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Abstract Transcription of acetyl-CoA carboxylase in avian liver is low during starvation or after consumption of a low-carbohydrate, high-fat diet and high during consumption of a high-carbohydrate, low-fat diet. The role of fatty acids or metabolites derived from fatty acids in the nutritional control of acetyl-CoA carboxylase transcription was investigated by determining the effects of long- and medium-chain fatty acids on acetyl-CoA carboxylase expression in primary cultures of chick embryo hepatocytes. Palmitate, oleate, and arachidonate caused a decrease in acetyl-CoA carboxylase activity in hepatocytes incubated with triiodothyronine (T3). The inhibition of acetyl-CoA carboxylase activity caused by arachidonate was accompanied by a similar decrease in transcription of the acetyl-CoA carboxylase gene. In contrast, neither palmitate nor oleate were effective in modulating acetyl-CoA carboxylase transcription. These results are consistent with arachidonate or a metabolite derived therefrom mediating the effects of diets containing high levels of n–6 polyunsaturated fatty acids on acetyl-CoA carboxylase transcription in liver. Hexanoate and octanoate also inhibited acetyl-CoA carboxylase activity in the presence of T3. The magnitude of the hexanoate- or octanoate-induced decrease in acetyl-CoA carboxylase activity was greater than that observed for long-chain fatty acids. Hexanoate and octanoate inhibited acetyl-CoA carboxylase activity at a transcriptional step, and did so within 2 h of addition of fatty acid. Addition of carnitine partially reversed the inhibitory effects of octanoate on acetyl-CoA carboxylase expression, suggesting that a metabolite of octanoate is involved in mediating this response. 2-Bromooctanoate was a more potent inhibitor of acetyl-CoA carboxylase expression than octanoate or hexanoate. We postulate that a metabolite of hexanoate and octanoate, possibly a six or eight carbon acyl-CoA, plays a role in the nutritional regulation of acetyl-CoA carboxylase transcription.—**Hillgartner, F. B., and T. Charron.** Arachidonate and medium-chain fatty acids inhibit transcription of the acetyl-CoA carboxylase gene in hepatocytes in culture. *J. Lipid Res.* 1997. **38:** 2548–2557.

Supplementary key words transcription • fatty acid synthesis • thyroid hormone •liver • avian

Acetyl-CoA carboxylase (EC 6.4.1.2) catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA which is the donor of all but two (omega) of the carbon atoms for the synthesis of long-chain fatty acids. This reaction is considered a pace-setting step of the fatty acid synthesis pathway (1). In the liver of avians and mammals, the activity of acetyl-CoA carboxylase is regulated by alterations in nutritional status. Short-term changes in enzyme activity are mediated by allosteric and covalent modification mechanisms (1–3). Acetyl-CoA carboxylase activity is also controlled by long-term alterations in enzyme concentration. For example, the concentration of acetyl-CoA carboxylase in liver is low in starved chickens and rats; feeding a high-carbohydrate, low-fat diet stimulates an 8- to 20-fold increase in the amount of the enzyme (4–8). The composition of the diet also regulates the concentration of hepatic acetyl-CoA carboxylase. In chickens and rats fed a low-carbohydrate, high-fat diet, the level of acetyl-CoA carboxylase is substantially reduced compared to that of animals fed a high-carbohydrate, low-fat diet (9–12). Changes in acetyl-CoA carboxylase concentration caused by nutritional manipulation are accompanied by comparable alterations in the synthesis rate and mRNA abundance for acetyl-CoA carboxylase, indicating that regulation occurs at a pretranslational step (5–9, 13–16). The effects of dietary manipulation on acetyl-CoA carboxylase mRNA abundance are mediated primarily by changes in the transcription rate of the acetyl-CoA carboxylase gene (14).

Several lines of evidence indicate that glucose, insulin, 3,5,3′-triiodothyronine (T3), and glucagon are humoral factors that communicate changes in nutritional status to the liver, thereby mediating nutritional regulation of hepatic acetyl-CoA carboxylase. First, the stimulation in acetyl-CoA carboxylase transcription caused by

Abbreviations: T3, 3,5,3′-triiodo-L-thyronine.

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feeding a high-carbohydrate, low-fat diet is preceded or paralleled by increases in the molar ratio of insulin/glucagon and the levels of glucose and T3 in the blood (17). Second, glucose, insulin, T3, and glucagon regulate acetyl-CoA carboxylase transcription in primary cultures of chick embryo hepatocytes. T3 stimulates a 3-fold increase in the transcription of acetyl-CoA carboxylase gene (18). Glucose (20 mM) has no effect by itself on acetyl-CoA carboxylase transcription but amplifies the effect of T3 by 3-fold. Insulin accelerates the increase in acetyl-CoA carboxylase transcription caused by T3 and glucose, whereas glucagon inhibits the stimulatory effect of T3 and glucose by 65%.

Fatty acids or metabolites derived from fatty acids may also participate in the nutritional regulation of acetyl-CoA carboxylase. Saturated, monounsaturated, and polyunsaturated long-chain fatty acids cause a decrease in the concentration and rate of synthesis of acetyl-CoA carboxylase in rat hepatocyte cultures (19, 20). This finding coupled with the observation that levels of unesterified long-chain fatty acids in the blood and liver are elevated during starvation or high-fat feeding (21, 22) suggest that these agents may signal alterations in nutritional state to the liver. In the present study, we investigated this possibility further by determining the effects of palmitate, oleate, and arachidonate on the transcription rate of the acetyl-CoA carboxylase gene in chick embryo hepatocytes. We show that arachidonate inhibits transcription of acetyl-CoA carboxylase in the presence of T3, whereas, palmitate and oleate are not effective in regulating the transcription of this gene. We also demonstrate that six- and eight-carbon fatty acids are potent inhibitors of acetyl-CoA carboxylase transcription. We postulate that a metabolite derived from these fatty acids plays a role in the nutritional regulation of acetyl-CoA carboxylase transcription.

