

Medium chain triglycerides dose-dependently prevent liver pathology in a rat model of non-alcoholic fatty liver disease

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Abstract

Metabolic syndrome is often accompanied by development of hepatic steatosis and less frequently by non-alcoholic fatty liver disease (NAFLD) leading to non-alcoholic steatohepatitis (NASH). Replacement of corn oil with medium chain triacylglycerols (MCT) in the diets of alcohol-fed rats has been shown to protect against steatosis and alcoholic liver injury. The current study was designed to determine if a similar beneficial effect of MCT occurs in a rat model of NAFLD. Groups of male rats were isocalorically overfed diets containing 10%, 35% or 70% total energy as corn oil or a 70% fat diet in which corn oil was replaced with increasing concentrations of saturated fat (18:2, beef tallow:MCT oil) from 20% to 65% for 21 days using total enteral nutrition (TEN). As dietary content of corn oil increased, hepatic steatosis and serum alanine amino transferases were elevated ($P < 0.05$). This was accompanied by greater expression of cytochrome P450 enzyme CYP2E1 ($P < 0.05$) and higher concentrations of polyunsaturated 18:2 and 20:4 fatty acids (FA) in the hepatic lipid fractions ($P < 0.05$). Keeping the total dietary fat at 70%, but increasing the proportion of MCT-enriched saturated fat resulted in a dose-dependent reduction in steatosis and necrosis without affecting CYP2E1 induction. There was no incorporation of C8–C10 FAs into liver lipids, but increasing the ratio of MCT to corn oil: reduced liver lipid 18:2 and 20:4 concentrations; reduced membrane susceptibility to radical attack; stimulated FA β - and ω -oxidation as a result of activation of peroxisomal proliferator activated receptor (PPAR) α , and appeared to increase mitochondrial respiration through complex III. These data suggest that replacing unsaturated fats like corn oil with MCT oil in the diet could be utilized as a potential treatment for NAFLD.

Keywords: NAFLD, NASH, steatosis, medium chain triacylglycerols, fatty acid

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Introduction

Medium chain triacylglycerols (MCT oil) consists of saturated fatty acids (FA) of chain length consisting of predominantly 8–10 carbons.¹ Unlike long-chain fats, MCTs are absorbed directly from the diet into the hepatic portal vein, and are thought to enter the liver cells and mitochondria by minimal use of normal FA transport systems or by diffusion where they are rapidly degraded to increase thermogenesis.^{2,3} In addition, MCT FAs are poorly esterified into cellular triacylglycerols in adipose tissue and do not induce adipocyte differentiation² and have been suggested to increase satiety and reduce food intake.⁴ As a result, diets high in MCT have been utilized clinically to prevent obesity.^{2–6} Interestingly, MCT has also been shown to prevent alcohol-induced liver injury in animal models^{7–10}

and clinically in patients with cirrhosis.¹¹ The protective effect of MCT on alcoholic liver injury has variously been suggested to involve increased FA degradation, decreased lipid peroxidation, reduced gut permeability and inhibition of endotoxin-induced Kupffer cell activation.^{7–10} Since alcoholic steatohepatitis and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH) share a very similar pathology, it has been suggested that dietary supplementation with MCT might have a similarly beneficial effect on NAFLD/NASH pathology. However, to date, this has not been extensively investigated. A study by Charles Lieber's group in 2008¹² suggested that complete replacement of dietary long-chain triacylglycerols (LCT) with MCT could dramatically ameliorate NASH development produced by *ad libitum* feeding of a liquid diet

containing 70% fat for 21 days as a result of improved insulin and adipokine status, reduced steatosis, reduced inflammation and increased hepatocyte proliferation. However, interpretation of these data was complicated by the substitution of MCT for two different sources of LCT, corn oil and olive oil, and by reductions in caloric intake produced by MCT.¹² In this same study, the authors observed increased rather than decreased hepatotoxicity when MCT only partly replaced LCT unless the MCT substitution was accompanied by caloric restriction.¹²

We recently developed a new rat model of NAFLD in which overfeeding a liquid diet high in the polyunsaturated LCT corn oil via an intragastric cannula (total enteral nutrition, TEN), resulted in dose- and time-dependent progression of NAFLD to NASH.¹³ The advantage of this model is that diets of substantially different composition can be overfed isocalorically to produce obesity, metabolic syndrome and NASH while eliminating the variable of different levels of caloric intake between groups. We utilized the model to demonstrate the importance of oxidative stress in NASH progression,¹⁴ and herein examine the effects of isocaloric substitution of corn oil with an MCT-enriched mixture of saturated fats (MCT:beef tallow, 82:18) on body composition, development of liver pathology, induction of CYP2E1, liver FA composition and hepatic lipid homeostasis.

Materials and methods

Animals and experimental design

Male Sprague-Dawley rats (175 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA). Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved animal facility. Animal maintenance and experimental treatments were conducted in accordance with the ethical guidelines for animal research established and were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Rats had an intragastric cannula surgically inserted and were allowed seven days to recover before infusion of TEN diets as described previously.¹³ Animals had *ad libitum* access to water throughout the experiments. Rats were randomly assigned to groups of $n = 7-8$ and were overfed 220 kcal/kg^{3/4}/d diets (17% above that required to match the growth of *ad libitum* chow fed rats) containing 10%, 35% or 70% corn oil (CO) for 21 days to produce obesity and dose-dependent development of NAFLD. In the other experimental groups the total % energy from fat was kept constant at 70%, but a saturated fat mixture of 82:18 MCT oil:beef tallow (MCT) was substituted for CO at a level of 20%, 35%, 50% or 65%. MCT oil was obtained from Nestle Nutrition and the beef tallow was from Bio-Serv. Fat was isocalorically substituted for carbohydrate calories as described previously such that 10% CO diets contained 71% carbohydrate, 35% CO diets contained 46% carbohydrate and 70% fat diets contained 11% carbohydrate in the form of 1:3 maltodextrin:dextrose.^{13,14} There were a total of seven diets and each had a caloric density of 890 kcal/L (isocaloric). Protein content (19% whey protein),

vitamin and mineral contents were the same in all diets.¹³ Diets were formulated to meet the caloric and nutritional recommendations established by the National Research Council (NRC), but were fed at a level that exceeded the recommended caloric intake by 17% to increase weight gain and adiposity and produce steatohepatitis. Body weight gains were measured twice a week and at the beginning and end of the study. Total body fat composition was assessed by nuclear magnetic resonance (Echo, Houston, TX, USA). At sacrifice serum, fat pads and livers were collected and stored at -20°C and -70°C , respectively.

Liver pathology

Steatosis as determined by Oil Red O staining of lipid droplets was quantified by image analysis as described previously.¹³ Triglyceride was extracted from whole liver homogenates with chloroform:methanol (2:1, v:v) and analyzed using Triglyceride Reagent (IR141; Synermed, Westfield, IN, USA). Inflammation was assessed by measurement of tumor necrosis factor (TNF α).

mRNA expression using realtime reverse transcriptase polymerase chain reaction (RT-PCR) as described below.¹⁴ Necrosis was assessed biochemically by measurement of serum alanine amino transferase (ALT) activity at sacrifice using the Infinity ALT liquid stable reagent (Thermo Electron Corp., Waltham, MA, USA) according to the manufacturer's protocols. Cellular proliferation was measured indirectly by realtime RT-PCR assessment of mRNA encoding Ki67 which is only expressed in dividing cells¹⁵ and by Western immunoblot analysis of the hepatic expression of proliferating cell nuclear antigen (PCNA) as described previously.¹⁶

Biochemical analysis

Western immunoblot analysis of apoprotein expression for CYP2E1 and CYP4A1 was conducted as previously described and normalized against total protein loaded based on ponceau staining of the membranes.¹⁶ CYP2E1 was a gift from the laboratory of Dr Magnus Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden).¹⁷ CYP4A1 was detected using a polyclonal sheep antibody to rat CYP4A1¹⁸ which was a gift from Dr Gordon Gibson (University of Surrey, Guildford, UK). CYP2E1 activity was assessed directly by measurement of microsomal *p*-nitrophenol hydroxylase activity.¹⁹ Susceptibility of cellular membranes to attack by free radicals generated by CYP2E1 was determined by assessing the rate of formation of thiobarbituric reactive lipid peroxidation products in microsomes incubated with carbon tetrachloride.²⁰ Lipid FA profile analysis of liver homogenates was conducted by gas chromatography mass spectrometry as previously described in our laboratory.²¹ Peroxidizability index of liver free FA, triglycerides and phospholipid fractions was calculated based on FA profiles as described by Lambert *et al.*²² Mitochondrial protein extracts were prepared using a Mitochondrial Isolation kit for Tissue (Pierce, Rockford, IL, USA) and immunoblotting was performed for oxidative phosphorylation complexes I-V (MitoSciences, Eugene, OR,

USA). Mitochondrial numbers were estimated indirectly by realtime RT-PCR analysis of expression of the mitochondrial transcription factor mtTFAM mRNA. In addition, sirtuin (SIRT)3 mRNA expression and expression of the uncoupling protein UCP2 mRNA were analyzed by realtime RT-PCR.²³

Realtime RT-PCR

Total RNA was extracted from livers using TRI Reagent and cleaned using RNeasy mini columns (Qiagen, Valencia, CA, USA). RNA quality was ascertained spectrophotometrically (ratio of A_{260}/A_{280}) and also by checking ratio of 28S to 18S ribosomal RNA using the RNA Nano Chip on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Total RNA (1 μ g) was reverse transcribed using the iScript Reverse Transcription kit (Biorad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The reverse transcribed cDNA (10 ng) was utilized for *real-time* PCR using the 2 $\times$ SYBR green master mix and monitored on a ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Gene-specific probes described previously^{13,14,20,21,23,24} were designed using Primer Express™ Software (Applied Biosystems, CA) and the relative amounts of gene expression were quantitated using a standard curve according to the manufacturer's instructions. Gene expression was normalized to 18S rRNA as described previously.¹³

EMSA for assessment of peroxisome proliferator activated receptor alpha (PPAR α) binding

Complementary oligonucleotide probes specific for the peroxisome proliferator response element (PPRE) on the acyl CoA oxidase gene were synthesized by Integrated DNA Technologies Inc. (Coralville, IA, USA). Hepatic nuclear extracts were used for electrophoretic mobility shift assay (EMSA) analysis of PPAR α binding as described previously.²⁴

Statistical analysis

Data for continuous outcomes are presented as mean $\pm$ standard errors of the mean (SE). One-way analysis of

variance (ANOVA) was used to test overall mean differences of each outcome across corn oil concentrations. *Post hoc* comparisons of means were performed using an all-pairs-Tukey-Kramer comparison test and considered significant if $P \leq 0.05$. The Jonckheere-Terpstra test, a non-parametric trend test, was used to test for a significant overall increasing or decreasing change in each outcome with varying corn oil concentrations. Statistical analyses were performed using the Stata statistical package version 12.0 (Stata Corp, College Station, TX, USA).

