Octanoate Inhibits Triglyceride Synthesis in 3T3-L1 and Human Adipocytes[1]

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ABSTRACT To understand how medium-chain fatty acids (FA) influence lipid metabolism in adipocytes, we studied the effects of octanoate on the oxidation of glucose and endogenous palmitate, cellular O_2 consumption, mitochondrial membrane potential, lipid synthesis from long-chain FA, glucose and lactate. We found that octanoate significantly suppressed the esterification of oleate into triglycerides (TG) in both 3T3-L1 and human adipocytes. Octanoate also significantly suppressed de novo FA synthesis. These effects were associated with octanoate-mediated reductions in the activities of acyl CoA:1,2-diacylglycerol acyltransferase (DGAT) and acetyl CoA carboxylase (ACC). Cells pretreated with octanoate had reduced mRNA levels for a number of lipid metabolism genes, including of DGAT, ACC and stearoyl CoA desaturase-1. On the other hand, octanoate did not acutely perturb cellular O_2 consumption or mitochondrial membrane potential. Together, these results suggest that octanoate affected adipocyte function by reducing TG synthesis but not by enhancing oxidation. J. Nutr. 133: 2512–2518, 2003.

KEY WORDS: • *medium-chain fatty acids* • *triglyceride synthesis* • *adipocytes*

Medium-chain fatty acids (FA)[3] are stored less efficiently in adipocytes than long-chain FA (1). Nevertheless, they are recovered in adipocytes from both visceral and subcutaneous depots in animals and humans fed medium-chain triglycerides (MCT) (2–4). Recent clinical trials showed that MCT diets effectively reduce fat mass in humans (5–9). In light of this new evidence, a better understanding of the mechanisms by which MCT influence fat tissue metabolism is clearly warranted. Early work in this area attributed the MCT effects to rapid absorption and hepatic oxidation of medium-chain FA (10–13). However, this might be only one of many possible mechanisms. Using a modified FA analysis protocol, we found that medium-chain FA accumulated to nearly 30 mol/100 mol in adipose triglycerides (TG) in MCT-fed animals (14). Because medium-chain FA have been shown to mobilize more quickly than long-chain FA (15–17), a moderate accumulation of medium-chain FA in adipocytes would indicate a substantial influx of these FA into cells in vivo. Hence, they might have a greater effect on adipocyte metabolism than previously appreciated. Elucidation of these effects in vitro will be an important step toward understanding the metabolic roles of MCT in vivo.

Early studies in this area focused on how medium-chain FA are metabolized rather than how they affect overall fuel metabolism (1,18). Poor long-term viability of isolated adipocytes made it difficult to study the effects of medium-chain FA on cellular function in vitro. In this work, we used 3T3-L1 adipocytes to investigate how medium-chain octanoate affected different aspects of energy metabolism. Some of the findings were also tested in primary cultured human adipocytes. Compared with directly isolated fat cells, cultured adipocytes can tolerate metabolic manipulations for longer periods of time and might yield new information that is otherwise difficult to acquire from isolated fat cells.

MATERIALS AND METHODS

Cell culture. 3T3-L1 preadipocytes were prepared as described previously (19), except that insulin was withdrawn 6 d before the experiments were conducted. Human subcutaneous adipocytes differentiated in primary culture were purchased from Zen-Bio (Chapel Hill, NC), and maintained using vendor-provided medium and protocol.

Incorporation of glucose, lactate and H_2O into cellular lipids. Cells were washed with warm PBS and incubated in DMEM for 60 min before use. [U-^{14}C] glucose (5 mmol/L, 2 mCi/L) or [U-^{14}C] lactate (10 mmol/L, 5 mCi/L) was added with fresh medium with appropriate FA concentrations (see below) and incubated for 3 h. Preliminary tests showed that the amount of glucose and lactate incorporated into cellular lipids was linear for at least 6 h. By the end of 3 h incubation, the recovery rate in the cellular lipids was <1% of the total glucose or lactate present in the medium. Hence, the substrate concentration was considered to be constant during the incubation period.

Cellular TG were isolated, and FA were separated from glycerol as described (19). The incorporation of ^{14}C into each phase was quantified by scintillation counting. Incorporation of ^3H_2O into FA was measured similarly, except that ^3H_2O was used as the tracing isotope.

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[3] Abbreviations used: ACC, acetyl CoA carboxylase; BSA, bovine serum albumin; DGAT, acyl CoA:1,2-diacylglycerol acyltransferase; FA, fatty acid(s); FAS, fatty acid synthase; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; KRB, Krebs-Ringer bicarbonate; MCT, medium-chain triglycerides; TG, triglycerides; TPP, tetraphenylphosphonium.

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Incorporation of exogenous FA into cellular lipids. Octanoate and oleate solutions were prepared in complex with bovine serum albumin (BSA; 5:1, mol:mol) (19). This ratio was used to promote net transfer of oleate from BSA-binding sites to cells (20). BSA has a weak affinity for octanoate (21) and changing the FA/BSA ratio from 2.0 to 10.0 did not affect the esterification of octanoate (data not shown).