MATERIALS AND METHODS

Chemicals

Nucleotides (Pharmacia Biotechnology), proteinase K (Boehringer Mannheim), fatty acids (Nu Chek Prep), [α-^{32}P]UTP and [α-^{32}P]dCTP (ICN Biomedicals) were purchased from the indicated sources. Crystalline bovine insulin was a gift from Lilly. Bovine serum albumin deficient in unesterified fatty acids was obtained from ICN Biomedicals. All other chemicals were from Sigma or of the highest purity commercially available.

Preparation and maintenance of isolated hepatocytes

Unincubated embryonated eggs from white Leghorn chickens were obtained from Truslow Farms, Chestertown, MD and incubated in an electric forced draft incubator at 37.5 ± 0.5°C and 80% relative humidity. Embryos (19 days of age) were killed by decapitation, the livers were removed, and hepatocytes were isolated (23). The isolated hepatocytes were incubated in Waymouth's medium MD705/1 (GIBCO) containing penicillin (60 μg/ml) and streptomycin (100 μg/ml) on untreated plastic Petri dishes (Fisher) at 40°C in a humidified atmosphere of 5% CO_2 and 95% air. One 90-mm Petri dish contained 1 ml of cell suspension (2–3 mg total protein, approximately 1×10^7 cells) and 9 ml of medium. The medium on all plates was changed after about 18 h of incubation. Hormone and other additions were as described in the legends to figures and tables. Palmitate, oleate, and arachidonate were bound to albumin as described by Mooney and Lane (24). Acetyl-CoA carboxylase activity was measured as described by Salati and Clarke (19). The concentration of citrate (10 mM) in this assay yields optimal product formation and reverses the inhibitory effects of long-chain fatty acids on acetyl-CoA carboxylase activity (25, 26). Protein was measured as described (27). We chose arachidonate to study the effects of polyunsaturated fatty acids on acetyl-CoA carboxylase expression because previous studies have shown that hepatocytes in primary culture have a limited ability to desaturate and/or elongate linoleate which is essential for linoleate-induced inhibition of expression of lipogenic enzymes (28, 29).

Isolation of RNA and quantitation of mRNA levels

Medium was removed and RNA was extracted from cells by the guanidinium thiocyanate/phenol/chloroform method (30). Total RNA (15 μg) was separated by size in 0.9% agarose, 0.7 M formaldehyde gels, and then transferred to a Nytran membrane (Schleicher & Schuell) using a vacuum blotting apparatus (Pharmacia Biotechnology). The RNA was crosslinked to the membrane by UV and baked at 80°C for 30–60 min. RNA blots were hybridized with ^{32}P-labeled DNA probes labeled by random priming (31). Hybridization and washes were as described (32). Membranes were subjected to storage phosphor autoradiography. Hybridization signals were quantified using ImageQuant software (Molecular Dynamics).

Nuclear run-on assay of transcription rates

Nuclei were isolated from the pooled cells of 15 90-mm plates (33, 34). Approximately 5×10^7 nuclei were stored at −80°C in 100-μl aliquots containing 50 mM

HEPES, pH 7.4, 75 mM NaCl, 0.1 mM EDTA, 5 mM dithiothreitol, 0.125 mM PMSF, and 50% glycerol. The in vitro elongation reactions were carried out as described (14, 35). ^{32}P-labeled RNA transcripts were purified by the method of Linial, Gunderson, and Groudine (36), using NICK columns (Pharmacia Biotechnology) as described in the manufacturer's instructions. Denaturation of DNA probes, application of DNA to GeneScreen membranes, hybridization of ^{32}P-labeled RNA transcripts to membrane-bound DNA, and posthybridization washes were carried out as described (14). Hybridization signals were quantified as described above.

DNA probes

Three non-overlapping cDNA fragments of chicken acetyl-CoA carboxylase were used as transcription probes in the nuclear run-on assay. ACC-1, ACC-2, and ACC-3 correspond to bases 151 to 2075, 2283 to 4486, and 5019 to 6926, respectively, of the chicken acetyl-CoA carboxylase cDNA sequence reported by Takai et al. (37). These cDNA fragments are located within the coding region (see Fig. 1C) and encode the 265 kDa isoform of acetyl-CoA carboxylase, the only isoform of the enzyme expressed in chicken liver (38). All three acetyl-CoA carboxylase cDNA probes hybridize to two mRNAs of 10 and 11 kb on Northern blots of chick embryo hepatocyte RNA. Based on Southern analysis of genomic DNA, ACC-1, ACC-2, and ACC-3 are unique and do not contain repetitive sequences (14). Nuclear transcripts generated during in vitro elongation hybridized only to the coding strand of each acetyl-CoA carboxylase probe, indicating that transcription was strand-specific. Each probe was subcloned into a Bluescript (Stratagene) vector. The chicken cDNAs for glyceraldehyde-3-phosphate dehydrogenase, and β-actin were generously provided by Robert Schwartz (Baylor College of Medicine) and Don W. Cleveland (Johns Hopkins University), respectively.

