LCSFA-HFD (-20%, $p=0.06$). In contrast, non-oxidative glucose disposal was increased by 20% in MCSFA-HFD

compared with CON (figure 2N), which likely contributed to the preserved glucose disposal in MCSFA-HFD.

The resting basal muscle glycogen content was unchanged in LCSFA-HFD, but reduced by 27% after MCSFA-HFD (figure 3A), despite similar energy and carbohydrate (9E%) intake during the interventions. The lower muscle glycogen content after MCSFA-HFD was accompanied by increased basal and insulin-stimulated muscle GS activity compared with CON (figure 3C). This was not the case after intake of LCSFA (figure 3B). These observations support the idea of an improved non-oxidative glucose disposal following MCSFA-HFD. During the clamp, the inhibitory phosphorylation of PDH-E1 α at Ser³⁰⁰ in skeletal muscle was higher and PDH-E1 α protein content lower following both interventions (figure 3D+E). High-fat intake thus increased the potential for β -oxidation derived acetyl-CoA influx into the tricarboxylic acid cycle, potentially via inhibition of PDH and attenuated availability of acetyl-CoA from glycolysis, supporting the lower oxidative glucose disposal during the clamp. In MCSFA-HFD, a 42% increase compared with CON in leg lactate venous-arterial difference was observed (figure 3F), indicating maintained glycolytic flux despite PDH inhibition. There were no changes in insulin-stimulated AKT Ser⁴⁷³ phosphorylation with the interventions (figure 3G+H), indicating that proximal insulin signaling fails to explain the reduced glucose disposal in LCSFA-HFD.

Discussion

Here, we demonstrate that substitution of only ~30 g (5E%) saturated LCFAs with saturated MCFAs prevents both whole body insulin resistance and impaired insulin-stimulated muscle glucose uptake induced by a hypercaloric saturated LCFA-rich diet in humans. Remarkably, both in the basal postabsorbtivestate and during the clamp, glucose metabolism and insulin sensitivity were preserved at the level of the eucaloric control diet, despite intake of ~82E% fat and 75% caloric excess. The basal FA oxidation was increased with MCSFA inclusion. The main difference between the hypercaloric

saturated high-fat diets was a greater availability of C8:0, C10:0 and C12:0 FAs from coconut oil, palm kernel oil and dairy products in the MCSFA-HFD group, with a corresponding greater intake of C16:0 and C18:0 FAs by the LCSFA-HFD subjects. The type of FAs ingested thus had marked impact on substrate oxidation and insulin action.

In one other human study, insulin sensitivity was evaluated following substantial MCFA intake from MCT oil. In that study, whole body insulin sensitivity was reported to be 17 and 30% greater in lean healthy and obese type 2 diabetic individuals, respectively, following five days eucaloric MCFA-rich diet with 40% fat and a large percentage of MCFA (77% of the fat was MCFA) when compared to a eucaloric LCFA-rich diet also with 40% fat in a cross-over design (24).

In the present study, the basal FA oxidation was markedly increased in MCFA-HFD compared with both CON and LCSFA-HFD. At the same time, the increase in RER during the clamp was actually larger in MCSFA-HFD compared to CON, while in LCSFA-HFD the increase in RER was attenuated compared to CON. This suggests preservation of glucose metabolic flexibility in MCSFA-HFD, which could play a role in maintenance of glucose disposal. Due to the almost 100% FA oxidation at basal conditions following MCSFA-HFD, absolute glucose oxidation during the clamp was still lower compared with CON (as in LCSFA-HFD). However, during the clamp non-oxidative glucose disposal was higher in MCSFA-HFD than in CON, likely linked to the lower basal muscle glycogen content and greater GS activity compared to CON. The substantial contribution of glycogen storage to insulin-stimulated glucose disposal is evident from tracer and magnetic resonance spectroscopy (MRS) studies (30;31), and an inverse relationship between basal glycogen content and insulin-stimulated glucose uptake in muscle has been described in rat muscle (32). In the context of non-oxidative glucose disposal, a 42% greater leg lactate venous-arterial difference was observed following MCSFA-HFD compared to CON. This indicates that while some G6P was directed towards glycogen, glycolysis rate was also maintained, giving rise to lactate release. Preserved muscle insulin action after MCFA intake thus appeared due to both high glycogen synthesis and glycolytic flux.