3T3-L1 adipocytes were incubated with FA for 5 h in Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4, 5 mmol/L glucose). [1-^{13}C] FA were used to label the esterification products, predominantly TG, in 3T3-L1 adipocytes (19). Human adipocytes were incubated with FA for 5 h in the vendor-provided medium, and [1-^{14}C] FA was used for isotope tracing. At the end of the incubation, cells were washed with PBS containing 10 g/L BSA and the lipid fraction was extracted and quantified by NMR or by scintillation counting as described (19). The 5-h incubation time was within the linear range (19).

Although a normal plasma FA concentration is ~0.5–0.8 mmol/L and higher with fasting (22), much higher FA levels might exist in local regions during acute hydrolysis of TG. Therefore, we used FA of 0.25–4 mmol/L to encompass the possible physiological range (23).

Measurement of oxidation products. Cells were incubated with [U-^{14}C] glucose in DMEM (5 mmol/L glucose) for 2 h without (control) or with added FA. CO_2 production from cell respiration was measured (19). To measure the oxidation of endogenous FA, cells were incubated with a trace amount of [9,10-^{3}H] palmitate for 24 h. Cells were then maintained in normal medium for an additional 7 d to allow equilibration between the labeled and unlabeled pools. Cells were then incubated in KRB buffer for 5 h with or without added FA. The $^{3}H_2O$ liberated from endogenous palmitate was measured (24).

O_2 consumption measurement. Differentiated 3T3-L1 adipocytes were dissociated from culture plates using an enzyme-free cell dissociation solution containing 2 mmol/L glucose. The same solution was used as the buffer throughout the respiration measurements. O_2 consumption was measured as described previously (25). About 1×10^5 cells were used for each experiment. After recording the basal O_2 consumption, FA were added by injection through a pinhole while the O_2 consumption was monitored continuously. At the end of the experiment, 2 nmol of carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP) was added to uncouple the oxidative phosphorylation, and the maximum O_2 consumption was recorded. Results were calculated by dividing the O_2 consumption rate before and after adding FCCP as the percentage of maximum respiration.

Membrane potential measurement. The mitochondrial membrane potential was estimated by incubating the cells for 15 min with a depolarizing buffer (pH 7.4) containing ^{3}H-tetraphenylphosphonium (TPP^+; 5 nmol/L, 0.01 mCi/L) (26). Cells were then washed with ice-cold PBS solution and solubilized with 0.1 mol/L NaOH. Because TPP^+ partitions into the mitochondrial and cytosolic compartments in adipocytes in a potential-dependent fashion by dipolarizing the plasma membrane, the amount of TPP^+ retained in the cells represents the amount entrapped in the mitochondria, and reflects the changes in mitochondrial membrane potential.

Assays for acyl CoA:1,2-diacylglycerol acyltransferase (DGAT) and acetyl CoA carboxylase (ACC). DGAT activity was measured using a protocol modification of an assay of Berge et al. (27). The reaction buffer was combined with 30 μmol/L acyl CoA oleoyl CoA, 250 μmol/L 1,2-dioleoyl-*sn*-glycerol (25 mmol/L stock in ethanol/water, 1:1, v/v), and 30 μg crude protein, in a total volume of 0.5 mL. The concentration of [1-^{14}C] oleoyl CoA was kept stable in all experiments (0.2 mCi/L). Preliminary tests confirmed the reaction linearity to crude protein between 25 to 50 μg, incubation time between 10 to 30 min and acyl CoA between 25 to 40 μmol/L. After incubation at 37°C for 15 min, 2.5 mL stop solution (heptane/isopropanol/NH_2SO_4; 40:10:1) was added, followed by 3 mL water and 3 mL heptane. The organic phase was then washed 3 times with 5 mL washing solution (isopropanol/water, 2:3). After the final wash, >95% of the background radioactivity was removed, and the incorporation of [1-^{14}C] oleoyl CoA into TG was measured by scintillation counting.

ACC activity was determined using a modified protocol of Thampy et al. (28). The reaction was initiated by adding ~25 μg cell crude protein (25 μL) to the 140 μL assay buffer (pH 7.4) containing 67 mmol/L Tris, 5 mmol/L EDTA, 5 mmol/L dithiothreitol, 20 mmol/L Mg-acetate, 0.4 g/L lactate dehydrogenase, 1 mmol/L NADH, 5 g/L FA-free BSA, 5 mmol/L ATP, 0.5 mmol/L acetyl CoA, 20 mmol/L ^{14}C-$NaHCO_3$ (20 mCi/L) and 13.2 mmol/L sodium citrate. The reaction was allowed to proceed for 8 min at 37°C. The incorporation of ^{14}C bicarbonate into malonyl CoA was measured.