RESULTS

Action of long-chain fatty acids

Palmitate and oleate are the major species of nonesterified free fatty acids present in the plasma and liver of avians (22). The levels of these fatty acids are regulated by changes in nutritional status. The concentrations of palmitate and oleate in plasma and liver are high in starved chicks and are low in chicks fed a high-carbohydrate, low-fat diet. These alterations in the levels of palmitate and oleate are mediated primarily by changes in the rate of lipolysis in adipose tissue (39). To investigate whether palmitate and oleate are involved in mediating changes in hepatic acetyl-CoA carboxylase expression caused by starvation/refeeding, we determined the effects of these fatty acids on the activity of acetyl-CoA carboxylase in chick embryo hepatocytes incubated in the presence of insulin (50 nM) and glucose (27.5 mM) with or without T3 (1.5 μM). Addition of albumin-bound palmitate or oleate to the culture medium inhibited the T3-induced increase in acetyl-CoA carboxylase activity in a concentration-dependent manner (**Table 1**). At a concentration of 250 μM, the inhibition by palmitate and oleate was about 35%. Palmitate and oleate had no effect on acetyl-CoA carboxylase activity in the absence of T3. We also investigated the effects of arachidonate on acetyl-CoA carboxylase activity because increasing the level of polyunsaturated fat in the diet causes a decrease in acetyl-CoA carboxylase expression in chicks and rats (14, 40). Addition of arachidonate at 100 and 250 μM inhibited the T3-induced increase in acetyl-CoA carboxylase activity by 32 and 45%, respectively (Table 1). Arachidonate had no effect on acetyl-CoA carboxylase activity in the absence of T3. The above data indicate that long-chain fatty acids of differing levels of saturation are able to inhibit acetyl-CoA carboxylase activity in chicken embryo hepatocyte cultures. These changes in acetyl-CoA carboxylase activity caused

TABLE 1. Long-chain fatty acids inhibit the T3-induced increase in acetyl-CoA Carboxylase Activity

	Acetyl-CoA Carboxylase Activity	
Addition	−T3	+T3
Fatty acid-free albumin (17 μM)	ND	100
Palmitate (100 μM)	ND	81 ± 5[a]
Oleate (100 μM)	ND	79 ± 8
Arachidonate (100 μM)	ND	68 ± 4[a]
Fatty acid-free albumin (42 μM)	16.2 ± 3	96 ± 8
Palmitate (250 μM)	13.8 ± 2	62 ± 7[b]
Oleate (250 μM)	15.7 ± 2	65 ± 10[b]
Arachidonate (250 μM)	16.8 ± 1	53 ± 4[b]

Hepatocytes were isolated as described under Materials and Methods and incubated in Waymouth's medium containing insulin (50 nM) and 27.5 mM glucose. At 18 h of incubation, the medium was changed to one of the same composition with or without T3 (1.5 μM). Palmitate, oleate, and arachidonate were added at this time. The concentration of the fatty acids was 100 and 250 μM. The corresponding albumin concentrations were 17 and 42 μM, respectively. Controls were treated with fatty acid-free albumin alone. At about 42 h of incubation, the medium was changed to one of the same composition. The cells were harvested at 66 h of incubation and acetyl-CoA carboxylase activity was measured. The results are expressed as percentages of the value for cells incubated with T3 and 17 μM albumin ± SE of three experiments. 100 = 32 ± 2 mU/mg protein.

Superscript letters indicate significant differences ($P < 0.05$) as determined by Student's *t*-test: [a]versus 17 μM fatty acid-free albumin; [b]versus 42 μM fatty acid-free albumin. ND, not determined.

by long-chain fatty acids reflect alterations in the amount of acetyl-CoA carboxylase because the activity of the enzyme was measured in the presence of citrate under optimal conditions (19).

To determine the mechanism for changes in acetyl-CoA carboxylase activity caused by long-chain fatty acids, mRNA abundance and transcription of acetyl-CoA carboxylase was measured. Incubating hepatocytes with palmitate or oleate at a concentration of 250 μM for 48 h had no effect on the accumulation of acetyl-CoA carboxylase mRNA caused by T3 (**Fig. 1A**). Palmitate and oleate also had no effect of the transcription rate of the acetyl-CoA carboxylase gene under conditions where hepatocytes were incubated with T3 for 29 h and fatty acids were added at a concentration of 250 μM (Fig. 1B) or 500 μM (results not shown) during the last 5 h of the incubation with T3. Similar results were obtained when palmitate or oleate (250 μM) were added at the same time as T3 and incubated for 29 h (results not shown). These data indicate that the inhibitory effects of palmitate and oleate on the level of acetyl-CoA carboxylase are not mediated by a pretranslational mechanism. The lack of effect of palmitate on the mRNA abundance and transcription of acetyl-CoA carboxylase is not due to a lack of metabolism of this fatty acid because palmitate in the medium is rapidly converted to CO_2 and intracellular lipids in chick embryo hepatocytes (23). In contrast to the actions of palmitate and oleate, addition of arachidonate (250 μM) inhibited the T3-induced accumulation of acetyl-CoA carboxylase mRNA by 44 ± 6% (Fig. 1A). Arachidonate also decreased the transcription of the acetyl-CoA carboxylase gene when it was added during the last 5 h of a 29-h incubation with T3. The extent of the decrease in acetyl-CoA carboxylase transcription as detected by the 5′-most acetyl-CoA carboxylase cDNA probe (ACC-1) was 47 ± 3 and 51 ± 8% when arachidonate was added at a concentration of 250 μM (Fig. 1B) and 500 μM (results not shown), respectively. Similar changes in acetyl-CoA carboxylase transcription were observed with acetyl-CoA carboxylase probes from the middle (ACC-2) and 3′ (ACC-3) portions of the cDNA. These data indicate that the effects of arachidonate on acetyl-CoA carboxylase activity are accounted for by changes in acetyl-CoA carboxylase mRNA abundance, which in turn, are due primarily to changes in acetyl-CoA carboxylase transcription. The effects of arachidonate on acetyl-CoA carboxylase transcription were specific because transcription of the genes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were not affected by this fatty acid (Fig. 1B).