Results

Isocaloric feeding of MCT dose-dependently reduces adiposity

Overfeeding diets containing different proportions of CO resulted in comparable body weight and obesity with an ending body composition of $\sim 20\%$ body fat (Table 1). Isocaloric substitution of MCT for CO resulted in a dose-dependent decrease in weight gain and body fat as measured by both reduced fat pad weights and directly by nuclear magnetic resonance ($P < 0.0001$; Table 1).

Liver pathology is reduced by increasing dietary content of MCT

Increasing the %CO in the diet resulted in a dose-dependent development of NAFLD, as indicated by elevated hepatosteatosis and serum ALT concentrations (Tables 1 and 2). Although liver size was slightly reduced ($P < 0.02$), appearance of lipid droplets as measured by Oil Red O staining (Figure 1) was paralleled by increased liver triglyceride content in 70% CO fed rats compared with 10% CO fed rats (trend: $P < 0.01$; Table 2). Analysis of alterations in the fatty acid (FA) composition of hepatic lipid fractions: free FA, triglycerides and phospholipids is shown in Table 3. The major FAs in the free FA and triglyceride fractions were 16:0, 18:1, 18:2 and 20:4. In the phospholipid fraction, 18:0 was also present in a significant proportion of the lipid. Other FA species made up only a small portion of the lipid fractions (data not shown). Increasing dietary %CO

Table 1 Effects of dietary fat composition on body weight and adiposity

Treatment body	Weight (g)	Gonadal fat (g)	Abdominal fat (g)	% fat mass*	% lean mass*
10% CO	370.9 $\pm$ 18.3 ^a	4.4 $\pm$ 0.8 ^a	5.9 $\pm$ 0.9 ^a	19.8 $\pm$ 0.7 ^a	70.4 $\pm$ 0.9 ^a
35% CO	359.0 $\pm$ 14.8 ^a	3.9 $\pm$ 0.6 ^a	5.3 $\pm$ 0.7 ^a	20.4 $\pm$ 1.0 ^a	70.6 $\pm$ 1.2 ^a
70% CO	358.3 $\pm$ 10.1 ^a	4.0 $\pm$ 0.4 ^a	6.2 $\pm$ 0.6 ^a	19.7 $\pm$ 0.7 ^a	70.0 $\pm$ 1.0 ^a
50% CO/20% MCT	336.2 $\pm$ 8.3 ^{a,b}	2.8 $\pm$ 0.2 ^b	3.7 $\pm$ 0.3 ^b	14.9 $\pm$ 0.9 ^b	73.4 $\pm$ 0.9 ^{a,b}
35% CO/35% MCT	335.1 $\pm$ 8.8 ^{a,b}	3.0 $\pm$ 0.2 ^b	2.9 $\pm$ 0.4 ^{b,c}	14.8 $\pm$ 0.8 ^b	75.1 $\pm$ 1.3 ^b
20% CO/50% MCT	300.1 $\pm$ 9.1 ^b	2.0 $\pm$ 0.2 ^{b,c}	2.4 $\pm$ 0.2 ^c	15.3 $\pm$ 0.3 ^b	74.1 $\pm$ 0.3 ^b
5% CO/65% MCT	304.7 $\pm$ 9.7 ^b	1.7 $\pm$ 0.3 ^c	1.6 $\pm$ 0.2 ^c	10.4 $\pm$ 0.6 ^c	73.3 $\pm$ 1.0 ^{a,b}
ANOVA (<i>P</i> value) [†]	0.79	0.86	0.63	0.69	0.89
Trend (<i>P</i> value) [†]	0.25	0.43	0.80	0.96	0.63
ANOVA (<i>P</i> value) [‡]	0.002	<0.0001	<0.0001	<0.0001	0.013
Trend (<i>P</i> value) [‡]	<0.0001	<0.0001	<0.0001	<0.0001	0.010

CO, corn oil, MCT, 82:18 MCT:beef tallow mix

Data are mean $\pm$ SEM. Means with different superscripts are significantly different $P < 0.05$ a < b < c

*Determined by nuclear magnetic resonance

[†]CO dose-response

[‡]MCT dose-response after isocaloric substitution for CO

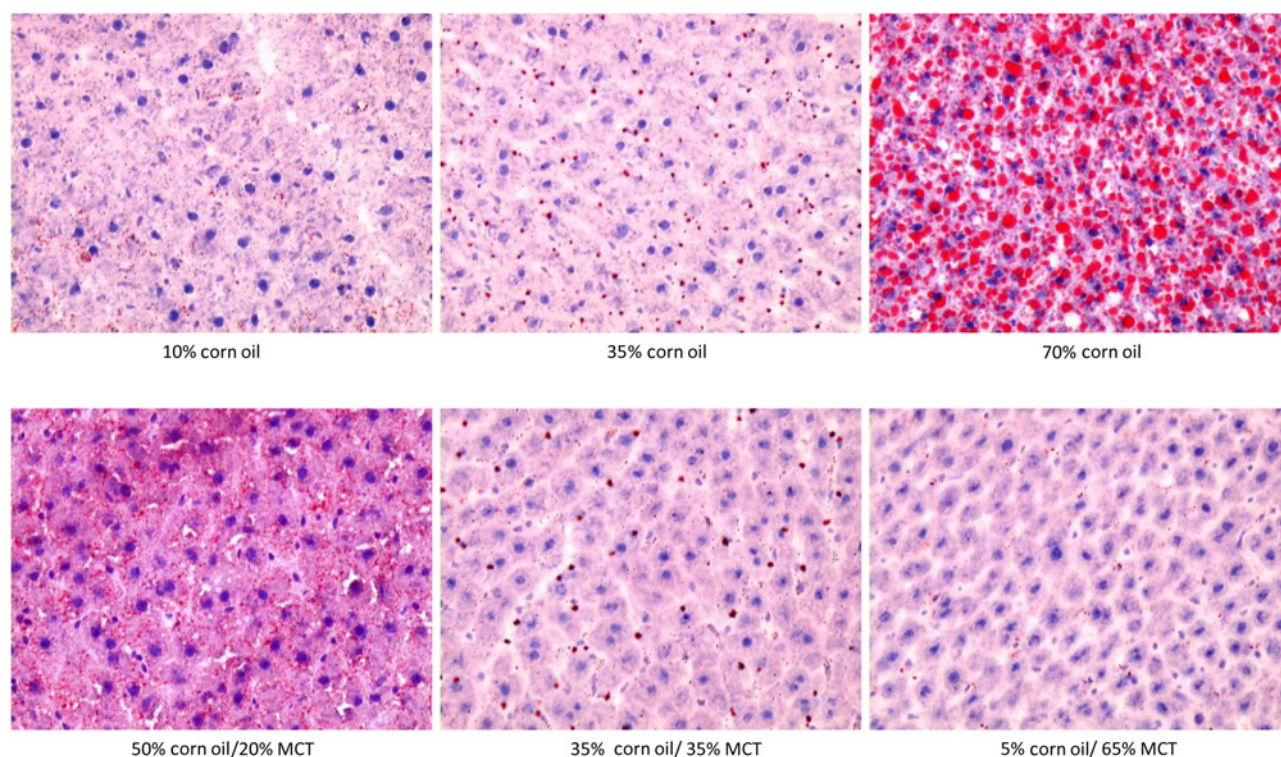


Figure 1 Representative Oil Red O stained liver sections illustrating the effects of dietary fat composition on accumulation of hepatic lipid droplets in the TEN model

