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Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species-dependent: Potent tissue-specific effects of medium chain fatty acids

Running Title: Medium chain fatty acids and insulin action

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Objective: Medium-chain fatty acids (MCFA) have been reported to be less obesogenic than long-chain fatty acids (LCFA), however relatively little is known regarding their effect on insulin action. Here we examined the tissue-specific effects of MCFA on lipid metabolism and insulin action.

Methods: C57BL6/J mice and Wistar rats were fed either a low-fat (LF) control diet or high-fat diets rich in MCFA or LCFA for 4-5 weeks and markers of mitochondrial oxidative capacity, lipid levels and insulin action were measured.

Results: Mice fed the MCFA diet displayed reduced adiposity and better glucose tolerance than LCFA-fed animals. In skeletal muscle, triglyceride levels were increased by the LCFA diet (77%, $P<0.01$), but remained at LF control levels in the MCFA-fed animals. The LCFA-diet increased (20-50%, $P<0.05$) markers of mitochondrial metabolism in muscle compared to LF controls, however the increase in oxidative capacity was substantially greater in MCFA-fed animals (50-140% vs. LF controls, $P<0.01$). The MCFA diet induced a greater accumulation of liver triglycerides than the LCFA diet, likely due to an upregulation of several lipogenic enzymes. In rats, isocaloric feeding of MCFA or LCFA HF diets induced hepatic insulin resistance to a similar degree, however insulin action was preserved at the level of LF controls in muscle and adipose from MCFA-fed animals.

Conclusions: MCFA reduce adiposity and preserve insulin action in muscle and adipose, despite inducing steatosis and insulin resistance in the liver. Dietary supplementation with MCFA may therefore be beneficial for preventing obesity and peripheral insulin resistance.

Insulin resistance, defined as an impaired ability of insulin to regulate carbohydrate and lipid metabolism in target tissues, is one of the major metabolic defects of obesity and type 2 diabetes (T2D). It is closely linked with excess lipid deposition in non-adipose tissues, particularly skeletal muscle and liver, and several mechanisms have been proposed describing how lipid metabolites antagonize insulin action (1; 2). Although the precise factors which cause inappropriate lipid accumulation are still not completely resolved, a number of studies have suggested that reduced mitochondrial capacity for lipid oxidation, particularly in skeletal muscle, may lead to partitioning of FA into lipid storage pathways and a subsequent deterioration in insulin sensitivity (1; 3).

Given the close link between lipid accumulation and reduced insulin action, one of the primary experimental paradigms for investigating the etiology of insulin resistance is high-fat (HF) feeding (e.g. 45-60% of calories) in rodents. Many studies have demonstrated that consumption of a diet high in long-chain fatty acids (LCFA) induces widespread insulin resistance in muscle, liver and adipose tissue of both rats and mice (4-7). Under these conditions of excess LCFA availability however, we (8) and others (9) have demonstrated that mitochondrial content and fatty acid oxidative capacity are actually increased in muscle, suggesting that there is a compensatory response to increase fatty acid utilization pathways which is insufficient to prevent lipid overload and insulin resistance. Indeed we have recently shown that acute overexpression of carnitine palmitoyltransferase-1 (CPT-1) in muscle, increases fatty acid oxidative capacity above that induced by high-fat feeding alone and this partially protects against lipid-induced insulin resistance (10).

While high-fat diets containing most classes of LCFA (e.g. saturated, monounsaturated and omega-6) lead to obesity and insulin resistance (4; 6; 11), an interesting group of fatty acids that have been suggested to have anti-obesity potential, are medium chain (C8-12) fatty acids (MCFA) (12; 13). Studies in humans and rodents have shown that MCFA induce higher energy expenditure and fatty acid oxidation compared with LCFA, and this is associated with lower adipose mass (14-17). Compared to LCFA however, less is known regarding the effect of MCFA on insulin sensitivity. In rats, HF diets rich in MCFAs have been reported to be less deleterious for glucose and insulin tolerance compared with LCFAs (11; 18; 19). A limited number of studies in humans have also suggested that MCFA may not have detrimental effects on insulin action (20; 21). Whether specific tissues are involved in the favourable effects of MCFA on insulin action is currently unclear, particularly as several studies have shown that MCFA induce hepatic steatosis (11; 17), which would be expected to have a negative impact on insulin sensitivity in this tissue. Therefore our aim in this study was to investigate the tissue-specific effects of HF diets containing MCFA on lipid metabolism and insulin action.

MATERIALS AND METHODS

Animals. Eight-week old male C57BL6/J mice and male Wistar rats were purchased from the Animal Resources Centre (Perth, Australia). The animals were kept in a temperature-controlled room (22±1°C) on a 12-hour light/dark cycle with free access to water. Mice and rats were fed *ad libitum* for one week on a standard low-fat laboratory diet (LF; 8% calories from fat, 21% calories from protein, 71% calories from carbohydrate, Gordon's Specialty Stock Feeds, Yanderra, NSW, Australia) and were then randomly allocated to remain on the LF diet or to receive a HF diet, enriched with

either LCFA from lard or MCFA from hydrogenated coconut oil. The dietary FA composition was determined as described below and is presented in Table 1. For mice, the HF diets were based on Rodent Diet #D12451 from Research Diets Inc. (New Brunswick, NJ; containing 45% of calories from fat) and animals were fed *ad libitum* for a period of 5 wks. For the rat studies, animals were pair-fed LCFA and MCFA HF diets (59% of calories from fat) as previously described (6). All experiments were carried out with the approval of the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee, following guidelines issued by the National Health and Medical Research Council of Australia.