Long-chain fatty acids have been reported to inhibit the binding of T3 to its nuclear receptor in vitro by a competitive mechanism (41, 42). In the experiments described above, hepatocytes were incubated with a concentration of T3 (1.5 μM) that was about 10^3 times higher than that required to saturate the nuclear T3 re-

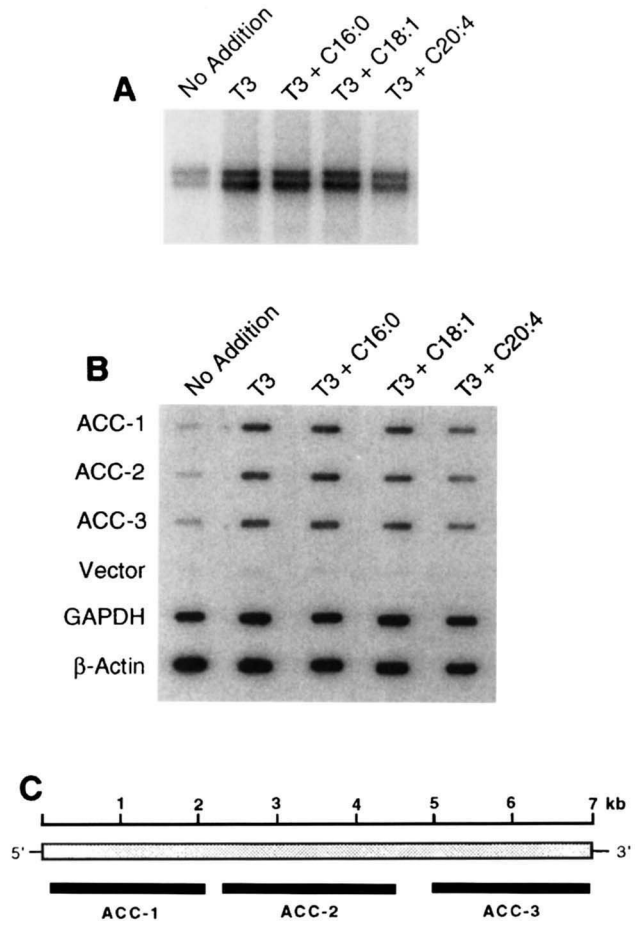

Fig. 1. Effects of long-chain fatty acids on the mRNA abundance (A) and transcription (B) of acetyl-CoA carboxylase. Hepatocytes were isolated and incubated in Waymouth's medium containing insulin and 27.5 mM glucose. At 18 h of incubation, the medium was changed to one of the same composition with or without T3 (1.5 μM). In A, albumin-bound palmitate (C16:0), oleate (C18:1), or arachidonate (C20:4) were added at this time. The concentration of the fatty acids was 250 μM. Controls were treated with fatty acid-free albumin (42 μM). At about 42 h of incubation, the medium was changed to one of the same composition. The cells were harvested at 66 h of incubation and acetyl-CoA carboxylase mRNA levels were measured by Northern analysis as described under Material and Methods. In B, fatty acids (250 μM) were added at 42 h of incubation without medium change and the incubation was continued for 5 h. Nuclei were isolated, and acetyl-CoA carboxylase transcription was measured by nuclear run-on assay as described under Materials and Methods. The acetyl-CoA carboxylase cDNA fragments that were used as probes in the nuclear run-on assay are shown in C. The stippled rectangle represents the coding sequence of the acetyl-CoA carboxylase cDNA. The experiments in A and B were repeated three times with similar results.

ceptor. This level of hormone was used in long-term incubations to ensure that optimal levels of T3 were present throughout the incubation as T3 is degraded rapidly in serum-free Waymouth's medium (43). Because high T3 concentrations may mask a possible inhibitory effect of long-chain fatty acids on acetyl-CoA carboxylase expression, the effect of palmitate on acetyl-CoA carboxylase transcription was also determined in hepatocytes incubated with 20 nM T3. The transcription rate of acetyl-CoA carboxylase in hepatocytes incubated with 20 nM T3 for 29 h was 49% of that observed in cells incubated with 1.5 μM T3 (**Fig. 2**). Addition of palmitate (250 μM) during the last 5 h of the incubation with 20 nM T3 had no effect on acetyl-CoA carboxylase transcription. Thus, palmitate is not effective in regulating acetyl-CoA carboxylase transcription in the presence of optimal or suboptimal T3 levels. Competitive interactions between T3 and palmitate for binding to nuclear T3 receptors do not appear to be involved in the regulation of acetyl-CoA carboxylase in intact hepatocytes.