Table 2 Effects of dietary fat composition on liver pathology

Treatment	Liver (% body wt)	Steatosis ^a	Triglycerides (mg/g)	ALT (U/mL)	TNF α [†] mRNA	K κ 67 [†] mRNA
10% CO	3.7 $\pm$ 0.2 ^b	0.06 $\pm$ 0.02 ^a	213 $\pm$ 8 ^b	47 $\pm$ 3 ^{a,b}	12.0 $\pm$ 2.4	9.1 $\pm$ 1.3
35% CO	3.4 $\pm$ 0.1 ^{a,b}	0.15 $\pm$ 0.02 ^a	186 $\pm$ 31 ^b	48 $\pm$ 2 ^b	9.1 $\pm$ 0.7	21.5 $\pm$ 6.3
70% CO	3.4 $\pm$ 0.1 ^a	0.33 $\pm$ 0.03 ^b	286 $\pm$ 29 ^c	69 $\pm$ 4 ^c	15.5 $\pm$ 3.1	14.0 $\pm$ 2.5
50% CO/20% MCT	3.8 $\pm$ 0.2 ^b	0.24 $\pm$ 0.04 ^b	153 $\pm$ 12 ^b	59 $\pm$ 5 ^{b,c}	10.8 $\pm$ 1.2	9.7 $\pm$ 2.0
35% CO/35% MCT	4.1 $\pm$ 0.1 ^{b,c}	0.11 $\pm$ 0.003 ^a	85 $\pm$ 15 ^{a,b}	42 $\pm$ 3 ^a	13.1 $\pm$ 1.5	11.8 $\pm$ 2.3
20% CO/50% MCT	4.2 $\pm$ 0.2 ^{b,c}	0.08 $\pm$ 0.03 ^a	121 $\pm$ 17 ^{a,b}	38 $\pm$ 1 ^a	15.7 $\pm$ 2.1	6.7 $\pm$ 0.9
5% CO/65% MCT	4.5 $\pm$ 0.2 ^c	0.02 $\pm$ 0.01 ^a	58 $\pm$ 9 ^a	45 $\pm$ 4 ^{a,b}	15.7 $\pm$ 2.8	5.9 $\pm$ 1.3
ANOVA (<i>P</i> value) [‡]	0.02	<0.0001	0.03	0.001	0.20	0.10
Trend (<i>P</i> value) [‡]	0.01	<0.0001	0.05	0.003	0.08	0.25
ANOVA (<i>P</i> value) [§]	<0.0001	<0.0001	<0.0001	<0.0001	0.97	0.18
Trend (<i>P</i> value) [§]	<0.0001	<0.0001	<0.0001	<0.0001	0.52	0.01

ALT, serum alanine aminotransferase activity; TNF α , tumor necrosis factor α ; CO, corn oil; **MCT**, 82:18 MCT:beef tallow mix

Data are mean $\pm$ SEM. Means with different superscripts are significantly different $P < 0.05$, a < b < c

^aData are digital image analysis of Oil Red O staining of lipid droplets

[†]mRNA expression data are realtime RT-PCR values normalized to expression of 18S

[‡]CO dose-response

[§]**MCT** dose-response after isocaloric substitution for CO

resulted in a dose-dependent rise in polyunsaturated 18:2 (linoleate) content in all three lipid fractions (trend: $P < 0.01$). In addition, the amount of arachidonate (20:4) was greater in the free FA and triglyceride fractions (trend: $P < 0.02$). Small elevations were observed in the total amount of 16:0 and 18:1 FA triglycerides and 18:0 phospholipids as the %CO in the diet increased ($P < 0.05$) along with a greater total triglyceride concentration in the liver. However, the proportion of 16:0 and 18:1 FA were reduced relative to the proportion of 18:2 FA. This resulted in dose-dependent increases in the peroxidizability index of the free FA and triglyceride fractions (trend: $P < 0.0001$)

and greater peroxidizability of the phospholipid fraction from the 70% CO group relative to the 35% CO group ($P < 0.05$; Table 4). Other FA species were present in hepatic lipid fractions in small amounts, but even when combined, contributed to only a small percentage of the total (data not shown). Development of steatosis was accompanied by induction of the cytochrome P450 enzyme CYP2E1 at the level of apoprotein and activity, which is a known source of reactive oxygen species²⁵ ($P < 0.05$; Figures 2 and 3). Steatosis was also associated with greater serum ALT values, indicative of the development of necrosis ($P < 0.05$; Table 2). After this relatively short

Table 3 Effects of dietary fat composition on hepatic FA composition ($\mu\text{g/g}$ liver)

FA	Free FA				Triglycerides				Phospholipids			
	16:0	18:1	18:2	20:4	16:0	18:1	18:2	20:4	16:0	18:1	18:2	20:4
10% CO	330 $\pm$ 16	136 $\pm$ 18	54 $\pm$ 4	38 $\pm$ 3	2479 $\pm$ 193 ^b	2127 $\pm$ 164 ^b	552 $\pm$ 216 ^a	21 $\pm$ 2 ^a	1728 $\pm$ 73 ^{a,b}	1952 $\pm$ 139	626 $\pm$ 40	1401 $\pm$ 63
35% CO	381 $\pm$ 42	176 $\pm$ 20	205 $\pm$ 26	93 $\pm$ 47	2414 $\pm$ 350 ^b	2105 $\pm$ 292 ^b	2415 $\pm$ 295 ^a	102 $\pm$ 14 ^a	2020 $\pm$ 209 ^{a,b}	2440 $\pm$ 210	822 $\pm$ 154	1176 $\pm$ 376
70% CO	533 $\pm$ 197	327 $\pm$ 130	639 $\pm$ 299	83 $\pm$ 13	4260 $\pm$ 632 ^c	3956 $\pm$ 637 ^c	7364 $\pm$ 1207 ^b	566 $\pm$ 106 ^b	1787 $\pm$ 254 ^{a,b}	2929 $\pm$ 268	1223 $\pm$ 214	2271 $\pm$ 409
50% CO/20% MCT	257 $\pm$ 42	182 $\pm$ 31	252 $\pm$ 43	114 $\pm$ 36	1645 $\pm$ 262 ^a	2045 $\pm$ 359 ^a	2591 $\pm$ 491 ^a	203 $\pm$ 44 ^a	1456 $\pm$ 209 ^a	2323 $\pm$ 337	1133 $\pm$ 157	2280 $\pm$ 363
35% CO/35% MCT	208 $\pm$ 19	131 $\pm$ 27	147 $\pm$ 38	74 $\pm$ 6	1345 $\pm$ 72 ^a	1532 $\pm$ 60 ^a	1265 $\pm$ 51 ^a	157 $\pm$ 18 ^a	1654 $\pm$ 75 ^{a,b}	2284 $\pm$ 77	956 $\pm$ 88	1997 $\pm$ 125
20% CO/50% MCT	467 $\pm$ 99	301 $\pm$ 80	174 $\pm$ 40	69 $\pm$ 13	1259 $\pm$ 192 ^a	1460 $\pm$ 214 ^a	646 $\pm$ 82 ^a	57 $\pm$ 10 ^a	2481 $\pm$ 329 ^{b,c}	2714 $\pm$ 300	1033 $\pm$ 251	2411 $\pm$ 468
5% CO/65% MCT	488 $\pm$ 92	317 $\pm$ 73	84 $\pm$ 14	53 $\pm$ 3	1487 $\pm$ 338 ^a	1670 $\pm$ 341 ^a	251 $\pm$ 70 ^a	21 $\pm$ 5 ^a	2812 $\pm$ 338 ^c	2799 $\pm$ 379	880 $\pm$ 90	2478 $\pm$ 366
ANOVA (<i>P</i> value) [*]	0.54	0.29	0.12	0.33	0.02	0.01	<0.0001	0.05	0.61	0.07	0.05	0.07
Trend (<i>P</i> value) [*]	0.98	0.10	<0.0001	0.02	0.03	0.04	<0.0001	0.02	0.93	0.03	0.01	0.44
ANOVA (<i>P</i> value) [†]	0.16	0.28	0.06	0.22	0.003	0.002	<0.0001	<0.0001	0.002	0.47	0.63	0.89
Trend (<i>P</i> value) [†]	0.66	0.47	<0.0001	0.07	<0.0001	<0.0001	<0.0001	<0.0001	0.001	0.57	0.07	0.85

CO, corn oil; MCT, 82:18 MCT:beef tallow mix; FA, fatty acid

Data are mean $\pm$ SEM. Means with different superscripts are significantly different $P < 0.05$, $a < b < c$ ^{*}CO dose-response[†]MCT dose-response after isocaloric substitution for COTable 4 Effects of dietary fat composition on peroxidizability index of hepatic lipids^{*}

Treatment	Free FA	Triglyceride	Phospholipid
10% CO	29.2 $\pm$ 1.4 ^a	11.5 $\pm$ 2.8 ^a	130.4 $\pm$ 5.0 ^b
35% CO	51.8 $\pm$ 9.6 ^c	41.8 $\pm$ 1.5 ^c	82.0 $\pm$ 14.0 ^a
70% CO	59.8 $\pm$ 2.2 ^c	69.6 $\pm$ 1.8 ^e	137.0 $\pm$ 7.0 ^{b,c}
50% CO/20% MCT	71.5 $\pm$ 6.8 ^c	59.2 $\pm$ 2.5 ^d	157.6 $\pm$ 5.0 ^c
35% CO/35% MCT	65.8 $\pm$ 2.6 ^c	49.1 $\pm$ 2.3 ^c	139.9 $\pm$ 2.5 ^{b,c}
20% CO/50% MCT	44.3 $\pm$ 6.9 ^{a,b}	29.0 $\pm$ 3.6 ^b	125.4 $\pm$ 12.0 ^b
5% CO/65% MCT	31.8 $\pm$ 4.4 ^a	11.4 $\pm$ 2.1 ^a	129.0 $\pm$ 3.3 ^b
ANOVA (<i>P</i> value) [†]	0.002	<0.0001	0.03
Trend (<i>P</i> value) [†]	<0.0001	<0.0001	0.44
ANOVA (<i>P</i> value) [‡]	<0.0001	<0.0001	0.02
Trend (<i>P</i> value) [‡]	<0.0001	<0.0001	0.03