Determination of body composition and energy expenditure. Fat and lean body mass were measured in mice using dual-energy x-ray absorptiometry (DXA) (Lunar PIXImus2 mouse densitometer; GE Healthcare) in accordance with the manufacturer's instructions. Oxygen consumption rate (VO_2) of individual mice was measured using an 8-chamber indirect calorimeter (Oxymax series, Columbus Instruments, Columbus, OH) as previously described (8).

In vivo glucose metabolism. Glucose tolerance tests (2 g/kg glucose *i.p.*) were performed in overnight-fasted mice. Blood samples were obtained from the tail tip at the indicated times and glucose levels were measured using a glucometer (Accu-Check, Roche, NSW, Australia). For euglycaemic-hyperinsulinaemic clamps in rats (insulin infusion 0.25 units/kg·hr), double jugular cannulae were implanted 7 days prior to experiments and animals (5h fasted) were studied over 2 hr in the conscious state as previously described (22).

Fatty acid composition, triglyceride and insulin levels. Lipids were extracted from tissues and diets by standard methods (23). For tissue lipid extracts, neutral lipids were separated from phospholipids by solid-phase extraction on Waters Sep-Pak silica columns (Milford, MA). Lipid fractions were transmethylated (24) and fatty acid methyl esters separated by gas-liquid chromatography on a Shimadzu 17A gas chromatograph (NSW, Australia) with a Restek FAMEWAX capillary column (Bellefonte, PA). Plasma, muscle and liver triglyceride contents were determined using a colorimetric assay kit (Triglycerides GPO-PAP; Roche Diagnostics, Indianapolis, IN) as previously described (22). Plasma insulin was determined by radioimmunoassay using a rat-specific kit (Linco Research, Inc., St. Charles, MO).

Enzyme activity measurements. Muscle and liver samples were homogenized 1:19 (wt/vol) in 50 mmol/l Tris-HCl, 1 mmol/l EDTA, and 0.1% Triton X-100, pH 7.2, using a Polytron instrument (Kinematica, Littau-Lucerne, Switzerland) and were subjected to three freeze-thaw cycles. Citrate synthase (CS), β-hydroxyacyl CoA dehydrogenase (HAD) and medium-chain acyl-CoA dehydrogenase (MCAD) were determined at 30°C as described previously (8) using a Spectra Max 250 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA).

Immunoblotting. Muscle and liver samples were resuspended in RIPA buffer (65 mM Tris (pH 7.4), 150 mM NaCl, 1% Nonidet NP-40, 0.5% sodium Deoxy-cholate, 0.1% SDS), supplemented with protease and phosphatase inhibitors (10 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin and 10 μg/ml leupeptin, 1 mmol/l Na_3VO_4, 10 mmol/l NaF) and solubilised for 2 h at 4°C. Equal amounts of tissue lysates (10-20 μg protein) were resolved by SDS-PAGE and immunoblotted with antibodies against PGC-1α (Chemicon International Inc., Temecula, CA), muscle and liver carnitine palmitoyltransferase-1 (CPT-1; Alpha Diagnostic International, San Antonio, TX),

uncoupling protein 3 (UCP3, Affinity Bioreagents Inc. Golden, CO), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD-1) and acetyl-CoA carboxylase (ACC) (Cell Signaling Tech., Beverly, MA), cytochrome oxidase (Complex IV) subunit 1 (Invitrogen, Victoria, Australia), and an antibody cocktail that recognizes several subunits of the mitochondrial respiratory chain (MS601, Mitosciences, Eugene, OR). Immunolabelled bands were quantitated by densitometry.

Statistical analyses. Data are presented as mean ± SE. One-way ANOVA with Fisher's PLSD post-hoc test was used to assess statistical significance between groups. Differences at $P<0.05$ were considered to be statistically significant.

RESULTS

Body composition, glucose tolerance and tissue triglyceride levels in mice. At the completion of the 5 wk feeding regime, body mass was not different among mice fed the LF, MCFA and LCFA diets (Table 2). Whole body adiposity measured by DXA scanning, was not significantly different between MCFA-fed mice and LF controls, but was substantially increased ($P<0.01$) in LCFA-fed mice (Table 2). The reduced level of adipose accumulation in MCFA-fed mice compared with LCFA-fed mice, appeared to be primarily due to an increased energy expenditure (VO_2) in MCFA-fed animals (MCFA 3.64 ± 1.3 vs. LCFA 3.34 ± 0.8 ml O_2/g/hr, n=7, $P<0.05$), as although the caloric intake was increased ($P<0.01$) in both fat-fed groups compared to LF controls (11.5 ± 0.5 kcal/day, n=6), no difference was observed between the MCFA and LCFA diets (MCFA 14.2 ± 0.1 vs. LCFA 14.6 ± 0.4 kcal/day, n=5-6).