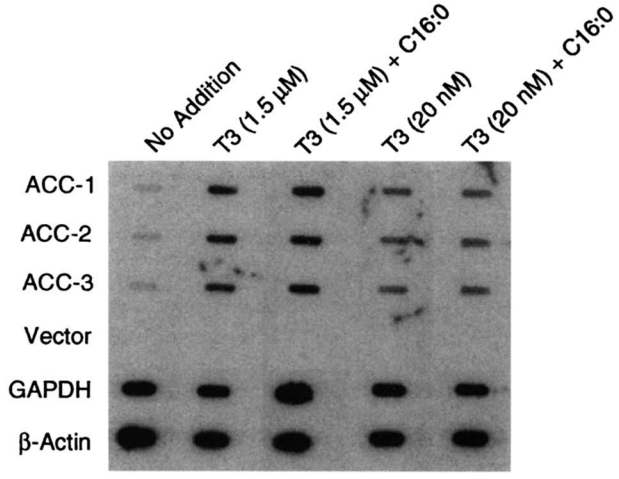

Fig. 2. Effect of palmitate on the transcription of the acetyl-CoA carboxylase gene in hepatocytes incubated with 20 nM or 1.5 μM T3. Hepatocytes were isolated and incubated in Waymouth's medium containing insulin and 27.5 mM glucose. At 18 h of incubation, the medium was changed to one of the same composition with or without T3. The concentration of T3 was 20 nM or 1.5 μM. At 30 h of incubation, the medium was changed to one of the same composition. At 42 h of incubation, albumin-bound palmitate (250 μM) was added without medium change. Controls were treated with fatty-acid free albumin (42 μM). At 47 h of incubation, nuclei were isolated, and acetyl-CoA carboxylase transcription was measured by nuclear run-on assay as described under Materials and Methods. All probes on each strip were hybridized with the same preparation of ^{32}P-labeled RNA transcripts at the same time; strips of prints of the autoradiograph were reorganized to maintain the same order of probes in all figures. This experiment was repeated twice with similar results.

Action of medium-chain fatty acids

In a previous report (44), unesterified medium-chain fatty acids were shown to cause a marked inhibition in the expression of the genes for malic enzyme and fatty acid synthase. This prompted us to investigate the effects of medium-chain fatty acids on the activity of acetyl-CoA carboxylase in chick embryo hepatocytes. Medium-chain fatty acids are much more soluble in aqueous medium than long-chain fatty acids. Thus, we were able to test the effects of medium-chain fatty acids without binding them to albumin. Incubating hepatocytes with hexanoate and octanoate (1 mM) in the presence of T3, insulin, and glucose (27.5 mM) for 72 h caused a 70 and 79% decrease in acetyl-CoA carboxylase activity, respectively (**Table 2**). Butanoate and decanoate also inhibited the T3-induced increase in acetyl-CoA carboxylase activity, but to a significantly lesser extent. In the absence of T3, addition of medium-chain fatty acids had no effect on acetyl-CoA carboxylase activity. Thus, medium-chain fatty acids inhibit the T3-induced increase in acetyl-CoA carboxylase activity, and fatty acids of six to eight carbons in chain length are most effective in causing this effect.

To determine the mechanism for the effects of hexanoate and octanoate on acetyl-CoA carboxylase activity, acetyl-CoA carboxylase mRNA levels were measured in chicken embryo hepatocytes incubated with different concentrations of these fatty acids. Both hexanoate and octanoate caused a dose-dependent decrease in the accumulation of acetyl-CoA carboxylase mRNA caused by T3 (**Fig. 3A**). Maximal inhibition of acetyl-CoA carboxylase mRNA abundance by hexanoate and octanoate was 75 ± 9 and 85 ± 6%, respectively,

TABLE 2. Medium-chain fatty acids inhibit the T3-induced increase in acetyl-CoA carboxylase activity

	Acetyl-CoA Carboxylase Activity	
Addition	−T3	+T3
None	10 ± 1	100
Butanoate	12 ± 1	56 ± 4[a]
Hexanoate	9 ± 1	30 ± 5[a]
Octanoate	8 ± 1	21 ± 2[a]
Decanoate	11 ± 2	67 ± 5[a]

Hepatocytes were isolated and incubated in Waymouth's medium containing insulin (50 nM) and 27.5 mM glucose. At 18 h of incubation, the medium was changed to one of the same composition, with or without T3 (1.5 μM) or the indicated fatty acids (1 mM). At about 66 h of incubation, the medium was changed to one of the same composition. The cells were harvested at 90 h of incubation and acetyl-CoA carboxylase activity was measured. The results are expressed as percentages of the value for cells incubated with T3 and no fatty acids ± SE of three experiments. 100 = 38 ± 3 mU/mg protein.

[a]Mean is different ($P < 0.01$) from that of cells treated with no fatty acids as determined by Student's *t*-test.