RNA analysis. Total RNA was prepared and analyzed as previously described (29). The primer sequences used were (sense, ss) TGAGGAGGACCGCATTTATC and (antisense, as) GAAGCTTCCTTCGTGACCAG for ACC; (ss) CTCTACACTGCCCTCTTCGG and (as) AGACATGTCCAGTTTTCCGC for SCD-1; (ss) GGCTACTGGGATCTGAGGTG and (as) GAGACAGCTTTGGCCTTGAC for DGAT1; (ss) TCTCAGCCCTCCAAGACATC and (as) GATGCCTCCAGACATCAGGT for DGAT2; (ss) TTGCTGGCACTACAGAATGC and (as) AACAGCCTCAGAGCGACAAT for fatty acid synthase (FAS); (ss) CTGACAAGCCAGAAAAAGCC and (as) TTTGGGGTTGCCTGTAGTTC for acyl CoA synthase; (ss) GAAGGCTCCTCACTTTGCAC and (as) ACATTCAATGCCCAATCGTT for lipoprotein lipase.

Materials. Unless otherwise indicated, all cell culture supplies were purchased from Fisher (Agawa, MA), radioisotopes from NEN (Boston, MA), ^{13}C-labeled FA from CIL (Cambridge, MA) and other chemicals from Sigma (St. Louis, MO).

Statistics. Data are shown as means ± SEM. Two groups were compared using Student's *t* test (see Fig. 3*B*). Other results were analyzed using one-way ANOVA and Duncan's multiple comparison tests. Differences were considered significant when $P < 0.05$.

RESULTS

Effects of octanoate on the oxidation of glucose and endogenous FA. When added to the exogenous medium, both octanoate and oleate suppressed the oxidation of glucose (**Fig. 1***A*). The suppression by octanoate was greater at 1 mmol/L than at 0.5 mmol/L ($P < 0.05$), whereas there was no difference between the two concentrations of oleate. At 0.5 mmol/L, octanoate suppressed glucose oxidation to about the same extent as oleate. Similarly, both octanoate and oleate

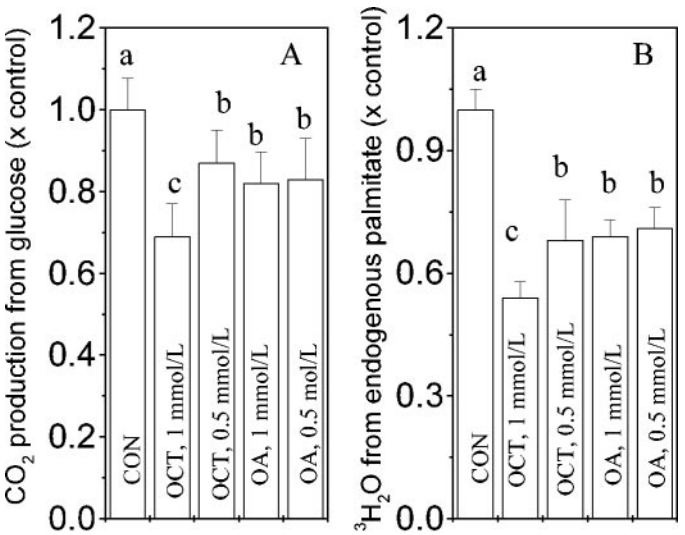

FIGURE 1 Effects of octanoate (OCT) and oleate (OA) on glucose and fatty acid (FA) oxidation in 3T3-L1 adipocytes. (*A*) $^{14}CO_2$ released from glucose [control (CON): 130 ± 10 pmol/(h · μg DNA)]. (*B*) $^{3}H_2O$ release from endogenous [9,10-^{3}H] palmitate [CON: 103 ± 6 pmol/(h · μg DNA)]. The species and concentrations of added FA are given in the figure. Data are means ± SEM ($n = 5$). Means for a variable without a common letter differ, $P < 0.05$.

suppressed the oxidation of endogenous palmitate (Fig. 1*B*). Suppression by octanoate, but not oleate, was greater at the higher concentration.

To determine whether exogenous FA interfered with the overall fuel economy, total cellular O_2 consumption was measured. When added acutely, octanoate did not affect, whereas oleate slightly increased, the O_2 consumption rate (**Fig. 2***A*). Therefore, octanoate did not appear to affect energy oxidation as it does in hepatocytes (30). Consistent with this, TPP^+ uptake in cells treated with octanoate was not different from that of control. Oleate increased TPP^+ uptake slightly, corresponding to a decrease in membrane potential, mirroring its effect on O_2 consumption.

Esterification of exogenous FA into TG. Esterification of oleate increased linearly with its concentration from 0.25 to 1.5 mmol/L, with or without insulin (**Fig. 3***A*). Any further linear increase in oleate esterification required insulin. Without insulin, esterification of oleate at high concentrations was saturated. In contrast, octanoate esterified less than oleate, and was not affected by its concentration, but was substantially increased by insulin at all FA concentrations (Fig. 3*A*).