To determine the effect of the MCFA and LCFA diets on whole-body glucose metabolism, we examined glucose clearance during an intraperitoneal glucose tolerance test (Fig 1). Mice fed the LCFA diet displayed a substantial impairment in glucose tolerance compared to LF controls as represented by the 88% ($P<0.01$) increase in the glucose area under the curve (AUC). Animals fed the MCFA diet exhibited a much milder impairment in glucose tolerance (19% increase in AUC ($P<0.05$) compared to LF controls; Fig 1). Given the established link between excess intracellular lipid and insulin resistance (2), we examined tissue triglyceride levels to determine if the difference in glucose tolerance in response to MCFA and LCFA may have been linked to a differential effect of the HF diets on liver and muscle lipid levels (Table 2). Muscle triglyceride content was not different between LF controls and MCFA-fed mice, but was significantly elevated in muscle from the LCFA-fed mice compared to both other groups (Table 2). In contrast to this, liver triglycerides were elevated by approximately 2.5-fold ($P<0.01$) in LCFA-fed animals compared to LF controls (Table 2), while MCFA-fed animals displayed liver triglyceride levels that were significantly higher ($P<0.01$) than both LF controls and LCFA-fed mice.

Markers of mitochondrial metabolism and lipogenesis. Mitochondria are a major site for lipid oxidation and therefore we examined several markers of mitochondrial metabolism to determine if the contrasting effects of MCFA and LCFA on intracellular lipid levels may be related to differences in fatty acid utilization. In skeletal muscle we observed a significant upregulation (30-40%) of CS, HAD and MCAD activity in LCFA-fed mice compared with LF control animals (Fig 2). Interestingly, the MCFA diet induced a much greater increase in muscle oxidative enzyme activity (90-140% higher than LF controls, Fig 2). We also examined the protein expression of subunits of the mitochondrial respiratory chain, as well as CPT-1, UCP3 and the transcriptional coactivator PGC-1α. Similar to the enzyme

activities, increased expression of mitochondrial proteins was observed in muscle of the LCFA-fed mice compared to LF controls, with a substantially greater increase seen in the protein levels of respiratory chain subunits, CPT-1 and UCP3 in MCFA-fed animals (Fig 3). Collectively these findings suggest a more potent stimulation of mitochondrial biogenic pathways in MCFA-fed mice compared to the LCFA-fed animals, however this difference was not due to greater PGC-1α expression, as this was increased to a similar extent in both groups (Fig 3).

In liver we observed no difference in the activity of CS, HAD or MCAD in either the MCFA or LCFA group compared to LF controls (Fig 2). There was no difference between the three dietary groups in the expression of CPT-1 or subunits from Complex III and Complex V of the respiratory chain, however LCFA-fed animals displayed a significant reduction in the expression of subunits from Complex II and Complex IV (Fig 3). As there was marked hepatic steatosis in MCFA-fed mice, without a consistent decrease in markers of fatty acid oxidative capacity, we also examined the protein expression of several enzymes involved in lipogenesis, to determine if increased flux through lipogenic pathways may be underpinning the elevated triglyceride accumulation in these animals. This appeared to be the case as compared to LF controls, MCFA-fed mice exhibited a 2.4-fold, 2.5-fold and 12-fold increase ($P<0.01$, n=6) in the protein expression of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and stearoyl-CoA desaturase-1 (SCD-1) respectively, while relative to LF controls LCFA-fed mice displayed a 43% increase ($P<0.05$, n=6) in FAS expression, with no difference observed for ACC or SCD-1 (Fig 4).

Body composition and insulin action in rats. Overall our studies in mice showed that MCFA have differential effects on lipid metabolism in liver and muscle and to investigate tissue-specific effect of MCFA on insulin action we conducted hyperinsulinemic-euglycemic clamps in rats pair-fed MCFA and LCFA high-fat diets. Following 4 wks of isocaloric HF feeding, rats fed the MCFA diet displayed reduced body weight and lower adiposity compared with the LCFA-fed animals (Table 3). Plasma glucose, insulin and triglyceride levels were significantly elevated in the LCFA-fed animals compared to the LF controls, while MCFA-fed rats displayed only minor elevations in insulin and triglyceride levels (Table 3). Similar to the pattern we observed in the mice, muscle triglyceride levels were not different between animals fed the LF and MCFA diets, but were increased in the LCFA-fed rats (Table 3). Liver triglyceride levels were also higher in the MCFA-fed animals, compared to both the LF controls and LCFA-fed rats (Table 3).