CO, corn oil; MCT, 82:18 MCT:beef tallow mix

Data are mean $\pm$ SEM. Means with different superscripts are significantly different $P < 0.05$, $a < b < c < d < e$ ^{*}Calculated based on FA profiles as described by Lambert *et al.*²²[†]CO dose-response[‡]MCT dose-response after isocaloric substitution for CO

period of overfeeding of high polyunsaturated fat diets, there was a tendency to increase expression of the inflammatory marker TNF α mRNA, but this did not reach statistical significance (trend: $P = 0.08$; Table 2). Increasing dietary CO from 10% to 70% had no significant effect on hepatic expression of Ki67 mRNA (Table 2) but significantly suppressed expression of PCNA protein ($P < 0.05$; Figure 4) suggesting a small decrease in cellular proliferation. Isocaloric substitution of CO in 70% fat diets with MCT oil-rich saturated fat resulted in a dose-dependent increase in liver weight (trend $P < 0.0001$), but a dose-dependent decrease in hepatic pathology including disappearance of lipid droplets (Figure 1, Table 2), decreased triglyceride content, reduced ALT values and decreased expression of Ki67 mRNA (trend: $P < 0.01$; Table 2) and PCNA protein (Figure 4). Interestingly, no incorporation of C8-C12 FAs was observed in any of the three hepatic lipid compartments after MCT substitution for CO, but there was a dose-dependent reduction of C18:2 and C20:4 content of the free FA and triglyceride fractions (trend $P < 0.0001$). Although dose-dependent decreases in 16:0 and C18:1 FAs were also observed in the triglyceride fraction with increasing MCT substitution ($P < 0.0001$), the relative composition of the free FA and triglyceride fractions shifted from 45% and 49% polyunsaturated FAs to 15% and 8%, respectively, in the 70% CO group compared with the 5% CO/65% MCT group. This coincided with a dose-dependent decrease in peroxidizability index (trend: $P < 0.0001$; Table 4). In the phospholipid fraction, substitution of MCT for CO resulted in dose-dependent elevations in 16:0 and 18:1 FAs (trend: $P < 0.001$; Table 3) and a decrease in peroxidizability index (trend $P < 0.03$; Table 4). No effects of MCT substitution were observed on expression of TNF α mRNA (Table 2) or on expression of CYP2E1 mRNA or microsomal apoprotein (Figure 2). A direct measurement of CYP2E1-dependent activity (*p*-nitrophenol hydroxylase¹⁹) was increased with increasing dietary %CO (trend $P = 0.003$), but was unchanged by MCT substitution, consistent with CYP2E1 protein expression (Figure 3). However, carbon tetrachloride dependent lipid peroxidation, a measure of microsomal membrane lipid peroxidation produced by

trichloroethyl radicals generated from the CYP2E1-catalyzed reductive dechlorination of carbon tetrachloride,²⁰ was significantly decreased by MCT substitution ($P < 0.05$; Figure 3).

Hepatic FA homeostasis is altered by increasing dietary CO and substitution with MCT

Overfeeding of diets with increasing %CO resulted in greater expression of mRNAs encoding the hepatic FA transporters CD36²⁶ and FATP2²⁷ (trend: $P < 0.05$; Table 5). In addition, as %CO increased there was a dramatic suppression in expression of adiponutrin (PNPLA3) mRNA (trend: $P < 0.0001$; Table 5). Adiponutrin is a hepatic triglyceride hydrolase for which inactivating mutations in humans have been linked to appearance of fatty liver disease.²⁸ In contrast, as hepatic steatosis appeared with increasing %CO, expression of hepatic mRNA for the transcription factor sterol regulatory element binding protein (SREBP-1c) and downstream genes encoding enzymes involved in *de novo* FA synthesis and desaturation²⁹ (fatty acid synthase [FASN], acyl CoA carboxylase [ACC1] and steroyl CoA desaturase [SCD-1]) appeared to be highly suppressed (trend: $P < 0.002$; Table 5). No effects of increasing %CO were observed on expression of CYP4A1

which catalyzes FA ω -oxidation¹⁸ (Figure 2), but there was a two-fold greater expression of mRNA encoding ACO, the rate-limiting enzyme in the peroxisomal β -oxidation of FAs,³⁰ in the 70% CO group relative to 10% and 35% CO (trend: $P < 0.01$; Table 5). Although there were no significant effects on expression of apolipoprotein B100 (ApoB100) mRNA, increasing %CO did elevate expression of the mRNA encoding microsomal triglyceride transport protein which regulates formation of very low-density lipoprotein (VLDL) for triglyceride export out of the liver³¹ (trend: $P < 0.003$; Table 5). Substitution of MCT for CO at a constant level of 70% dietary fat had no further effect on expression of FA transporter mRNAs or mRNAs linked to VLDL secretion. However, increasing the proportion of MCT at the expense of CO resulted in greater expression of genes involved in FA synthesis and of adiponutrin compared with the 70% CO group (trend: $P < 0.0001$; Table 5). Increasing substitution of MCT also produced a substantial induction of CYP4A1 mRNA and protein and ACO mRNA (trend: $P < 0.0005$; Figure 2, Table 5) both of which are regulated via the transcription factor PPAR α .^{30,32} Significantly increased nuclear protein binding to the PPARE on the ACO promoter was observed in EMSA analysis comparing the 70% CO group to the 5% CO/65% MCT group (Figure 5).

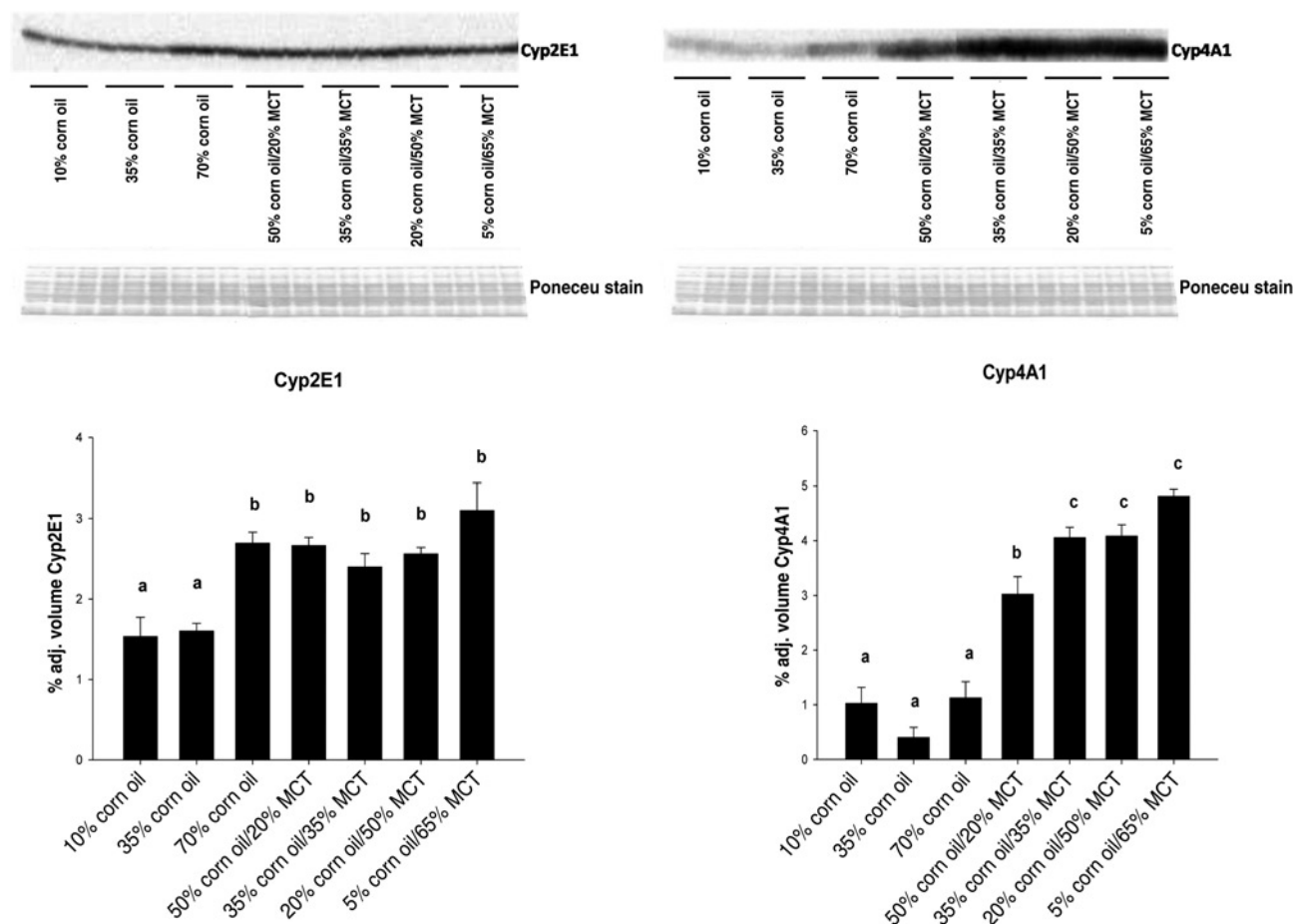


Figure 2 Effects of increasing dietary corn oil (CO) content and substitution of medium chain triacylglycerol (MCT) for CO on expression of CYP2E1 and CYP4A1 apoproteins in hepatic microsomes from rats overfed via total enteral nutrition. Immunoquantitation data are mean $\pm$ SEM densitometric data normalized for protein loading. Means with differing letters are significantly different $P < 0.05$, a < b < c