A direct metabolic consequence of insulin was increased glucose uptake and increased glucose incorporation into de novo synthesized FA (data not shown). To test whether this was associated with insulin-stimulated esterification of octanoate, lactate was tested as an insulin-independent substrate for de novo FA synthesis. The addition of lactate substantially increased the esterification of octanoate but had little effect on oleate (Fig. 3*B*). In parallel, lactate-derived isotope was largely incorporated into de novo synthesized FA with a minor fraction into the glycerol moiety (Fig. 3C). Both octanoate and oleate suppressed the utilization of lactate in the lipid synthesis, and octanoate reduced the FA synthesis from lactate slightly more than oleate (Fig. 3C).

Octanoate caused a concentration-dependent suppression

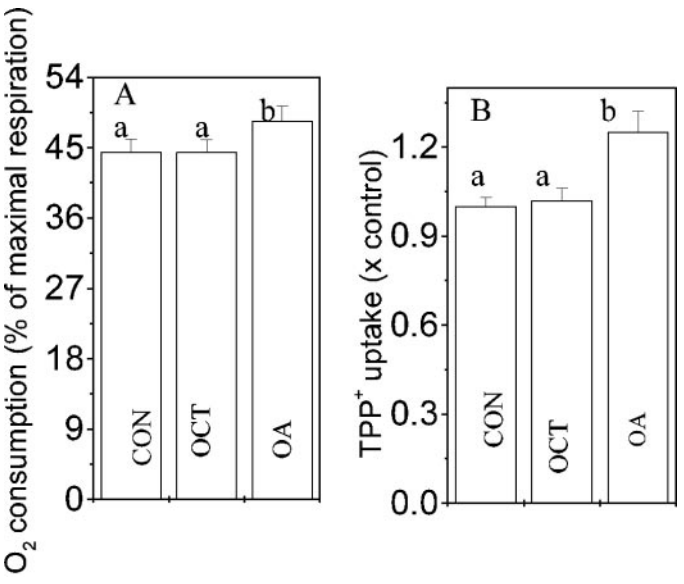

FIGURE 2 Effects of octanoate (OCT) and oleate (OA) on O_2 consumption (*A*) and mitochondrial membrane potential in 3T3-L1 adipocytes measured by tetraphenylphosphonium (TPP^+) uptake (*B*). In both experiments, fatty acids (FA) were added to the assay to a final concentration of 1 mmol/L and the effects were measured within the first 15 min of exposure to the added FA. Values are means ± SEM, $n = 3$. Means for a variable without a common letter differ, $P < 0.05$.

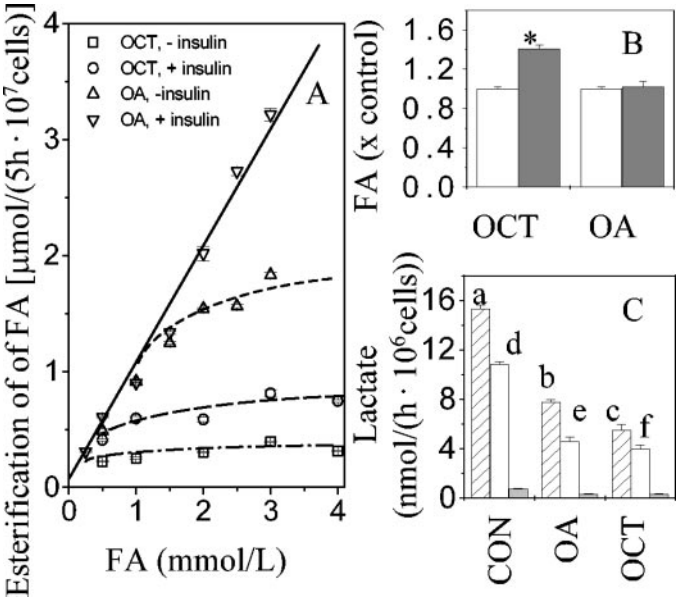

FIGURE 3 Effects of insulin (*A*) and lactate (*B*) on the esterification of exogenous fatty acids (FA) in 3T3-L1 adipocytes. (*A*) Cells were incubated with labeled FA at different concentrations for 5 h with or without insulin (10 nmol/L). The lines connecting the data points are for visual assistance only. (*B*) Cells were incubated with 1 mmol/L labeled FA the same as in A except that lactate (10 mmol/L) was added in place of insulin. The amount of FA esterified into triglycerides (TG) in the absence of lactate (open bars) was used as control. Values are means ± SEM, $n = 3$.*$P < 0.05$). (*C*) The incorporation of lactate into cellular lipids (hatched bars), FA (open bars), and glycerol (gray bars) fraction in the absence (CON) and presence of 1 mmol/L oleate (OA) or octanoate (OCT). Means for a variable without a common letter differ, $P < 0.05$.

of oleate esterification (**Fig. 4**A). To test the physiological relevance of this finding, we repeated this experiment using human adipocytes. Similar to that in 3T3-L1 adipocytes, esterification of medium-chain FA (octanoate, decanoate and laurate) into human adipocytes was much less compared with oleate (data not shown). Octanoate suppressed the esterification of oleate in human adipocytes similar to that found in 3T3-L1 adipocytes (Fig. 4*B*). Such suppression reached a plateau beyond the molar ratio of octanoate:oleate of 2:1.