Whole-body insulin sensitivity, measured as the glucose infusion rate (*GIR*) during a hyperinsulinemic-euglycemic clamp, was 44% ($P<0.01$) lower in the LCFA-fed rats compared to LF controls, while MCFA-fed animals displayed an 18% ($P<0.01$) reduction in *GIR* compared to LF controls (Table 4). The reduced *GIR* in LCFA-fed rats compared to LF controls was the result of both a reduced rate of insulin-stimulated glucose disposal into peripheral tissues (R_d) and a decreased suppression of hepatic glucose output (*HGO*) (Table 4). In MCFA-fed rats, the effect of insulin to suppress *HGO* was impaired to a similar degree to the LCFA-fed rats, but strikingly R_d was not different from LF controls. Skeletal muscle is the major peripheral tissue responsible for glucose disposal and accordingly we examined the uptake of ^{3}H-2-deoxyglucose tracer (Rg´) into red and white muscles during the clamp. Compared to LF controls, LCFA-fed rats exhibited a reduction (25-40%, $P<0.01$) in

R_g' in both red and white muscles (Table 4). Consistent with the results observed for R_d, MCFA-fed animals exhibited similar (red muscle) or even slightly increased (white muscle) R_g' values compared with LF controls (Table 4). In the epididymal adipose depot we also observed a 45% reduction ($P<0.01$) in R_g' as a result of the LCFA diet, while the MCFA diet preserved insulin action in this tissue (Table 4). Of interest, we analyzed the fatty acid composition of liver, muscle and epididymal adipose tissue and found that in MCFA-fed animals, MCFA accounted for ~20% of the total fatty acids in the neutral lipid fraction in muscle and adipose (i.e. the two tissues that did not develop insulin resistance), but <5% of the total fatty acids in liver, where insulin action was diminished (data not shown). In LF and LCFA-fed animals MCFA accounted for <1% of the total fatty acids in all tissues analyzed.

DISCUSSION

Previous studies examining the effect of different fatty acids on insulin action have reported improved glucose tolerance and insulin tolerance in rodents fed high-fat diets rich in MCFA, compared with LCFA (11; 18; 19). Our current study reveals the tissues responsible for the favourable effect of MCFA on whole-body glucose metabolism, as well as a mechanistic basis for these effects. We have made the intriguing observation that insulin action in skeletal muscle and adipose tissue is preserved at the level of LF controls when animals consume a HF diet rich in MCFA. In muscle, the lack of induction of insulin resistance with MCFA HF feeding is associated with a substantial increase in mitochondrial oxidative capacity, which is sufficient to prevent lipid accumulation in this tissue. However the liver of MCFA-fed animals accumulated greater amounts of triglyceride, likely due to upregulation of lipogenic pathways, and as such hepatic insulin action was reduced following MCFA HF feeding.

It is likely our findings have clinical relevance, as several studies have suggested that MCFA may be beneficial for insulin action in humans. Eckel et al. (20) showed in a small (n=3) cohort of subjects with T2D, that acute treatment with MCFA (40% fat for 5 days) resulted in a beneficial effect on insulin-stimulated glucose disposal, without consistent effects on insulin-mediated suppression of HGO. Furthermore a 3-month trial in patients with T2D reported improved homeostatic model assessment of insulin resistance (HOMA-IR) in subjects consuming MCFA compared with LCFA (21). The above human studies and those in rodents (11; 18; 19) indicate that MCFA do not induce insulin resistance to the same degree as LCFA, however they have provided limited information regarding the tissue-specific effects of MCFA on insulin action *in vivo* and/or a mechanism for any observed beneficial effects. Our current study clearly shows that MCFA do not induce insulin resistance in either muscle and adipose tissue, and given the fact that muscle is the major tissue for insulin-stimulated glucose disposal (25), the reported favourable effects of MCFA on whole-body glucose metabolism (11; 18; 20; 21) are probably related to changes in insulin action in muscle. It is worth noting however that the daily caloric intake in MCFA-fed mice was approximately 25% higher than in LF controls, and whether with more prolonged HF feeding this elevated energy intake would eventually lead to some metabolic dysfunction in muscle and adipose tissue remains to be determined.

The strong association between lipid accumulation and insulin resistance is well documented (1; 2; 6) and our findings that MCFA do not induce lipid accumulation in muscle and concurrently preserve insulin action in this tissue, strongly support the above link. We and others have reported that

under conditions of increased lipid availability, either through high-fat feeding (with LCFA), acute lipid infusions or muscle-specific overexpression of lipoprotein lipase, mitochondrial content and fatty acid oxidative capacity are upregulated in muscle (8; 9; 26; 27). Such a response likely represents an attempt of the muscle to cope with additional fatty acid substrates, however the fact that lipid still accumulates in muscle in animals under these conditions suggests that the compensatory upregulation of oxidative pathways is unable to deal with the elevated uptake of LCFA that is observed with such manipulations (26; 28). In comparison with LCFA however, we have shown that MCFA induce a substantially greater upregulation of mitochondrial oxidative capacity in muscle, and this appears to be at a sufficient level to prevent the deleterious effects of lipid oversupply on insulin action in this tissue.