An intriguing question is why muscle glycogen content was reduced when non-oxidative glucose disposal during the clamp was not compromised. We did not obtain any change in resting metabolic rate in the morning following the interventions (data not shown), confirming another human study in which one week of overfeeding with as much as 40E% MCFAs did not affect the basal metabolic rate (33). However, several studies have demonstrated that MCFA intake leads to an increased postprandial metabolic rate of 5-10% following MCFA intake of 15-50 g in both lean and obese individuals when compared with saturated and unsaturated LCFAs (33-37). The mechanisms for this have not been revealed. In the liver, MCFAs could increase β -oxidation and ketogenesis (38), which could contribute to an increased postprandial energy expenditure. In rodents, acute MCFA administration (C8:0) was shown to activate hypothalamic POMC neurons and increase metabolic rate, pointing to additional central regulation (39). A slight increase in the metabolic rate following meals during the MCSFA-HFD intervention could thus be hypothesized to have increased total substrate oxidation and thereby reduced muscle glycogen considering the low absolute carbohydrate intake of \sim 100 g/day.

We did not observe intervention effects on endogenous glucose production with either high fat diets, neither during basal postabsorptive conditions nor during the clamp. Insulin-stimulated glucose production was evaluated in response to a high, but still physiological, insulin infusion rate which suppressed endogenous glucose production by an average of 60%. Since the different response of the glucose infusion rate during the clamp (GIR) following the 2 high fat diets was largely explained by differences in leg glucose uptake, and endogenous glucose production was only suppressed by 60%, it is unlikely that a lower insulin infusion rate would have revealed differences in hepatic insulin sensitivity following the 2 diets.

The downregulation of insulin action in LCSFA-HFD was likely related to the energy surplus, as we have previously shown that six weeks of a 64E% high-fat diet rich in saturated LCFAs did not alter insulin sensitivity in overweight men under eucaloric conditions (2). In the present study, the downregulation of glucose disposal was not related to impaired proximal insulin signalling. This observation is confirmed by other high-fat overfeeding studies in lean men and women showing intact insulin-stimulated AKT and TBC1 domain family member 4 (TBC1D4) phosphorylation, as well as phosphoinositide 3-kinase (PI3K) activity, following 3-5 days of high-fat diet (50-78E% fat) and 40-75% energy excess (40-42). This points towards a more downstream regulation of glucose uptake potentially at the step of GLUT4 transport or membrane association that is impaired with surplus LCSFA-HFD high-fat diet intake. We did find that the muscle insulin resistance in LCSFA-HFD was accompanied with an impaired glucose metabolic flexibility, 40% lower absolute glucose oxidation during the clamp and also a 20% reduced non-oxidative glucose disposal ($p=0.06$).

In conclusion, the findings in this study show that substitution of only 30 g of saturated LCFA with MCFAs led to full reversal of insulin resistance at the whole body level and specifically in the skeletal muscle induced by marked energy excess provided by a diet rich in saturated LCFAs. Minor intake of saturated MCFAs had metabolic impact on skeletal muscle in human individuals. Together the findings suggest a potential of MCFA supplementation in regulation of both lipid and glucose homeostasis, and also highlights the need for nuanced dietary guidelines on saturated FAs.

Author contributions

B.K, E.A.R, A-M.L, K.A.S designed the study and carried out the experiments. A-M.L, A.M.F, K.A.S contributed to the results. A-M.L, A.M.F, M.K and B.K wrote the manuscript. All authors contributed to the manuscript and approved the final version of the manuscript.