On the other hand, consistent with the effects of insulin and lactate, provision of oleate at appropriate concentrations increased the esterification of octanoate into TG, but became saturated (Fig. 4*A*) or suppressed (Fig. 4*B*) when oleate concentration exceeded that of octanoate.

Inhibition of DGAT activity by octanoate. Because octanoate is esterified primarily in the *sn*3 position in TG, especially in the presence of long-chain FA [(19) and data not shown], the above results suggest that DGAT might be the site at which octanoate interferes with oleate esterification. DGAT activity was indeed substantially reduced by the presence of octanoyl CoA (**Fig. 5**A). Preincubating cells with octanoate, but not oleate, for 24 h also reduced DGAT activity (Fig. 5*B*). This suggests that octanoate had both an acute and a sustained inhibitory effect on DGAT. The latter is probably related to a down-regulation of DGAT expression.

Effects of octanoate on de novo FA synthesis. TG synthesis in adipocytes uses FA from exogenous supply, de novo synthesis, or endogenous turnover (31). Because glucose plays a key role in all of these processes, we measured the incorporation of glucose into cellular TG in the presence of different

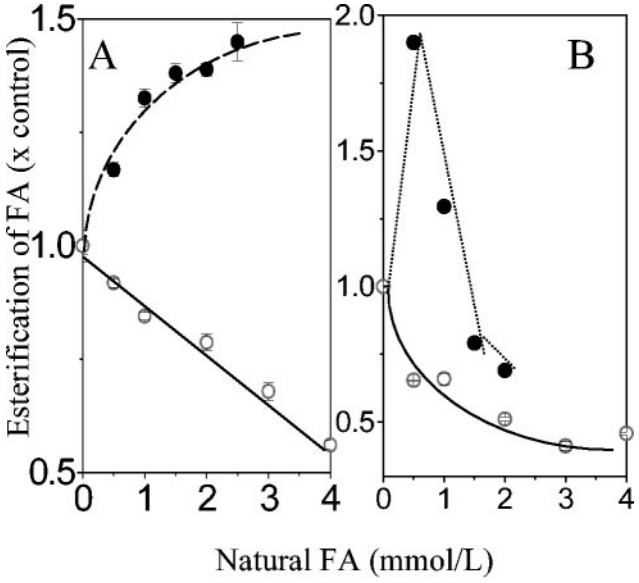

FIGURE 4 Effects of octanoate (OCT) on the esterification of oleate (OA) into triglycerides (TG) and vice versa in 3T3-L1 and human adipocytes. (*A*) 3T3-L1 adipocytes were incubated for 3 h in Krebs-Ringer bicarbonate buffer containing [1-^{13}C] octanoate (2 mmol/L, closed circle) or with [1-^{13}C] oleate (1 mmol/L, open circle). (*B*) Human adipocytes were incubated for 3 h in vendor provided medium with 1 mmol/L [1-^{14}C] octanoate (2 mCi/L, closed circle) or [1-^{14}C] oleate (0.5 mCi/L, open circle). Natural oleate was added to cells incubated with isotope-labeled octanoate, and natural octanoate was added to cells incubated with isotope-labeled oleate. The amounts of [1-^{14}C] fatty acids (FA) esterified into TG in the absence of added natural FA were used as the control values: (0.11 ± 0.02) μmol/(h · 10^{7}cells) for [1-^{13}C] octanoate and (0.47 ± 0.04) μmol/(h · 10^{7}cells) for [1-^{13}C] oleate in 3T3L1 adipocytes; (0.4 ± 0.02) nmol/(h · 10^{4}cells) for [1-^{14}C] octanoate and (19.5 ± 1.5) nmol/(h · 10^{4}cells) for [1-^{14}C] oleate in human adipocytes. Values are means ± SEM, n = 3 for 3T3-L1 and n = 2 for human adipocytes. The lines connecting the data points are for visual assistance only.

exogenous FA. Net TG synthesis (glucose incorporated into TG-glycerol) was not affected by octanoate, but was substantially increased by oleate or palmitate (**Fig. 6***A*). Replacement of part of the oleate or palmitate with octanoate reduced net TG synthesis (data not shown). On the other hand, glucose incorporation into de novo synthesized FA was not affected by oleate or palmitate but was substantially reduced by octanoate (Fig. 6*B*). A similar result was found when ^3H_2O was used as the substrate for de novo synthesis (not shown).

Sustained effects of octanoate on cellular lipid synthesis. To further determine whether the inhibitory effects of octanoate on TG synthesis were sustained, cells were pretreated with octanoate or oleate for 24 h. This did not affect the subsequent incorporation of glucose into TG-glycerol (**Fig. 7***A*) or the esterification of exogenous oleate (data not shown). However, de novo FA synthesis was largely reduced by pretreatment with octanoate, but not oleate (Fig. 7*B*).