The underlying molecular mechanism by which MCFA induce a more potent upregulation of mitochondrial biogenesis in muscle than LCFA is currently unclear. We observed a substantial accumulation of MCFA in the neutral lipid fraction of muscle from MCFA-fed animals and one major pathway through which fatty acids influence substrate metabolism in muscle is via activation of peroxisome proliferator-activated receptors (PPAR), particularly PPARδ. These transcription factors when activated by fatty acids, or other ligands, control genes involved in oxidative and fatty acid metabolism (29). Several studies have shown however that MCFA have low binding affinity for PPARs (30; 31), suggesting that a direct effect of MCFA on PPAR-dependent transcription is unlikely responsible for the increase in mitochondrial biogenesis. PPARs can also be activated via interaction with the transcriptional coactivator PGC-1α, which is considered a master controller of mitochondrial biogenesis in muscle. We observed similar upregulation of PGC-1α content in muscle with both the MCFA and LCFA diets. However, post-translational modification (e.g. acetylation) of PGC-1α is known to regulate its activity (32) and whether MCFA specifically affect this pathway or influence the activity of other transcription factors is currently unknown.

In addition to oxidative metabolism, there are a number of other pathways that influence lipid deposition in tissues, including lipid uptake from the circulation and for tissues such as the liver, the rate of *de novo* lipogenesis. With regard to these factors, MCFA differ from LCFA in a number of important ways. MCFA are more readily absorbed into the bloodstream and therefore a greater proportion of these fatty acids reach the liver through the portal vein (33). MCFA can also enter the mitochondrion for oxidation via CPT-1 independent mechanisms (34). These unusual physical properties are thought to largely explain the increase in energy expenditure and decreased adiposity observed with MCFA-rich diets, i.e. due to enhanced hepatic fatty acid oxidation, particularly in the post-prandial period (13). Our novel finding of a very potent upregulation of mitochondrial content in muscle by MCFA, suggests that enhanced flux of substrates through oxidative metabolism in muscle may also contribute to MCFA-induced changes in energy expenditure and adiposity, as well as improved muscle insulin action (current study and (13-15).

The other tissue in which MCFA were less deleterious than LCFA for insulin action was adipose tissue. Small adipocytes are more insulin-sensitive than large adipocytes (35; 36), and previous studies have demonstrated that adipocyte size is reduced with MCFA diets (16; 37), potentially due to a reduction in adipogenic gene expression (18). It is likely that the preserved insulin sensitivity we observed in adipose following MCFA high-fat feeding is

simply a consequence of reduced adipocyte size, although given the fact that MCFA accumulate significantly in adipose tissue following MCFA HF feeding (current study and (18)), we cannot rule out a more direct effect of MCFA on adipocyte function that may be beneficial for insulin action. Furthermore as adipose tissue secretes a number of adipokines that affect carbohydrate and lipid metabolism in other tissues, it remains to be determined if MCFA-induced changes in adipokine profile (19), partly contribute to the changes in mitochondrial content and insulin action observed in skeletal muscle with the MCFA high-fat diet.

Despite the favourable effects of MCFA on muscle and adipose metabolism, another important finding was that MCFA robustly induced insulin resistance in liver and caused a greater degree of hepatic steatosis than LCFA in both mice and rats. This elevation in liver triglyceride levels did not appear to be due to a decreased capacity for lipid oxidation as we observed generally similar levels of mitochondrial enzyme activity and protein expression in the different dietary groups. As mentioned above, the entry of MCFA into mitochondria is less dependent on CPT-1 than LCFA and a consequence of accelerated β-oxidation is an excess production of acetyl-CoA. Much of this acetyl-CoA is converted into ketone bodies, which have been reported to be elevated in MCFA-fed animals (17; 38). Acetyl-CoA is also a substrate for *de novo* lipogenesis, and in line with other reports (39; 40) we observed a substantial upregulation of lipogenic enzymes in liver from the MCFA-fed mice, presumably to deal with the excess acetyl-CoA, and this is likely a major contributor to the increased triglyceride levels in these animals. Consistent with this we only observed a small proportion of MCFA in the neutral lipid fraction of liver from MCFA-fed animals, suggesting metabolism of these fatty acids through lipogenic pathways.

There is controversy in the literature regarding the effects of MCFA on liver triglycerides. In rodents, a number of studies have reported increased liver triglyceride levels with MCFA feeding (11; 17), while others show no difference between high-fat diets containing MCFA or LCFA (16). Interestingly, one recent study in rats suggested that liver triglyceride content is significantly lower with a diet containing only MCFA compared with LCFA, but this effect was diminished in the presence of LCFA (41). In humans, a number of studies have reported that MCFA do not have adverse effects on liver lipid levels (42; 43), however inconsistent findings have been reported regarding the effect of MCFA on circulating lipid parameters (21; 44; 45). It is possible that methodological differences may underlie many of these seemingly disparate findings, such as the dietary fat content and composition, the length of dietary intervention and the composition of other constituents of the diet (e.g. carbohydrates and protein).