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	<i>CON LCSFA</i>	<i>LCSFA</i>	<i>CON MCSFA</i>	<i>MCSFA</i>	<i>main effects / interactions</i>
<i>Basal</i>					
Glucose, mmol·l ⁻¹	5.5±0.1	6.0±0.1**	5.6±0.1	5.5±0.1	int x group (p<0.01)
Insulin, uU·ml ⁻¹	4.0±0.7	7.1±0.4**	5.0±0.9	5.9±1.5	int x group (p<0.05)
Fatty acids, μmol·l ⁻¹	319±49	283±36	539±60	484±51	group (p<0.001)
Triacylglycerol, μmol·l ⁻¹	835±118	576±45	640±121	476±116	int (p<0.01)
Total cholesterol, mmol·l ⁻¹	4.25±0.36	4.48±0.38	4.21±0.31	4.09±0.40	
HDL cholesterol, mmol·l ⁻¹	1.13±0.06	1.40±0.11	1.20±0.10	1.30±0.09	int (p<0.05)
LDL cholesterol, mmol·l ⁻¹	2.51±0.33	2.53±0.35	2.42±0.29	2.23±0.31	
Epinephrine, nmol·l ⁻¹	0.55±0.06	0.63±0.11	0.27±0.08	0.39±0.09	int (p<0.05)
Norepinephrine, nmol·l ⁻¹	1.72±0.12	1.62±0.19	1.31±0.48	1.11±0.33	
TNFα, pg·ml ⁻¹	2.23±0.24	2.60±0.29*	1.64±0.59	1.30±0.22	int x group (p<0.05)
IL-6, pg·ml ⁻¹	0.51±0.07	0.54±0.06	0.79±0.14	1.11±0.34	
<i>Clamp</i>					
Glucose, mmol·l ⁻¹	5.5±0.1	5.5±0.1	5.6±0.1	5.5±0.1	
Insulin, uU·ml ⁻¹	86.4±5.2	82.2±5.6	93.0±3.1	85.8±2.4	int (p<0.05)
Insulin clearance, ml·min ⁻¹	18.5±1.2	17.4±1.1	16.0±0.5	18.8±1.4	

Table 1. Arterial plasma parameters. All parameters were obtained in the basal, fasted state. Data are means±SEM. TNFα: tumor-necrosis factor α, IL-6: interleukin 6. Two-way RM ANOVAs were applied to test for effect of diet type/group (LCSFA-HFD or MCSFA-HFD) and effect of intervention (FA surplus). When ANOVA revealed interaction, this was indicated by *intervention (int) x group*. α p<0.05, αα p<0.01, main effect of dietary FA surplus. * p<0.05, ** p<0.01 effect of intervention within the respective group. n=8 in LCSFA-HFD and CON for this group, n=9 in MCSFA-HFD and CON for this group. For the LCSFA-HFD group, data on plasma fatty acids and triacylglycerol have been previously published (27).

FIGURE LEGENDS

Figure 1. Fatty acid composition of the eucaloric control diet (CON), and the hypercaloric saturated long-chain (LCSFA-HFD) and medium-chain (MCSFA-HFD) high-fat diets.

Figure 2. Impaired postabsorptive insulin-glucose homeostasis and reduced whole body and peripheral glucose disposal following intake of a hypercaloric high-fat diet enriched in long-chain saturated FAs (LCSFA-HFD), but not following intake of long-chain saturated FAs including medium-chain FAs (MCSFA-HFD). A. randomized cross-over study design with two intervention groups. B. Homeostatic model assessment of insulin resistance (HOMA IR)-index in the basal state. C. Basal glucose rate of appearance (Ra) obtained in the fasted state prior to the clamp. D+E. Glucose infusion rate (GIR), expressed per kg body mass (BM). F. Glucose rate of appearance (Ra) during the last 20 min of the clamp. G. Glucose rate of disappearance (Rd) during the clamp. H+I. Insulin stimulated leg glucose uptake (expressed per kg leg mass (LM)). J+K. Basal and insulin stimulated respiratory exchange ratio (RER). L. Delta RER values, calculated as the increase in RER from basal to the end of the clamp. M. oxidative glucose disposal and N, non-oxidative glucose disposal during the clamp. Data in E, I, J, K, M, N show average values from the last 60 min of the clamp. Bar graphs show means \pm SEM with individual data plots. Two-way RM ANOVAs were applied to test for effect of group (MCSFA-HFD or LCSFA-HFD) and intervention, or effect of insulin and intervention within each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ effect of intervention (main effect in K, M). # $p < 0.05$, ### $p < 0.001$ effect of insulin (main effect in K). $n = 8$ in CON LCSFA-HFD and LCSFA-HFD, $n = 9$ in CON MCSFA-HFD and MCSFA-HFD. For the LCSFA-HFD group, data in E and I have been previously published (27).