Effects of octanoate on ACC activity. For cells preincubated with various FA, oleate had no effect, whereas octanoate and palmitate slightly but significantly inhibited ACC activity (**Fig. 8***A*). Adding octanoyl CoA or oleoyl CoA to the assay mixture had no effect on ACC activity (Fig. 8*B*), nor did the addition of free FA (data not shown). Palmitoyl CoA, on the other hand, suppressed ACC activity substantially, as previously reported (32–34).

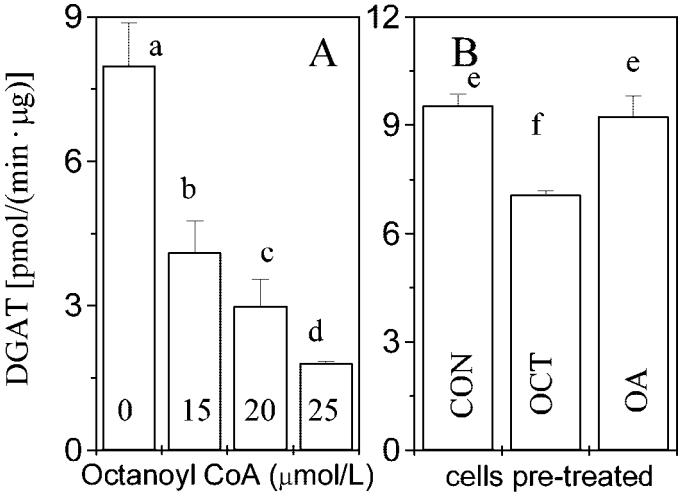

FIGURE 5 Effects of octanoate (OCT) on acyl CoA:1,2-diacylglycerol acyltransferase (DGAT) activity in 3T3-L1 adipocytes. (*A*) Octanoyl CoA was directly added to the assay mixture together with [1-^{14}C] oleoyl CoA. (*B*) Cells were preincubated in normal medium with 1 mmol/L octanoate or oleate (OA) for 24 h, and DGAT was assayed with [1-^{14}C] oleoyl CoA only. Values are means ± SEM, n = 4. Means for a variable without a common letter differ, $P < 0.05$.

Octanoate down-regulated the mRNA level of lipid synthetic genes. Cells pretreated with octanoate had reduced lipid metabolic enzyme activities (Figs. 5*B* and 7*B*). Because these experiments were performed after the removal of octanoate, we speculated that such inhibitory effects occurred at the enzyme expression level. To test this possibility, we measured the mRNA levels of several key enzymes involved in adipocyte lipid synthesis, Octanoate pretreatment significantly down-regulated the expression of a number of the key enzymes in lipid synthesis, including DGAT and ACC, which might correspond to the reduced enzyme activities in cells pretreated with octanoate (**Table 1**; Fig. 5*B*).

DISCUSSION

In this work, we showed that at low to moderately high concentrations, oleate esterification into TG was linearly de-

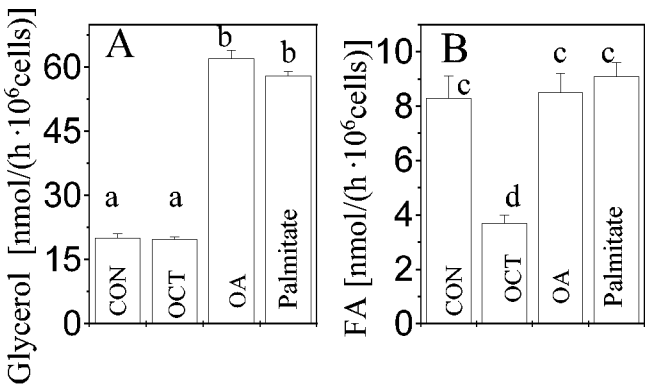

FIGURE 6 Effects of octanoate (OCT) on glucose incorporation into triglyceride (TG)-glycerol (*A*) and TG-fatty acids (FA) (*B*) moieties in 3T3-L1 adipocytes. Cells were incubated in Krebs-Ringer bicarbonate buffer with [U-^{14}C] glucose for 3 h with added 1 mmol/L FA as indicated. Data are mean ± SE, n = 3. Means for a variable without a common letter differ, $P < 0.05$.

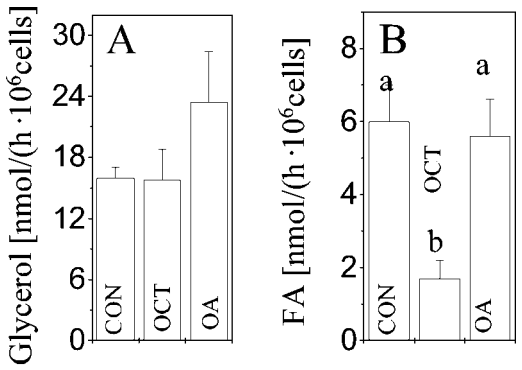

FIGURE 7 The sustained effects of octanoate (OCT) on glucose incorporation into triglyceride (TG)-glycerol (*A*) and TG-fatty acids (FA) (*B*) moieties in 3T3-L1 adipocytes. Cells were preincubated in normal medium with 1 mmol/L octanoate or oleate (OA) for 24 h. Control cells were pretreated with bovine serum albumin vehicle only. Cells were then incubated in Krebs-Ringer bicarbonate buffer with [U-^{14}C] glucose for 3 h without added FA. Values are means ± SEM, $n = 3$. Means for a variable without a common letter differ, $P < 0.05$.