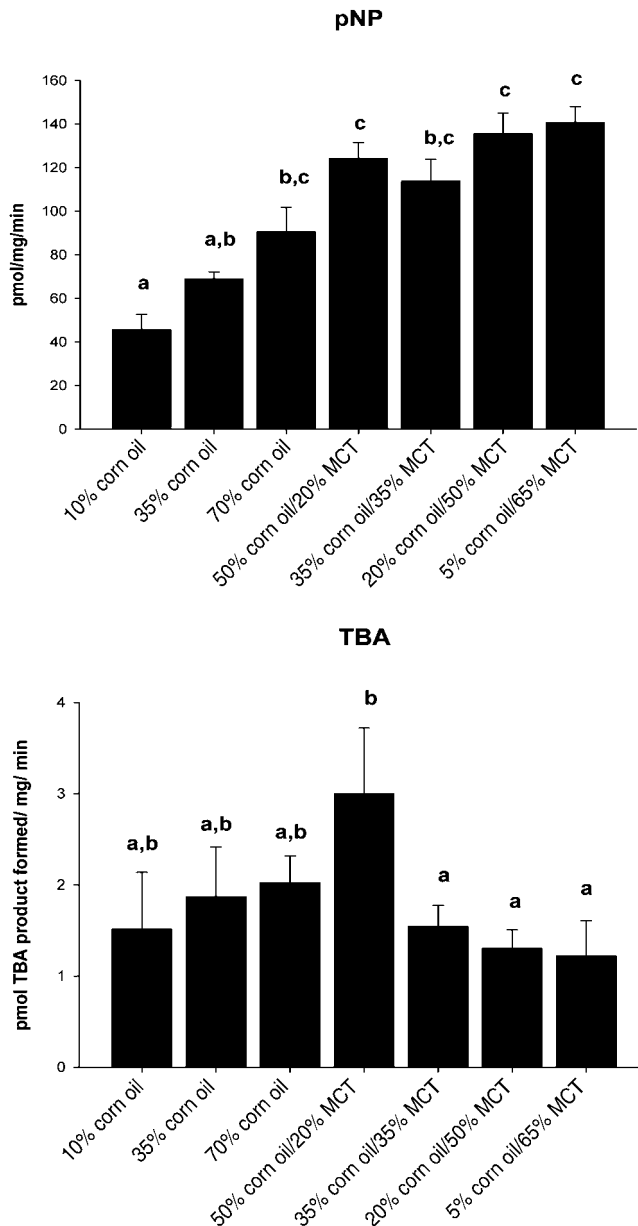


Figure 3 Effects of increasing dietary corn oil (CO) content and substitution of medium chain triacylglycerol (MCT) for CO on CYP2E1-dependent microsomal monooxygenase activity. Top panel: *p*-nitrophenol (pNP) hydroxylase activity; bottom panel: carbon tetrachloride-dependent lipid peroxidation as measured by generation of thiobarbituric acid reactive products (TBARS). Data are mean $\pm$ SEM. Means with differing letters are significantly different $P < 0.05$, $a < b < c$

Effects of increasing dietary CO and substitution with MCT on mitochondrial respiration

No effects of manipulating dietary fat composition with CO or MCT were observed on expression of the mitochondrial marker mtTFAM mRNA. Moreover, no significant effects were observed on expression of UCP2 mRNA (data not shown). However, significant effects of manipulating dietary fat composition were observed on expression of proteins comprising the mitochondrial respiratory complexes (Figures 6 and 7). Increasing the % of dietary fat as CO from 10% to 70% induced expression of Complex I, III and IV ($P < 0.05$), but a CO-dependent dose-response was only

observed for Complex III (trend $P < 0.035$). Isocaloric substitution of MCT for CO resulted in a dose-dependent decrease in expression of complex II (trend: $P < 0.0004$), but dose-dependent elevation in expression of both Complex III and Complex V (ATP synthase; trend: $P < 0.0001$) relative to the 70% CO group. These dietary effects on respiratory complexes occurred in the absence of changes in expression of SIRT3 mRNA (data not shown).

Discussion

Although the beneficial effects of dietary medium chain fatty acids (MCFA) on energy expenditure and body composition have been examined extensively in both animal models and clinical studies,^{1–6} fewer studies have examined the effects of MCFA on accumulation of hepatic triglycerides after the feeding of high-fat diets or the effects of MCFA on the development of obesity-associated NAFLD. In the current study we observed an almost complete loss of liver pathology, including a marked dose-dependent reduction in steatosis, as the fraction of dietary fat from MCFA in the form of MCT oil was isocalorically increased. Decreases in markers of hepatic injury, such as ALTs, are most likely secondary to the decrease in steatosis. Interestingly, in contrast to the previous study of Lieber *et al.*,¹² we observed no evidence of increased hepatotoxicity or increased hepatocyte proliferation associated with either feeding MCT alone or as a mixture containing long-chain FAs. In contrast, increasing substitution of MCT for corn oil appeared to slightly reduce proliferation based on expression of Ki67 mRNA and PCNA protein.

Previous studies of MCFA effects on liver triglyceride accumulation in the experimental animal literature have resulted in disparate and contradictory findings. Buettner *et al.*³³ and Turner *et al.*³⁴ have reported significantly increased steatosis in rats and mice fed MCFA as part of pelleted high-fat diets fed *ad libitum* relative to animals fed low-fat diets or diets high in long-chain fats. Others, such as Baba *et al.*,³⁵ reported no differences. In contrast, a recent report by Lieber *et al.*¹² found significantly reduced hepatic triglyceride accumulation using a liquid diet containing only MCFA compared with a liquid diet high in long-chain polyunsaturated fats. Part of the explanation for these inconsistent findings may lie in methodological differences such as use of solid versus liquid diets. Moreover, most previous studies have used *ad libitum* feeding where differences in food intake and caloric density result in significant differences in overall caloric intake. Additional studies in which TEN diets made with MCT are administered at different levels of calories to mimic the differences between *ad libitum* and isocaloric feeding would be required to address this. In addition, not all MCFA sources are the same. Both Buettner *et al.* and Turner *et al.* used hydrogenated coconut oil as their MCFA source and in this case the major dietary MCFAs are lauric acid (C12:0) and myristic acid (C14:0),³⁴ in contrast both Lieber *et al.* and the current study utilized MCT oil as the MCFA source. In this case the major dietary MCFAs are the shorter chain FAs C8:0 and C10:0.^{7,12}

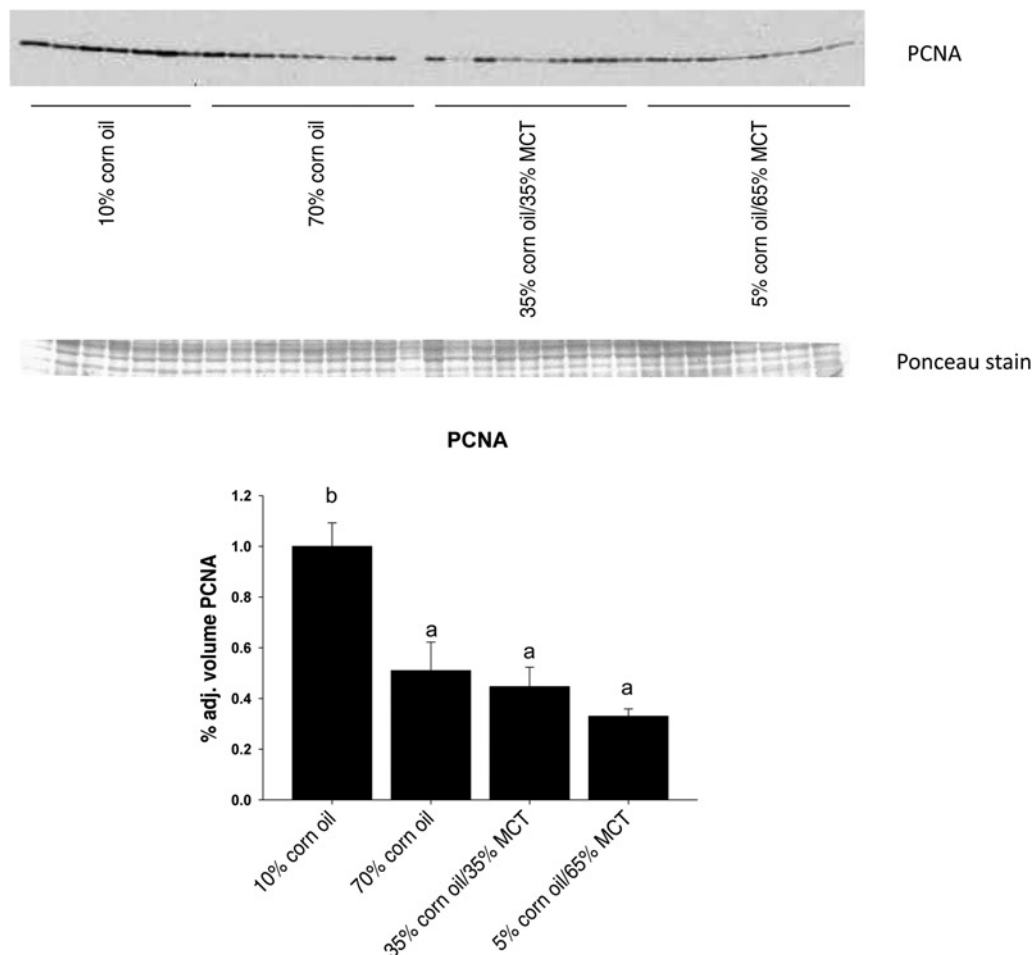


Figure 4 Effects of increasing dietary corn oil (CO) content and substitution of medium chain triacylglycerol (MCT) for CO on expression of proliferating cell nuclear antigen protein in liver homogenates from rats overfed via total enteral nutrition. Immunoquantitation data are mean $\pm$ SEM densitometric data normalized for protein loading. Means with differing letters are significantly different $P < 0.05$, $a < b$

MCFAs are known to have different pharmacokinetics and utilize different metabolic pathways than long-chain FAs. MCFAs are not incorporated as part of chylomicrons, but instead are absorbed directly into the hepatic portal vein and have been reported to not require transporters such as CD36 or FATPs to enter the liver.^{2,3} Thus, in this regard, we were surprised that these transporters remained elevated in all the 70% fat diet groups compared with the 10% and 35% CO groups regardless of dietary FA composition.