In summary our study shows that high-fat diets containing MCFA have divergent effects on tissue-specific insulin sensitivity, inducing insulin resistance to a similar degree as LCFA in liver, while preserving insulin action at the level of LF controls in muscle and adipose tissue. The preservation of muscle insulin action by MCFA is associated with a potent stimulation of mitochondrial biogenesis, which appears to be sufficient to prevent lipid accumulation in this tissue. Given that the total amount of dietary fat used in the current studies is relatively high (i.e. 45-60% of energy), it will be important to determine in future studies the amount of dietary MCFA (both in absolute terms and relative to dietary LCFA) required for beneficial effects on energy metabolism and insulin action, and whether

this amount of dietary MCFA avoids liver lipid accumulation. In this regard some human studies have reported positive effects on energy expenditure and body composition with relatively low dietary doses of MCFA (21; 46; 47). Additionally as some anti-diabetic therapies (e.g. metformin) are known to exert the majority of their insulin-sensitising effects via their actions in the liver (48), it will be of interest to determine if MCFA supplementation in conjunction with such agents, results in beneficial effects on insulin action in multiple insulin-target tissues.

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Table 1. Fatty acid composition of the LF and HF diets

FA	LF	MCFA	LCFA
8:0	0	7.3	0
10:0	0	5.7	0
12:0	0	36.7	0
14:0	0.6	16.5	1.3
16:0	13.1	9.8	24.6
18:0	4.0	10.5	15.3
18:1 (n-9)	38.7	3.0	32.1
18:2 (n-6)	31.7	9.8	21.7
18:3 (n-3)	6.7	0.3	0.9

Fatty acid (FA) composition was determined by gas chromatography. Values are expressed as the percentage of total fatty acids.

Table 2. Body mass, fat pad mass and tissue triglyceride levels in mice

	LF	MCFA	LCFA
Body mass (g)	28.2 ± 0.5	28.7 ± 0.6	29.1 ± 1.0
Fat mass (%)	14.7 ± 0.6	17.3 ± 1.7	25.6 ± 1.9†§
Muscle triglyceride (□mol/g)	11.1 ± 0.8	13.0 ± 2.8	19.7 ± 1.9†‡
Liver triglyceride (□mol/g)	8.5 ± 0.7	49.7 ± 4.4†	20.1 ± 2.7†§

Values are means ± SE for n = 6-11 animals. Fat mass (%) was determined by DXA scanning. *P < 0.05, †P < 0.01 vs. LF; ‡P < 0.05, §P < 0.01 vs. MCFA.

Table 3. Body mass, fat pad mass, circulating parameters and tissue triglyceride levels in rats

	LF	MCFA	LCFA
Body mass (g)	349 ± 7	337 ± 6	387 ± 5†§
Epididymal fat (%)	1.0 ± 0.1	1.3 ± 0.1†	1.6 ± 0.05†§
Retroperitoneal fat (%)	1.0 ± 0.1	1.3 ± 0.1	2.1 ± 0.2†§
Lumbar fat (%)	1.5 ± 0.1	1.5 ± 0.1	2.2 ± 0.1†§
Plasma glucose (mM)	7.2 ± 0.2	7.5 ± 0.2	8.1 ± 0.2*
Plasma insulin (mU/l)	24 ± 2	31 ± 3	66 ± 5†§
Plasma triglyceride (mM)	0.8 ± 0.1	0.9 ± 0.0	1.1 ± 0.2*
Muscle triglyceride (μmol/g)	1.7 ± 0.1	1.9 ± 0.2	2.9 ± 0.3†§
Liver triglyceride (μmol/g)	4.8 ± 0.3	18.4 ± 0.9†	14.4 ± 2.1†§

Values are means ± SE for n = 5-9 animals. Fat pad weights are expressed as a percentage of body mass. *P < 0.05, †P < 0.01 vs. LF; ‡P < 0.05, §P < 0.01 vs. MCFA.

Table 4. Metabolic parameters from hyperinsulinemic-euglycemic clamps in rats

	LF	MCFA	LCFA
GIR (mg/kg.min)	38.4 ± 1.6	31.4 ± 1.3†	21.5 ± 1.6†§
Rd (mg/kg.min)	36.9 ± 1.3	34.3 ± 1.1	25.9 ± 1.9†§
HGO (mg/kg.min)	-1.5 ± 0.63	3.0 ± 1.1†	4.3 ± 0.4†
Rg′ - red quadriceps	29.4 ± 2.0	26.5 ± 1.8	16.9 ± 2.7†§
Rg′ - red gastrocnemius	29.7 ± 2.8	26.8 ± 1.4	17.6 ± 0.8†§
Rg′- white gastrocnemius	7.1 ± 0.6	9.0 ± 0.6†	5.4 ± 0.4†§
Rg′- epididymal fat	1.8 ± 0.2	2.2 ± 0.2	1.0 ± 0.1†§

Values are means ± SE for n = 5-7 animals. Plasma levels of glucose and insulin were similar for all groups during the clamp (data not shown). GIR – glucose infusion rate; Rd – glucose disposal rate; HGO – hepatic glucose output; Rg′ – insulin-stimulated ^{3}H-2-deoxyglucose uptake in skeletal muscle or adipose tissue during the clamp (μmol/100g.min). *$P < 0.05$, $^{\dagger}P < 0.01$ vs. LF; $^{\ddagger}P < 0.05$, $^{\S}P < 0.01$ vs. MCFA.