TABLE 1

The effect of octanoate and oleate on the mRNA levels of selected metabolic genes[1–4]

Gene	Octanoate	Oleate
SCD-1	0.43 ± 0.02*	0.78 ± 0.04
ACC	0.71 ± 0.05*	0.90 ± 0.01
ACS-1	0.75 ± 0.04	1.1 ± 0.1
FAS	0.74 ± 0.04	0.84 ± 0.04
LPL	0.73 ± 0.13*	0.98 ± 0.03
DGAT2	0.65 ± 0.02*	1.1 ± 0.01
DGAT1	1.1 ± 0.13	0.90 ± 0.04

[1] Values are means ± SEM. * $P < 0.05$ vs. untreated cells.
[2] The results are shown as the ratio of treated/untreated cells. The raw data from RT-PCR were in arbitrary units in reference to 18S rRNA.
[3] Cells were pretreated with 1 mmol/L fatty acids for 24 h in normal medium before being used for mRNA analysis.
[4] Abbreviations: SCD-1, stearoyl CoA desaturase-1; ACC, acetyl CoA carboxylase; ACS-1, acyl CoA synthase-1; FAS, fatty acid synthase; LPL, lipoprotein lipase; DGAT, acyl CoA:1,2-diacylglycerol acyltransferase.

pendent on its exogenous availability, whereas octanoate esterification into TG was low and independent of its concentration (Fig. 3A). Similar results were found for decanoate and laurate (data not shown). Therefore, the rate-determining factors for the esterification of long-chain vs. medium-chain FA appear to be different. Insulin stimulation of oleate esterification occurred only at high oleate concentrations, likely because demand for the esterification of oleate in large quantity stimulated glucose uptake for α-glycerolphosphate synthesis. This enabled a continuous linear relationship between oleate esterification and its exogenous concentration. On the other hand, insulin nearly doubled the amount of octanoate esterified into TG, independent of its concentration. This insulin effect was partially mimicked by lactate (Fig. 3B).

In normal adipocytes, most TG synthesis is catalyzed sequentially by α-glycerophosphate acyltransferase, lysophosphatidate acyltransferase and DGAT. Because octanoate has no affinity for glycerophosphate acyltransferase (35), we suggest that the availability of long-chain FA to initiate the TG synthesis cascade might be an important regulatory factor for octanoate esterification into TG. Insulin stimulates de novo FA synthesis and increases the production of diacylglycerol, the precursor that reacts with octanoyl CoA to complete the final step in TG synthesis. Supplementing lactate might serve a similar purpose by increasing substrates for de novo FA synthesis (Fig. 3B,C). Similarly, adding oleate also increased octanoate esterification (Fig. 4), likely a result of oleate-stimulated diacylglycerol synthesis. At higher oleate concentrations, octanoate esterification became saturated (Fig. 4A) or even suppressed [Fig. 4B and (1)].

In spite of its low esterification rate, octanoate substantially inhibited the esterification of oleate (Fig. 4, A,B). Our data suggest that the octanoate-mediated reduction in DGAT activity might play an important role (Fig. 5A). Although octanoyl CoA has a certain affinity for DGAT [Fig. 3 (19,35)[, this affinity is nevertheless weaker than that of long-chain acyl CoA. This is caused in part by the mismatch between the strong hydrophobicity of neutral lipids (diacylglycerol and TG) and the strong hydrophilicity of octanoyl CoA. DGAT catalyzed the acylation reaction primarily at the surface of the endoplasmic reticulum (**Fig. 9**) (36). This requires that both substrates are bound to the enzyme and also that the substrates are suitably stabilized in the membrane. Assuming that oc-

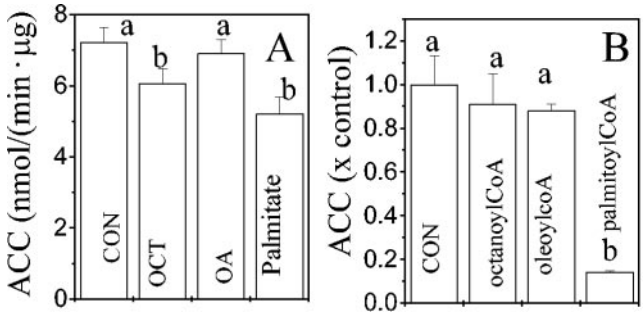

FIGURE 8 The effects of fatty acids (FA) preincubation (*A*) or directly added acyl CoA (*B*) on citrate-activated acetyl CoA carboxylase (ACC) activity in 3T3-L1 adipocytes. Cells were preincubated in normal medium with added octanoate (OCT), oleate (OA) or palmitate at 1 mmol/L for 24 h, and then used to assay for ACC activity. (*B*) ACC activity was assayed with 25 μmol/L of different added acyl CoA. Values are means ± SEM, $n = 3$. Means for a variable without a common letter differ, $P < 0.05$.