Figure 3. Data from skeletal muscle showing increased basal muscle glycogen content and increased basal and insulin-stimulated glycogen synthase activity in MCSFA-HFD compared with LCSFA-HFD, together with increased insulin-stimulated lactate v-a difference across the leg. CON, eucaloric control diet. LCSFA-HFD, hypercaloric diet enriched in long-chain saturated FAs. MCSFA-HFD, hypercaloric diet enriched in long-chain and medium-chain saturated FAs. A. Basal skeletal muscle glycogen content, expressed per kg dry weight (d.w.). B+C. Glycogen synthase (GS) activity, expressed as % of fractional velocity (FV %). D. PDH-E1 α Ser³⁰⁰ phosphorylation during insulin stimulation. E. PDH-E1 α protein content, measured in insulin-stimulated biopsies. F. Leg lactate v-a difference, shown as average values during the last 60 min of the clamp. G+H. Basal and insulin-stimulated Akt Ser⁴⁷³ phosphorylation. I. Representative western blots. RU: relative units, related to the basal condition in

CON for the LCSFA-HFD group (or respective CON in G+H). Bar graphs show mean \pm SEM with individual data plots. Two-way RM ANOVAs were applied to test for effect of group (MCSFA-HFD or LCSFA-HFD) and intervention, or effect of insulin and intervention within each group. * $p < 0.05$, ** $p < 0.01$ effect of intervention, or main effect of intervention (C, D, E). ### $p < 0.001$ main effect of insulin. $n=8$ in CON LCSFA-HFD and LCSFA-HFD, $n=9$ in CON MCSFA-HFD and MCSFA-HFD. For the LCSFA-HFD group, data in A and G have been previously published (27).