The hepatic fatty acyl synthase required for triglyceride re-esterification has a chain length preference for FAs of 14 or more carbons.⁵ Consistent with these data, there was little accumulation of MCFA into triglycerides or phospholipids in the current study. Increasing substitution of MCT for corn oil appeared to result in a substantial up-regulation of FA oxidation in the liver sufficient to significantly reduce the development of steatosis resulting from lipid oversupply. MCFA have been suggested to preferentially undergo mitochondrial and extra-mitochondrial oxidation compared with long-chain FAs.² Our data demonstrate a dose-dependent increase in expression of CYP 4A1 which catalyzes the microsomal ω -oxidation of FAs and

in expression of mRNA encoding ACO, the rate-limiting step in peroxisomal β -oxidation of FAs when MCT was substituted isocalorically for corn oil. The increase in CYP4A1 expression is similar to that we have previously observed in alcohol-treated rats fed MCT-rich diets.⁷ These enzymes are both downstream targets for the transcription factor PPAR α .^{30,32} Our EMSA data demonstrate for the first time that dietary MCT results in PPAR α activation. The peroxisomal proliferator response and cytochrome P450 induction associated with PPAR α activation probably also explains the greater liver weight associated with increasing dietary MCT since liver hypertrophy is generally associated with this response.³⁶ Several studies have shown that MCFAs have low-binding affinity for the PPARs;^{37,38} therefore, it is likely that PPAR α activation by MCT is indirect, and this mechanism will be pursued in future studies.

The major pathway for FA oxidation is mitochondrial β -oxidation. MCFAs have been demonstrated not to require rate-limiting transformation into acylcarnitine by carnitine palmitoyl transferase for transport into the mitochondria,³⁹ but instead penetrate the mitochondrial membrane directly and are rapidly oxidized to acetyl CoA.

Table 5 Effects of dietary fat composition on expression of genes involved in hepatic lipid homeostasis*

Treatment	FA transport		FA synthesis		SCD1	TG hydrolysis		FA degradation		VLDL secretion	
	CD36	FATP2	SREBP-1c	FASN	ACC1	Adiponutrin	ACO	MTTP	ApoB100		
10% CO	3.0 ± 0.5 ^{a,b}	5.6 ± 0.4 ^a	9.6 ± 1.1 ^b	18.5 ± 3.2 ^b	12.3 ± 1.5 ^c	13.4 ± 1.1 ^c	2.3 ± 0.3 ^a	6.2 ± 0.5 ^a	9.2 ± 0.5		
35% CO	2.0 ± 0.4 ^a	5.7 ± 0.3 ^a	7.6 ± 1.3 ^{a,b}	4.9 ± 1.1 ^a	5.5 ± 0.8 ^{a,b}	4.4 ± 1.0 ^b	2.8 ± 0.6 ^a	6.3 ± 0.3 ^a	9.3 ± 0.7		
70% CO	4.9 ± 0.6 ^b	8.9 ± 0.6 ^b	5.0 ± 0.4 ^a	2.0 ± 0.2 ^a	2.8 ± 0.3 ^a	0.3 ± 0.3 ^a	5.1 ± 0.9 ^b	9.5 ± 0.5 ^b	9.1 ± 0.4		
50% CO/20% MCT	5.4 ± 0.7 ^b	9.7 ± 1.1 ^b	7.1 ± 1.1 ^a	2.6 ± 0.3 ^a	2.7 ± 0.3 ^a	1.4 ± 0.2 ^a	9.7 ± 2.0 ^{b,c}	10.1 ± 1.2 ^b	8.5 ± 1.1		
35% CO/35% MCT	5.7 ± 0.6 ^b	10.2 ± 0.8 ^b	7.9 ± 1.1 ^{a,b}	2.4 ± 0.4 ^a	2.3 ± 0.3 ^a	2.2 ± 0.4 ^a	11.7 ± 1.3 ^{b,c}	9.6 ± 1.0 ^b	8.6 ± 0.9		
20% CO/50% MCT	7.2 ± 1.0 ^b	10.2 ± 0.9 ^b	6.7 ± 0.9 ^a	4.4 ± 1.5 ^a	3.1 ± 0.5 ^a	2.1 ± 0.1 ^a	13.9 ± 3.0 ^c	8.8 ± 1.0 ^b	8.5 ± 0.7		
5% CO/65% MCT	6.5 ± 0.9 ^b	10.1 ± 0.9 ^b	11.5 ± 1.1 ^b	16.6 ± 2.5 ^b	5.4 ± 0.6 ^b	12.0 ± 4.0 ^b	13.4 ± 2.0 ^c	9.0 ± 0.8 ^b	7.7 ± 0.5		
ANOVA (P value) [†]	0.004	0.002	0.008	<0.0001	<0.0001	<0.0001	0.02	<0.0001	0.96		
Trend (P value) [†]	0.04	0.009	0.002	<0.0001	<0.0001	<0.0001	0.01	0.003	0.88		
ANOVA (P value) [‡]	0.24	0.83	0.002	<0.0001	0.0001	<0.0001	0.009	0.87	0.82		
Trend (P value) [‡]	0.09	0.29	0.001	0.0002	0.016	0.014	0.0004	0.28	0.20		

CO, corn oil; MCT, 82:18 MCT:beef tallow mix; FA, fatty acid; TG, triglyceride; VLDL, very low density lipoprotein; CD36, scavenger receptor; FATP, fatty acid transport protein; SREBP-1c, sterol regulatory element binding protein; FASN, fatty acid synthase; ACC1, acyl CoA carboxylase; SCD-1, sterol Co A desaturase; ACO, acyl Co A oxidase; MTTP, microsomal triglyceride transfer protein; ApoB100, apolipoprotein B100

Data are mean ± SEM. Means with different superscripts are significantly different $P < 0.05$, $a < b < c$

*mRNA values determined by realtime RT-PCR normalized to 18S

[†]CO dose-response

[‡]MCT dose-response after isocaloric substitution for CO

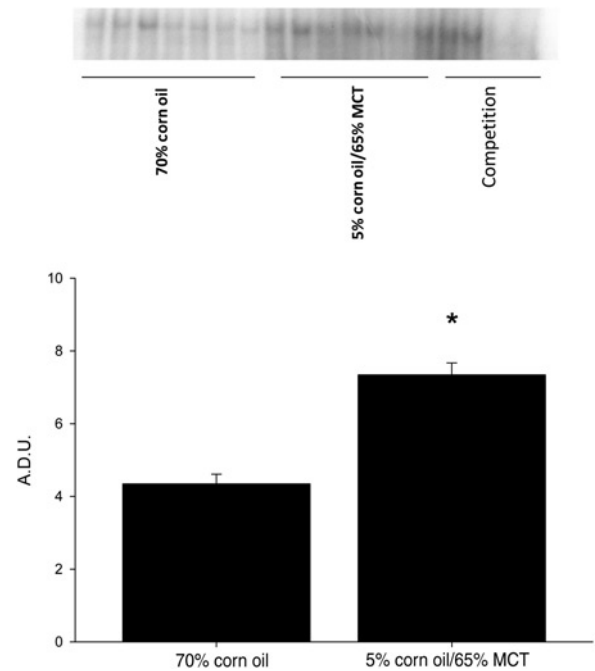


Figure 5 Top panel: Electrophoretic mobility shift (EMSA) assay of nuclear protein binding to the peroxisome proliferator response element on the acyl CoA oxidase (ACO) promoter in the 70% CO and 5% CO/65% MCT groups. Competition lanes show 5% CO/65% MCT samples with or without excess cold oligonucleotide to demonstrate specificity of binding. Bottom panel: densitometric quantitation of EMSA signal, * $P < 0.05$

Expression of both respiratory Complex III and V (ATP synthase) were substantially elevated in response to MCT substitution for corn oil in the 70% fat fed groups, while expression of complex II appeared to be suppressed. A likely explanation for these effects is that at this high level of dietary fat, oxaloacetate levels will be limiting since it will be shuttled into gluconeogenesis to supply the brain with glucose. In the absence of sufficient oxaloacetate to condense with the acetyl group of acetyl CoA to form citrate, acetyl CoA will be used to form ketone bodies which are known to be elevated after MCFA feeding⁴⁰ rather than fed through the Krebs cycle, and less FAD2 will be oxidized through Complex II. Since expression of respiratory complex I does not change, we speculate that electrons are being delivered to Complex III directly through Complex I and that additional electrons enter the respiratory chain at Complex III from MCFA derived glycerol 3-phosphate via glycerol-3-phosphate dehydrogenase and ubiquinone.^{41,42} The elevation of mitochondrial respiration did not appear to be associated with uncoupling of the respiratory chain or with increased mitochondrial numbers, since we observed no increase in expression of UCP2 or mtTFAM mRNAs. Moreover, although changes in SIRT3 expression have been linked to differences in expression of mitochondrial respiratory complexes,²³ we observed no effects of MCT on expression of this mitochondria-specific deacetylase.