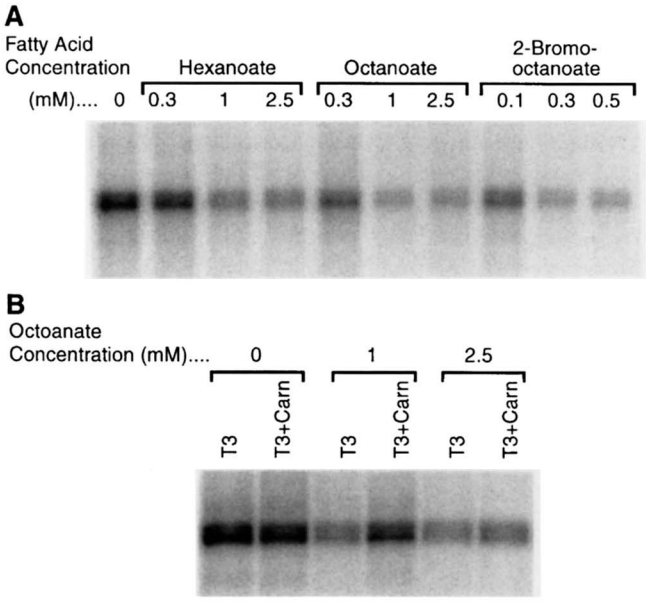

Fig. 3. Effects of six- and eight-carbon fatty acids on the abundance of acetyl-CoA carboxylase mRNA as function of concentration (A) and the presence or absence of carnitine (B). Hepatocytes were isolated and incubated in Waymouth's medium containing insulin and 27.5 mM glucose. At 18 h of incubation, the medium was changed to one of the same composition and T3 (1.5 μM) was added with or without hexanoate, octanoate, or 2-bromooctanoate at the indicated concentrations. In (B), carnitine (5 mM) was added at this time. The cells were harvested 48 h later and acetyl-CoA carboxylase mRNA levels were measured by Northern analysis as described under Materials and Methods. The experiments in A and B were conducted a total of four and three times, respectively, with similar results.

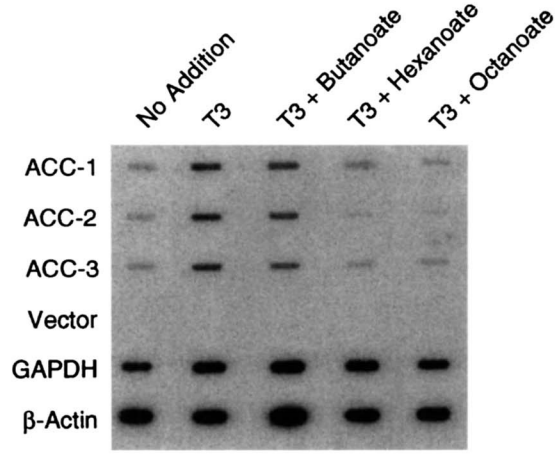

Fig. 4. Butanoate, hexanoate, and octanoate rapidly inhibit the transcription of the acetyl-CoA carboxylase gene. Hepatocytes were isolated and incubated in Waymouth's medium containing insulin and 27.5 mM glucose. At 18 h of incubation, the medium was changed to one of the same composition and T3 (1.5 μM) was added. At 44 h of incubation, the indicated fatty acids (1 mM) were added without medium change and the incubation was continued for 2 h. Nuclei were isolated, and acetyl-CoA carboxylase transcription was measured by nuclear run-on assay as described under Materials and Methods. This experiment was repeated twice with similar results.

and was observed at a concentration of 1 mM. These data indicate that hexanoate and octanoate act at a pretranslational a step to inhibit acetyl-CoA carboxylase levels.

To assess the role of transcription in mediating the inhibition of acetyl-CoA carboxylase mRNA levels by medium-chain fatty acids, nuclear run-on experiments were performed. Addition of hexanoate or octanoate (1 mM) during the last 2 h of a 28-h incubation with T3 caused a marked decrease in the acetyl-CoA carboxylase transcription (**Fig. 4**). The extent of the inhibition of transcription as detected by ACC-1 was 79 ± 9 and 85 ± 7% for hexanoate and octanoate, respectively. Similar changes in acetyl-CoA carboxylase transcription were detected by ACC-2 and ACC-3. Thus, rapid alterations in the rate of transcription initiation of the acetyl-CoA carboxylase gene account for all of the changes in acetyl-CoA carboxylase mRNA levels caused by hexanoate or octanoate. Butanoate (1 mM) also inhibited acetyl-CoA carboxylase transcription; the magnitude of this effect (35 ± 6% as detected by ACC-1) was less than that of hexanoate and octanoate. The effects of hexanoate, octanoate, and butanoate on acetyl-CoA carboxylase transcription were specific because transcription of the genes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were not affected by fatty acid treatment (Fig. 4).

Evidence that a metabolite mediates the inhibition of acetyl-CoA carboxylase expression by octanoate

To investigate whether inhibition of acetyl-CoA carboxylase expression by octanoate requires the metabolism of this fatty acid, we determined the effects of exogenous carnitine on acetyl-CoA carboxylase mRNA levels. Carnitine is a substrate in the transesterification of fatty acyl-CoAs to acylcarnitines, reactions catalyzed by acylcarnitine transferases. Various forms of this enzyme have been identified that utilize short-, medium-, or long-chain fatty acyl-CoAs (45, 46). Because these reactions are readily reversible, addition of carnitine should increase the levels of acylcarnitines and decrease the levels of fatty acyl-CoAs via mass action. Addition of 5 mM carnitine to chick embryo hepatocytes incubated in the presence of T3 and 1 mM octanoate increased the abundance of acetyl-CoA carboxylase mRNA by 2.3 ± 0.4-fold (Fig. 3B). Carnitine had little or no effect on acetyl-CoA carboxylase mRNA levels in cells incubated with 0 or 2.5 mM octanoate. These