FIGURE LEGENDS

Fig. 1. Glucose tolerance test in overnight fasted LF (black circles), MCFA (white circles) and LCFA-fed (grey circles) mice. **A)** Blood glucose levels after an intra-peritoneal glucose load (2 g/kg). **B)** Incremental areas under the curve as an indicator of glucose clearance. Data represent the means ± SE of 5-11 mice. *$P < 0.01$ vs. LF, $^{\dagger}P < 0.01$ vs. LF and MCFA.

Fig. 2. Oxidative enzyme activity in skeletal muscle and liver from mice fed the LF (black bars), MCFA (white bars) and LCFA (grey bars) diets. Data represent the means ± SE of 5-6 mice. *$P < 0.01$ vs. LF and LCFA, $^{\dagger}P < 0.01$ vs. LF.

Fig. 3. Immunoblots for markers of mitochondrial metabolism and biogenesis in skeletal muscle and liver from mice fed the LF, MCFA and LCFA diets. Equal amounts of muscle lysates (10-20 μg protein) were resolved by SDS PAGE and immunoblotted with specific antibodies for PGC-1α, CPT-1, UCP3 and mitochondrial respiratory chain subunits. Densitometric analysis (relative to LF controls) for n=6 animals is presented. *$P < 0.05$, $^{\dagger}P < 0.01$ vs LF; $^{\ddagger}P < 0.05$, $^{\S}P < 0.01$ vs MCFA.

Fig. 4. Immunoblots for enzymes involved in lipogenesis in liver from mice fed the LF, MCFA and LCFA diets. Equal amounts of liver lysates (10-20 μg protein) were resolved by SDS PAGE and immunoblotted with specific antibodies for FAS, ACC and SCD-1.