In addition to oxidation and formation of ketone bodies, published data suggest that acetyl CoA derived from MCFA may be used to synthesize longer chain FAs and esterified.^{2,34} Our data are consistent with this since

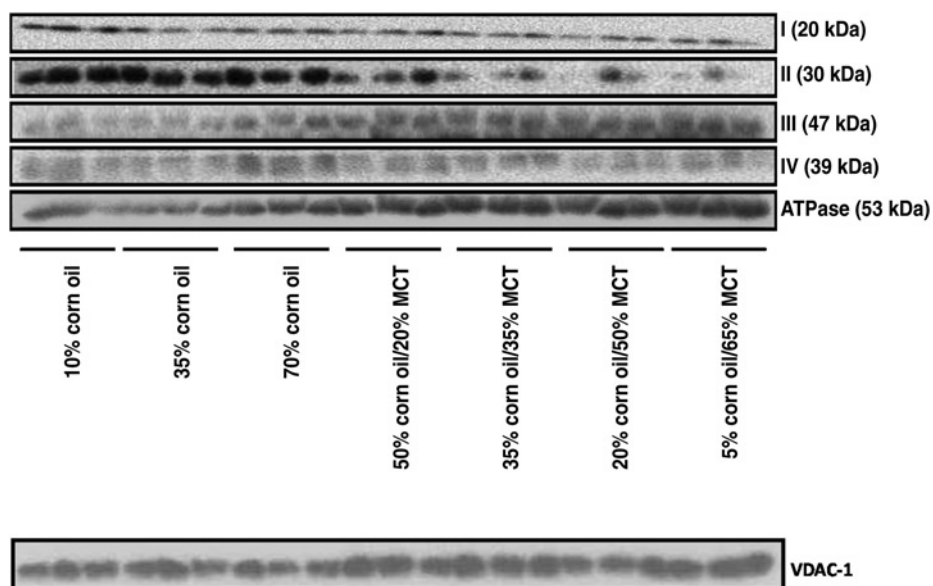


Figure 6 Representative Western immunoblots showing expression of hepatic mitochondrial respiratory complexes in rats overfed CO or CO/MCT diets via total enteral nutrition. Each lane represents a different animal (3/group). VDAC-1 expression was assessed as a loading control. CO, corn oil; MCT, medium chain triacylglycerol

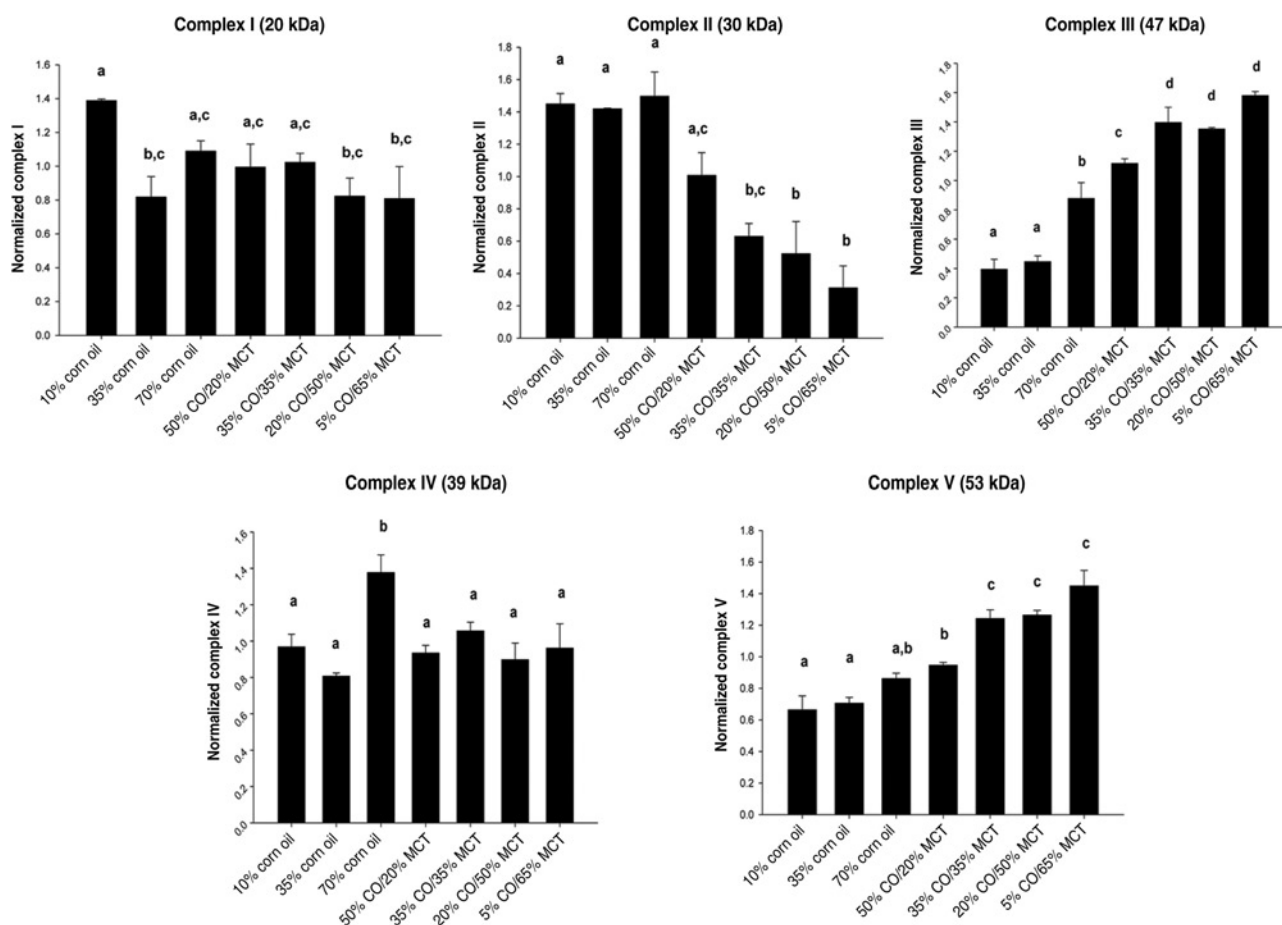


Figure 7 Immunoquantitation of hepatic mitochondrial respiratory complexes in rats overfed corn oil or corn oil/MCT diets via total enteral nutrition. Data are mean ± SEM. Means with differing letters are significantly different $P < 0.05$, $a < b < c < d$, CO, corn oil; MCT, medium chain triacylglycerol

increasing substitution of MCT for corn oil increased expression of FASN and ACC1, the rate-limiting enzymes in FA synthesis back to levels observed in low-fat-fed rats and restored the composition of hepatic free FAs and triglycerides.

Interestingly, our results differ substantially from those of Turner *et al.*,³⁴ who reported increased mitochondrial respiratory complexes in the muscle of male C57BL6/J mice-fed MCFA from hydrogenated coconut oil compared with mice fed low-fat diets or diets high in long-chain fats. However, they observed no effects on expression of mitochondrial respiratory complexes in the liver relative to a low-fat diet and higher expression of hepatic complexes II and IV relative to mice fed long-chain fats. In addition, this study reported increased hepatic triglyceride content in the coconut oil-fed group compared with the other groups associated with dramatic increases in expression of lipogenic enzymes and *de novo* lipogenesis well above levels observed in mice fed low-fat diets. It is possible that these differences in biochemical and physiological responses to feeding MCFA reflect species and strain differences. Alternatively, it is possible that the shorter chain C8:0 and C10:0 MCFAs in MCT oil have distinct and different metabolic effects than the longer chain C12 and C14 FAs in coconut oil.

In conclusion, using a rat model to study NAFLD, we have demonstrated that isocaloric replacement of dietary corn oil (long-chain polyunsaturated fats) with MCT as part of a high-fat diet results in a dose-dependent reduction in weight gain, adiposity and liver pathology. This was associated with increased PPAR α -dependent hepatic FA oxidation and increased mitochondrial respiration. These data suggest that saturated fat diets high in MCT oil may be health beneficial and have therapeutic potential in the treatment of NASH and other aspects of metabolic syndrome. Although it is not feasible to completely substitute long-chain triglycerides (LCT) in the diet with MCT oil due to the low smoking point of MCTs which limit their use as a cooking oil,⁴³ a new synthetic oil incorporating both 18:1 LCT and MCT with beneficial health effects on metabolic syndrome has recently been commercially introduced in Japan^{43,44} and similar products incorporating saturated LCTs such as 18:0 and MCT could be developed for the US market.

Author contributions: MJJR and TMB designed the study; MJR, JNB, NS, JV, MF, YT, XW and MAC carried out the study and analyzed and interpreted the data; MJJR wrote the manuscript and all the authors reviewed and edited the manuscript.