data are consistent with a metabolite mediating the inhibition of acetyl-CoA carboxylase expression by octanoate. This metabolite may be an acyl-CoA containing six or eight carbons whose concentration is reciprocally regulated by octanoate and carnitine. To investigate this possibility, we examined the effects of 2-bromooctanoate on acetyl-CoA carboxylase mRNA levels. In hepatocytes, 2-bromooctanoate is activated to 2-bromooctanoyl-CoA and then oxidized to 2-bromo-3-ketooctanoyl-CoA, an irreversible inactivator of mitochondrial and peroxisomal 3-ketothiolases I (47, 48). 2-Bromooctanoate also inhibits diacylglycerol acyltransferase in hepatocytes; 2-bromooctanoyl-CoA is the active inhibitor (49). Incubating chick embryo hepatocytes with 2-bromooctanoate in the presence of T3 caused a dose-dependent decrease in the accumulation of acetyl-CoA carboxylase mRNA (Fig. 3A). The maximal inhibition of acetyl-CoA carboxylase mRNA abundance caused by 2-bromooctanoate was 83 ± 12% and was observed at a concentration of 0.3 mM. Thus, 2-bromooctanoate was more potent than octanoate in inhibiting the expression of acetyl-CoA carboxylase. This result suggests that the active inhibitor may be an acyl-CoA containing six or eight carbons.

DISCUSSION

Unesterified long-chain fatty acids can regulate acetyl-CoA carboxylase activity in hepatocytes by modulating both the catalytic efficiency and level of expression of the enzyme. Regarding the former mode of regulation, long-chain fatty acyl-CoAs are potent allosteric inhibitors of acetyl-CoA carboxylase (25, 50, 51). Regarding the latter mode of regulation, long-chain fatty acids inhibit the expression of acetyl-CoA carboxylase in rat (19, 20) and avian (Table 1) hepatocytes. The results of the present study demonstrate that the mechanism by which long-chain fatty acids inhibit expression of acetyl-CoA carboxylase varies depending on the species of fatty acid. Arachidonate acts primarily at the level of transcription initiation to modulate acetyl-CoA carboxylase expression, whereas, palmitate and oleate appear to act at a translational step (Figs. 1 and 2). Because the effects of starvation and feeding a high-carbohydrate, low-fat diet on acetyl-CoA carboxylase expression are mediated primarily by changes in transcription of the acetyl-CoA carboxylase gene (14), we conclude that palmitate and oleate are not involved in mediating these responses. This finding was not expected when one considers that palmitate and oleate are the major species of unesterified free fatty acids present in the liver and blood and that the levels of these fatty acids are regulated by starvation/refeeding (22). Thus, the role of palmitate and oleate in the nutritional control of acetyl-CoA carboxylase activity may be limited to changes in the catalytic efficiency of the enzyme. Although arachidonate is effective in regulating transcription of acetyl-CoA carboxylase, its role in mediating the effects of starvation and refeeding a high-carbohydrate, low-fat diet on acetyl-CoA carboxylase expression is unclear because arachidonate levels in the blood and liver probably do not vary significantly during this nutritional manipulation. Arachidonate or metabolites derived from arachidonate may play a greater role in mediating the inhibition of acetyl-CoA carboxylase transcription caused by feeding high levels of polyunsaturated fatty acids from the n–6 family (14).

Long-chain fatty acids have been shown to regulate the expression of other genes involved in lipogenesis. For example, arachidonate and other polyunsaturated long-chain fatty acids of the n–3 and n–6 families cause a decrease in the activities and mRNA abundance for glucose-6-phosphate dehydrogenase (L. M. Salati, personal communication), stearoyl-CoA desaturase-1 (52, 53), malic enzyme (54), fatty acid synthase (29, 54), S14 protein (54, 55), and L-pyruvate kinase (54, 56) in hepatocyte cultures. In contrast, saturated and/or monounsaturated long-chain fatty acids have no effect on the expression of these proteins. Thus, the pattern of regulation of mRNA levels for acetyl-CoA carboxylase by long-chain fatty acids appears to be similar to that of other lipogenic enzymes. Further studies have shown that the effects of polyunsaturated fatty acids on mRNA levels for stearoyl-CoA desaturase-1, L-pyruvate kinase, and S14 protein are accounted for by changes in gene transcription (52, 53, 55, 56). The *cis*-acting sequence in the L-pyruvate kinase gene that mediates the inhibitory effect of polyunsaturated fatty acids on L-pyruvate kinase transcription maps to a hepatocyte nuclear factor-4 (HNF-4) binding site in the 5′-flanking region of the gene (56). Because this fatty acid regulatory element overlaps with sequences that mediate stimulation of L-pyruvate kinase transcription of glucose and insulin, polyunsaturated fatty acids may inhibit L-pyruvate kinase transcription by interfering with the action of the glucose/insulin signaling pathway. In contrast to L-pyruvate kinase, sequences in the S14 gene that confer inhibition of transcription by polyunsaturated fatty acids do not co-localize with a glucose/insulin response element (55). The factor(s) that interacts with S14 fatty acid response element has not yet been identified. The above studies suggest that several distinct mechanisms are involved in mediating the effects of polyunsaturated fatty acids on transcription of genes involved in lipogenesis. Further analyses aimed at identifying the *cis*-acting sequences and *trans*-acting factors that mediate

the inhibition of acetyl-CoA carboxylase transcription by arachidonate will provide additional evidence demonstrating whether common or distinct mechanisms are involved in the transcriptional regulation of lipogenic genes by polyunsaturated fatty acids.