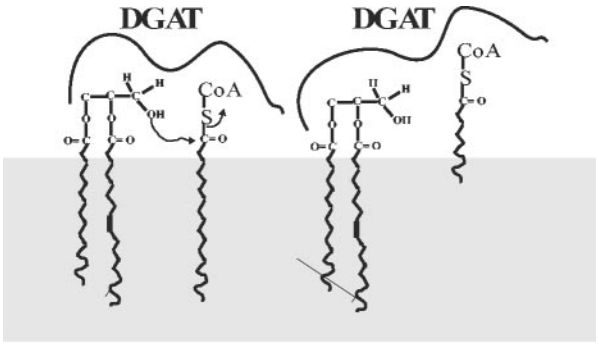

FIGURE 9 A schematic illustration of how a medium- chain CoA ester might inhibit acyl CoA:1,2-diacylglycerol acyltransferase (DGAT) activity by altering the enzyme conformation.

tanoyl CoA binds to DGAT similarly to long-chain CoA, it would less stably fit into the microsomal membrane due to its poor hydrophobicity. For this reason, it esterified less efficiently than long-chain CoA, but might serve as a competitive inhibitor for the enzyme. This view is supported by the fact that all other known natural inhibitors of DGAT, including eicosapentaenoic acid, also have highly hydrophilic molecular segments that prevent a stable fit in the membranes (27,37,38).

In addition to direct inhibition by octanoyl CoA, we found that DGAT activity was also reduced in cells pretreated with octanoate (Fig. 5*B*), suggesting a reduction of the enzyme mass in these cells. Two DGAT isoforms (DGAT1 and DGAT2) from different gene families have been identified in adipocytes (39,40). DGAT1 is involved mainly in basal TG synthesis, whereas DGAT2 is more responsive to physiological manipulations (39). We found that octanoate decreased the expression of DGAT2 but not DGAT1, and oleate did not have an effect on either (Table 1). The effects of octanoate incubation seem to mimic that of starvation, which also reduces the overall DGAT activity (41) and the expression of DGAT2 but not DGAT1 (42). Because octanoate or its derivatives are intermediates of long-chain FA β-oxidation, which increases substantially during starvation (41), we speculate that a rise in intracellular octanoate concentration might be related to signals of nutrient deficiency (43), which would suppress lipid synthetic enzymes. Consistent with this hypothesis, the mRNA levels of a number of other genes involved in lipid anabolism were reduced in cells pretreated with octanoate (Table 1). Many of these genes are also down-regulated by starvation (44,45), and suppression of ACC and FAS expression by octanoate has also been reported in hepatocytes (43,46,47).

The reduction in citrate-activated ACC activity in cells pretreated with octanoate was only moderate (Fig. 8*A*), and appeared not to be a direct inhibition by octanoyl CoA (Fig. 8*B*). Such a change might not be sufficient to account for the large decrease in de novo FA synthesis found in intact cells (Figs. 6,7). It is possible that the enzyme activity was substantially lower in live cells in which additional suppressive factors might be mediated by octanoate. For instance, intracellular ACC can be phosphorylated and inactivated by AMP kinase (48), and octanoate has been found to activate AMP kinase in hepatocytes (49). If this also applies to adipocytes, it might explain in part the suppression of de novo FA synthesis by octanoate. In addition, octanoate might suppress pyruvate dehydrogenase through a feedback inhibition by accumulation of octanoate-derived acetyl CoA as reported in hepatocytes (50). However, this would inhibit de novo synthesis only from glucose, not from ^3H_2O. Because we found that the latter was also inhibited, our data did not suggest inhibition of pyruvate dehydrogenase as a major mechanism for octanoate-mediated inhibition on de novo FA synthesis (1). On the other hand, although palmitoyl CoA substantially inhibited ACC activity in vitro (Fig. 8*B*), palmitate did not suppress de novo FA synthesis in intact cells (Fig. 6*B*). Palmitate also increased TG synthesis similarly to oleate (Fig. 6*A*). Therefore, although octanoate and palmitate are both saturated FA, the antilipogeneic effects of octanoate in adipocytes appear to be related to the acyl chain length rather than its saturation.

An unexpected result in this work is that although we observed suppression of the oxidation of glucose or endogenous palmitate by octanoate, such suppression was not drastic (Fig. 1) and did not affect cellular respiration or the mitochondrial membrane potential (Fig. 2). This was in contrast to earlier studies showing that octanoate potently increased O_2 consumption and diminished the mitochondrial membrane potential in hepatocytes (30,51), thus suggesting different metabolic roles of octanoate and oleate in the two cell types.

In summary, we showed that octanoate markedly inhibited TG synthesis in both 3T3-L1 and human adipocytes, both acutely and long term. These findings increase our knowledge of the mechanisms by which an MCT diet may mediate body fat mass reduction.

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