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REFERENCES

- 1 Leveille GA, Pardini RS, Tillotson JA. Influence of medium chain triglycerides on lipid metabolism. *Lipids* 1967;**2**:287–94
- 2 Papamandjaris AA, MacDougall DE, Jones PJH. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. *Life Sci* 1998;**62**:1203–15
- 3 Hill JO, Peters JC, Yang D, Sharp T, Kaler M, Abumrad NN, Greene HL. Thermogenesis in humans during overfeeding with medium chain triglycerides. *Metabolism* 1989;**38**:641–8
- 4 Rego Costa AC, Rosado EL, Soares-Mota M. Influence of the dietary intake of medium chain triglycerides on body composition, energy expenditure and satiety: a systemic review. *Nutr Hosp* 2012;**27**:103–8
- 5 Paust H, Keles T, Park W, Knoblach G. Fatty acid metabolism in infants. In: Chapman TE, ed. *Stable Isotopes in Paediatric Nutritional and Metabolic Research*. Great Britain: Intercept Ltd, 1990: 1–22
- 6 St Onge MP, Ross R, Parsons WD, Jones PJH. Medium chain triglycerides increase energy expenditure and decrease adiposity in overweight men. *Obes Res* 2003;**11**:395–402
- 7 Nanji AA, Zakim D, Rehemtulla T, Daly L, Miato S, Zhao S, Khwaja S, Tahan SR, Dannenberg AJ. Dietary saturated fatty acids down-regulate cyclooxygenase-2 and tumor necrosis factor alpha and reverse fibrosis in alcohol-induced liver disease in the rat. *Hepatology* 1997;**26**:1538–45
- 8 Nanji AA, Jokelainen K, Tipoe GL, Rahemtulla A, Dannenberg AJ. Dietary saturated fatty acids reverse inflammatory and fibrotic changes in rat liver despite continued alcohol administration. *J Pharmacol Exp Ther* 2001;**299**:638–44
- 9 Kono H, Enomoto N, Connor HD, Wheeler MD, Bradford BU, Rivera CA, Kadiiska MB, Mason RP, Thurman RG. Medium chain triglycerides inhibit free radical formation and TNF α production in rats given enteral ethanol. *Am J Physiol Gastrointest Liver Physiol* 2000;**278**:G467–76
- 10 Ronis MJJ, Korourian S, Zipperman M, Hakkak R, Badger TM. Dietary saturated fat reduces alcoholic hepatotoxicity in rats by altering fatty acid metabolism and membrane composition. *J Nutr* 2004;**134**:904–12
- 11 Fan ST. Review: nutritional support for patients with cirrhosis. *J Gastroenterol Hepatol* 1997;**12**:282–6
- 12 Lieber CS, DeCarli LM, Leo MA, Mak KM, Ponomarenko A, Ren C, Wang X. Beneficial effects versus toxicity of medium chain triacylglycerols in rats with NASH. *J Hepatol* 2008;**48**:318–26
- 13 Baumgardner JN, Shankar K, Hennings L, Badger TM, Ronis MJJ. A new model for nonalcoholic steatohepatitis in the rat utilizing total enteral nutrition to overfeed a high polyunsaturated fat diet. *Am J Physiol Gastrointest Liver Physiol* 2008;**294**:G27–38
- 14 Baumgardner JN, Shankar K, Hennings L, Badger TM, Ronis MJJ. N-acetylcysteine attenuates progression of liver pathology in a rat model of non-alcoholic steatohepatitis. *J Nutr* 2008;**138**:1872–9
- 15 Bachhi CE, Gown AM. Detection of cell proliferation in tissue sections. *Braz J Med Res* 1993;**26**:677–87
- 16 Ronis MJJ, Korourian S, Blackburn ML, Badeaux J, Badger TM. Then role of ethanol metabolism in development of alcoholic steatohepatitis in the rat. *Alcohol* 2010;**44**:157–69
- 17 Johansson I, Ingelman-Sundberg M. Benzene metabolism by ethanol-, acetone- and benzene-inducible P450 (IIE1) in rat and rabbit microsomes. *Cancer Res* 1988;**48**:5387–90
- 18 Tamburini PP, Masson HA, Bains SK, Makowski RJ, Morris B, Gibson GG. Multiple forms of hepatic cytochrome P450. Purification, characterization and comparison of a novel clofibrate-induced isozyme with other major forms of cytochrome P450. *Eur J Biochem* 1984;**139**:235–46
- 19 Koop DR. Hydroxylation of p-nitrophenol by rabbit ethanol-inducible cytochrome P450 isozyme 3a. *Mol Pharmacol* 1986;**29**:399–404
- 20 Johansson I, Ingelman-Sundberg M. Carbon tetrachloride-induced lipid peroxidation dependent on an ethanol-inducible form of rabbit liver microsomal cytochrome P450. *FEBS Lett* 1985;**183**:265–9
- 21 Wu X, Tong Y, Shankar K, Baumgardner JN, Kang J, Badeaux J, Badger TM, Ronis MJJ. Lipid fatty acid profile analysis in liver and serum in rats with nonalcoholic steatohepatitis using improved gas chromatography-mass spectrometry methodology. *J Agric Food Chem* 2011;**59**:747–54
- 22 Lambert AJ, Portero-Otin M, Pamplona R, Merry BJ. Effects of aging and caloric restriction on specific markers of protein oxidative damage and

- membrane peroxidizability in rat liver mitochondria. *Mech Aging Dev* 2004;**125**:529–38
- 23 Borengasser SJ, Lau F, Kang P, Blackburn ML, Ronis MJ, Badger TM, Shankar K. Maternal obesity during gestation impairs fatty acid oxidation and mitochondrial SIRT3 expression in rat offspring at weaning. *PLoS One* 2011;**6**:e24068
 - 24 Baumgardner JN, Shankar K, Korourian S, Badger TM, Ronis MJ. Undernutrition enhances alcohol-induced hepatocyte proliferation in the liver of rats fed via total enteral nutrition. *Am J Physiol Gastrointest Liver Physiol* 2007;**293**:G355–364
 - 25 Ronis MJ, Lindros K, Ingelman-Sundberg M. The CYP 2E1 family. In: Ioneddes C, ed. *Cytochrome P450: Biochemical and Toxicological Aspects*. CRC Press, 1996: 211–39
 - 26 Marecki JC, Shankar K, Ronis MJ, Badger TM. Ectopic fat disposition and insulin resistance develop after overfeeding of a high fat diet to prepubertal rats despite increased adiponectin signaling. *J Nutr Biochem* 2011;**22**:142–52
 - 27 Falcon A, Doege H, Fluit A, Tsang B, Watson N, Kay MA, Stahl A. FATP2 is a hepatic fatty acid transporter and peroxisomal very long chain acyl Co-A synthase. *Am J Physiol Endocrinol Metab* 2010;**299**:E384–93
 - 28 Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, NASH CRN. The association of genetic variability in patatin-like phospholipase domain containing protein (PNPLA3) with histological severity of nonalcoholic liver disease. *Hepatology* 2010;**52**:894–903
 - 29 Dentin R, Girard J, Postic C. Carbohydrate responsive element binding protein (ChREBP) and dextrol regulatory element binding protein (SREBP-1c) two key regulators of glucose metabolism and lipid synthesis in liver. *Biochimie* 2005;**87**:81–6
 - 30 Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisomal proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr* 2001;**21**:193–230
 - 31 Sugimoto T, Yamashita S, Ishigami M, Sakai N, Hirano K, Tahara M, Matsumoto K, Nakamura T, Matsuzawa Y. Decreased microsomal triglyceride transfer protein activity contributes to initiation of alcoholic liver steatosis in rats. *J Hepatol* 2002;**36**:157–62
 - 32 Johnson EF, Palmer CN, Griffin KJ, Hsu MH. Role of the peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. *FASEB J* 1996;**10**:1241–8
 - 33 Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Scholmerich J, Bollheimer LC. Defining high-fat diet rat models: metabolic and molecular effects of different fat types. *J Mol Endocrinol* 2006;**36**:485–501
 - 34 Turner N, Hariharan K, TidAng J, Frangioudakis G, Beale SM, Wright LE, Zeng XY, Leslie SJ, Li J-Y, Kraegen EW, Cooney GJ, Ye J-M. Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species dependent. *Diabetes* 2009;**58**:2547–54
 - 35 Baba N, Bracco EF, Hashim SA. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. *Am J Clin Nutr* 1982;**35**:678–82
 - 36 Fujimoto N, Inoue K, Yoshida M, Nishikawa A, Ozawa S, Gamou T, Nemoto K, Degawa M. Estrogen and androgen receptor status in hepatocellular hypertrophy induced by phenobarbital, clofibrate and piperonyl butoxide in F344 rats. *J Toxicol Sci* 2012;**37**:281–6
 - 37 Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci USA* 1997;**94**:4312–7
 - 38 Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, Sternbach DD, Lehmann JM, Wisely GB, Wilson TM, Klier SA, Milburn MV. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* 1999;**3**:397–403
 - 39 Friedman MI, Ramirez I, Bowden CR, Tordoff MG. Fuel partitioning and food intake: role for mitochondrial fatty acid transport. *Am J Physiol* 1990;**258**:R216–21
 - 40 Crozier G, Bois-Joyeux B, Chanez M, Girard J, Peret J. Metabolic effects induced by long-term feeding of medium chain tricerides in the rat. *Metabolism* 1987;**36**:807–14
 - 41 Lenaz G, Genova ML. Mobility and function of coenzyme Q (ubiquinone) in the mitochondrial respiratory chain. *Biochem Biophys Acta* 2009;**1787**:563–73
 - 42 Yeh JJ, Chinte U, Du S. Structure of glycerol-3-phosphate dehydrogenase, an essential monotopic membrane enzyme involved in respiration and metabolism. *Proc Natl Acad Sci USA* 2008;**105**:32803285
 - 43 Terada S, Yamamoto S, Sekine S, Aoyama T. Dietary intake of medium- and long-chain triacylglycerols ameliorates insulin resistance in rats fed a high fat diet. *Nutrition* 2012;**28**:92–7
 - 44 Negishi N, Shirasawa S, Arai Y, Suzuki J, Mukataka S. Activation of powdered lipase by cluster water and use of lipase powders for commercial esterification of food oils. *Enzyme Microb Technol* 2003;**32**:66–70

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