Previous work by other laboratories has shown that inhibitors of arachidonate metabolism are not effective in blocking the inhibitory effects of polyunsaturated fatty acids on expression of fatty acid synthase and glucose-6-phosphate dehydrogenase in liver (57, 58). Thus, products of eicosanoid synthesis do not appear to be involved in mediating the actions of polyunsaturated fatty acids on lipogenic enzyme expression. The identity of the active intermediate that mediates the effects of arachidonate on acetyl-CoA carboxylase transcription remains to be determined.

Another significant finding of the present report is that the medium-chain fatty acids, hexanoate and octanoate, are potent and selective inhibitors of T3-induced acetyl-CoA carboxylase expression in chick embryo hepatocytes (Table 2, Figs. 3 and 4). As with arachidonate, the effects of hexanoate and octanoate on the expression of acetyl-CoA carboxylase are mediated primarily by a transcriptional mechanism. The ability of carnitine to partially reverse the inhibition of acetyl-CoA carboxylase mRNA levels caused by octanoate suggests that the active agent is a metabolite derived from octanoate (Fig. 3B). This metabolite is likely to be an acyl-CoA containing six or eight carbons because 2-bromooctanoate was more effective than octanoate in inhibiting acetyl-CoA carboxylase expression (Fig. 3A). However, we cannot rule out the possibility that the active metabolite is derived from the metabolism of a six to eight carbon acyl-CoA. 2-Bromooctanoate inhibits and carnitine stimulates β-oxidation in hepatocytes (24, 47, 48). Thus, the effects of 2-bromooctanoate and carnitine on acetyl-CoA carboxylase expression may not necessarily be due to changes in the level of an acyl-CoA containing six to eight carbons but instead may be mediated by another metabolite whose concentration is controlled by rates of β-oxidation.

The mechanism(s) by which hexanoate and octanoate inhibit the T3-induced increase in transcription of acetyl-CoA carboxylase is presently unknown. Competitive interactions between these fatty acids and T3 for binding to the nuclear T3 receptor are not involved in mediating this response because hexanoate and octanoate have little or no effect on nuclear T3 binding activity in chick embryo hepatocytes (44). What are other possible mechanisms through which hexanoate and octanoate could inhibit transcription of acetyl-CoA carboxylase? One possibility is that the active metabolite of hexanoate and octanoate directly binds to the nuclear T3 receptor and regulates its function or interacts with a nuclear protein that modulates the transcriptional activity of the nuclear T3 receptor. For example, the active inhibitor may be a ligand for an as yet to be described orphan receptor that dimerizes with the nuclear T3 receptor. Another possible mechanism is that the active metabolite of hexanoate and octanoate triggers a phosphorylation/dephosphorylation cascade which causes an alteration in the phosphorylation state of the nuclear T3 receptor or a protein that modulates the activity of the nuclear T3 receptor. Protein phosphorylation is thought to play a role in regulating T3 action because protein kinase inhibitors inhibit and protein phosphatase inhibitors enhance the effects of T3 on gene transcription (59, 60).

Hexanoate and octanoate also inhibit transcription of fatty acid synthase and malic enzyme in chick embryo hepatocytes (44). In contrast to our results for acetyl-CoA carboxylase, 2-bromooctanoate stimulates the expression of fatty acid synthase and malic enzyme in the presence of T3. The reason for the different effects of 2-bromooctanoate on acetyl-CoA carboxylase relative to fatty acid synthase and malic enzyme is not clear. Possibly, different mechanisms are involved in mediating the inhibition of transcription of lipogenic enzymes by 2-bromooctanoate and octanoate.

Several lines of evidence indicate that additional factors besides glucose, insulin, T3, and glucagon are involved in mediating diet-induced changes in acetyl-CoA carboxylase expression. For example, alterations in acetyl-CoA carboxylase transcription caused by feeding previously starved chicks a high-carbohydrate, low-fat diet are significantly greater than those observed in chick embryo hepatocytes caused by manipulating the concentrations of glucose, insulin, T3, and glucagon in the culture medium (14, 18). A role for additional factors is also indicated by the observation that changes in the levels of insulin, T3, and glucagon in the blood caused by starvation/refeeding are substantially smaller than those that are needed to maximally regulate acetyl-CoA carboxylase transcription in chick embryo hepatocytes (17). Hexanoate and octanoate per se are not likely to play a role in mediating diet-induced changes in acetyl-CoA carboxylase transcription because these fatty acids are not present in significant amounts in cellular or dietary lipids (61). Nevertheless, the potent, rapid, and selective effects of hexanoate and octanoate on acetyl-CoA carboxylase transcription suggest that the active metabolite(s) mediating the action of these fatty acids may play a role in the physiological regulation of this enzyme. For example, the concentration of the active metabolite may be regulated by an unidentified humoral factor that is involved in mediating changes in acetyl-CoA carboxylase transcription caused by nutritional manipulation. To test this hypoth-

esis, the identity of the active intermediate must be further defined. One approach to this problem is to trace the intracellular signaling pathway for hexanoate and octanoate from target sequences in the acetyl-CoA carboxylase gene to the active metabolite. Studies aimed at characterizing the *cis*-acting elements mediating the effects of hexanoate and octanoate on acetyl-CoA carboxylase transcription in hepatocytes are currently in progress.

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