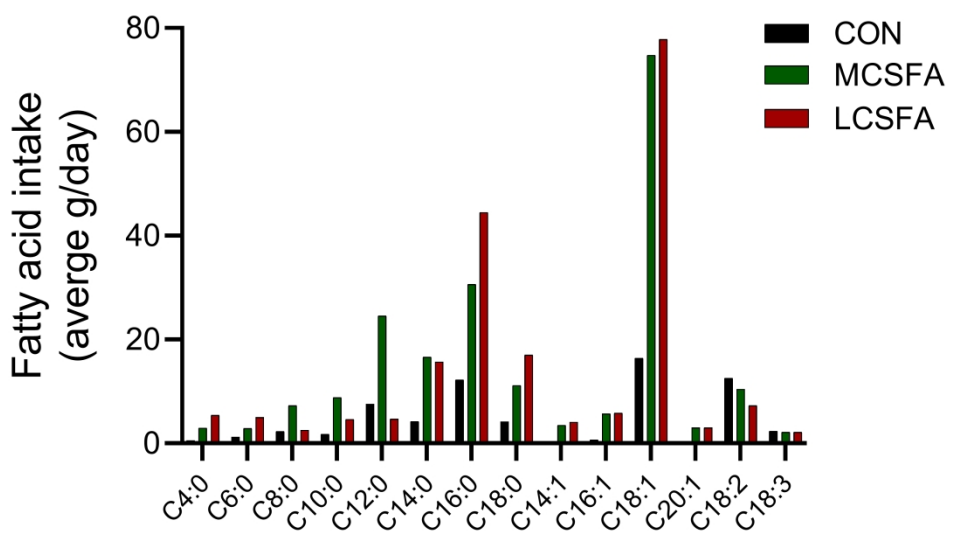


Figure 1

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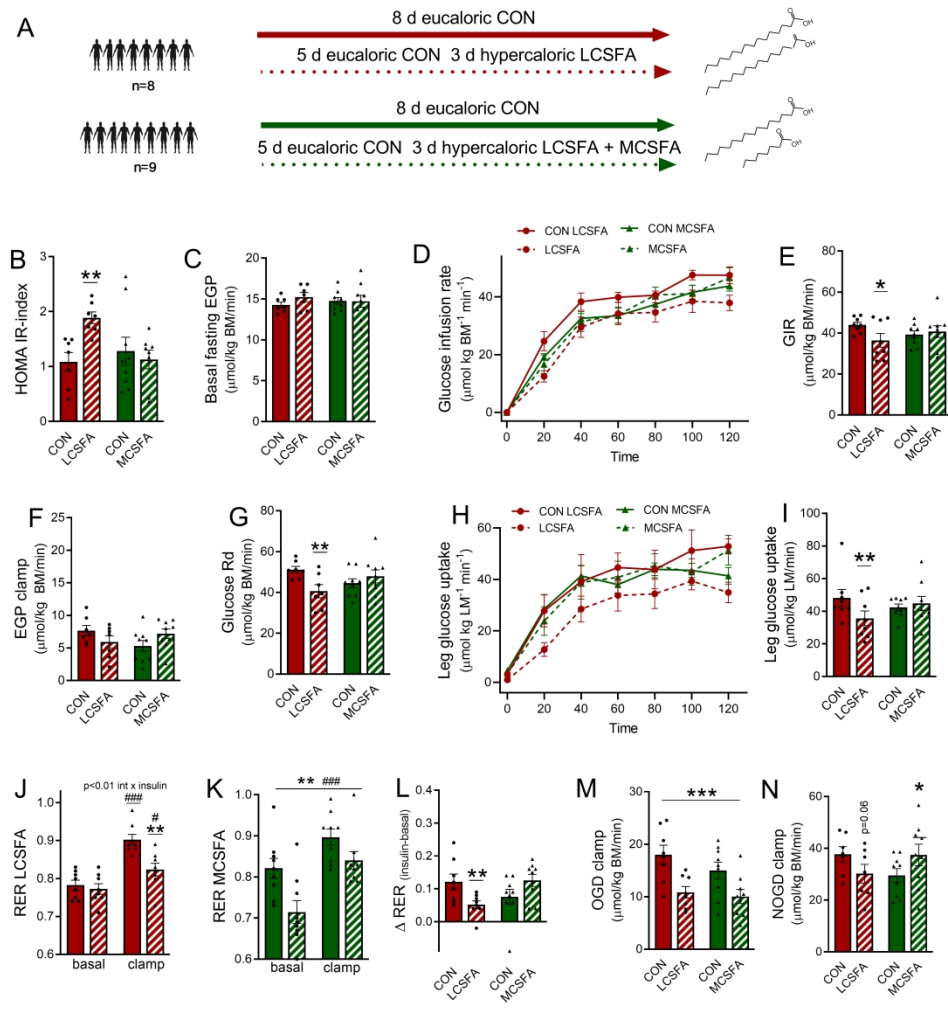


Figure 2

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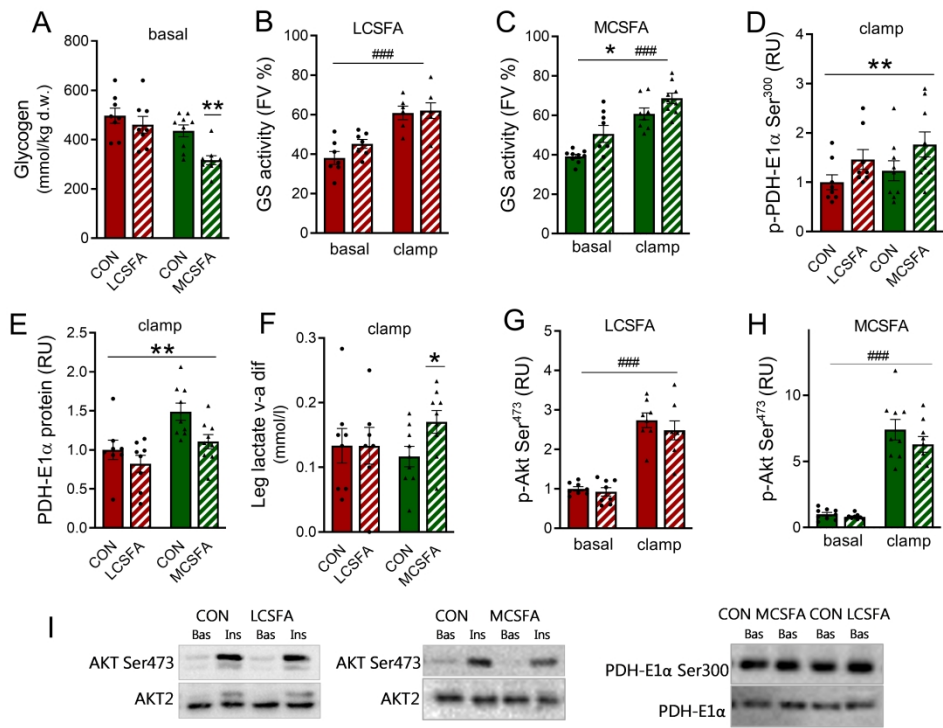


Figure 3

192x146mm (600 x 600 DPI)

	CON	LCSFA	MCSFA
Carbohydrate (E%)	63	9	9
Protein (E%)	14	8	10
Fat (E%)	24	83	81
SFA (E%)	13	56	62
MUFA (E%)	6	25	17
PUFA (E%)	5	2	2

Supplemental table S1. Macronutrient composition of the eucaloric control diet (CON) and the hypercaloric long- (LCSFA) and medium-chain (MCSFA) saturated high-fat diets.