Figure 1

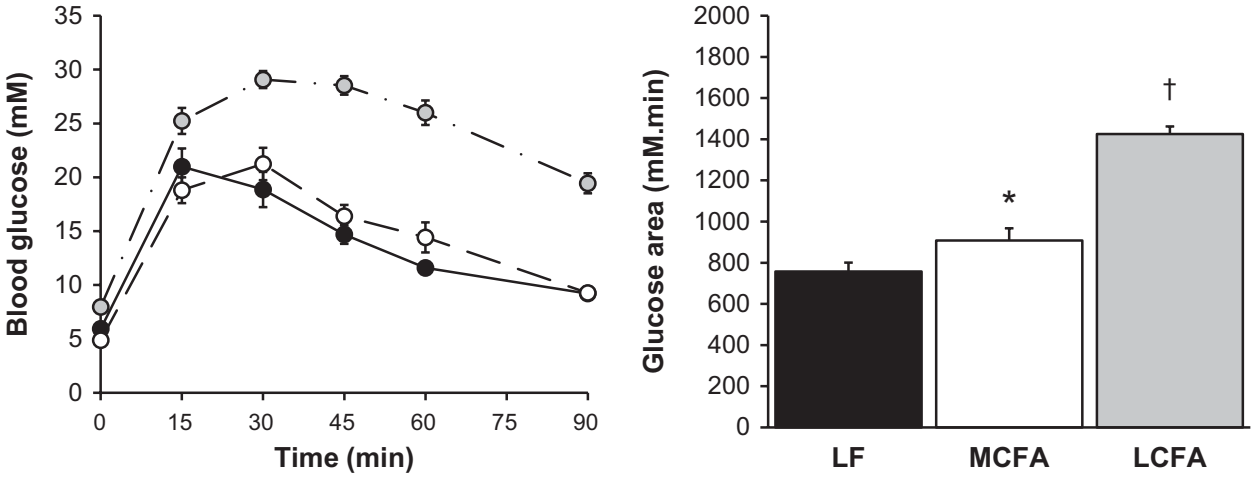

Figure 2

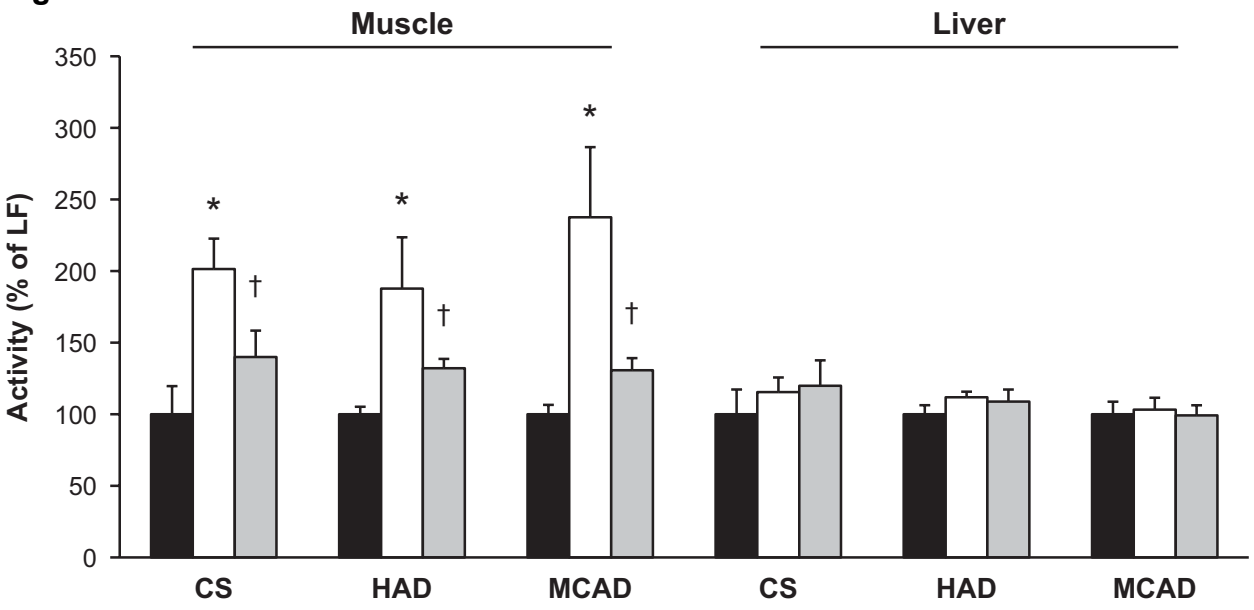

Figure 3

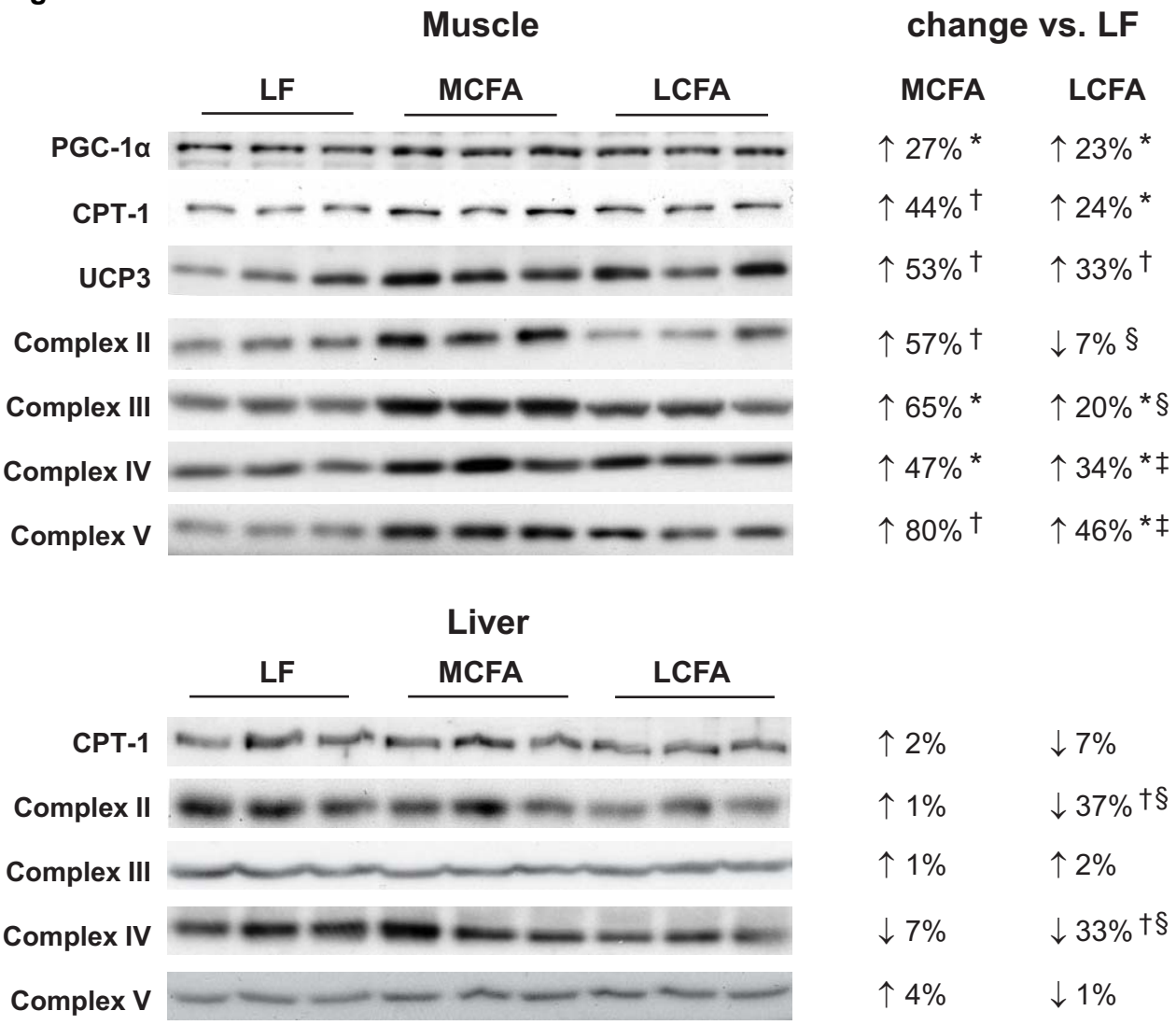

Figure 4