Metabolic Fate of Long-Chain Unsaturated Fatty Acids and Their Effects on Palmitic Acid Metabolism and Gluconeogenesis in Bovine Hepatocytes

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ABSTRACT

The objectives were to determine the metabolic fate of different long-chain fatty acids, and their effects on palmitic acid metabolism and gluconeogenesis in bovine hepatocytes. Hepatocytes were isolated from four ruminating calves and exposed in suspension for 3 h to one of the following treatments: 1 mM palmitic acid (1C16), 2 mM palmitic acid (2C16), or 1 mM palmitic acid plus either 1 mM oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosapentaenoic (C20:5), or docosahexaenoic acid (C22:6). Oxidation of [1-¹⁴C]palmitic acid or one of the [1-¹⁴C]-labeled treatment fatty acids to CO₂ or incorporation into cellular triglycerides (TG), phospholipids, cholesterol, and cholesterol esters were measured. Rates of oxidation to CO₂ were 3- to 4-fold higher for C22:6 than for other fatty acids, with the exception of C20:5, which had intermediate rates of oxidation to CO₂. In general, treatments 2C16 and C18:1 yielded the highest rates of incorporation into most cellular lipids, whereas the polyunsaturated fatty acids were poor substrates for incorporation into cellular lipids. The most pronounced change was a large reduction of polyunsaturated fatty acid incorporation into cellular TG compared to 1C16, 2C16, and C18:1. The unsaturated fatty acids also influenced palmitic acid metabolism. The addition of C20:5 yielded the highest rates of palmitic acid oxidation to CO₂ followed by addition of C18:1 and C22:6. Treatments containing polyunsaturated fatty acids decreased palmitic acid metabolism to TG and total cellular lipids compared with treatments 2C16 and C18:1. Rates of gluconeogenesis from propionate were significantly higher for the treatment containing C18:1. Long-chain fatty acids vary in their routes of metabolism and influence palmitic acid metabolism and gluconeogenesis in bovine hepatocytes. (**Key words:** fatty acids, hepatic metabolism, gluconeogenesis)

Abbreviation key: ASP = acid-soluble products, CPT-1 = carnitine palmitoyltransferase-I, DGAT = diacylglycerol acyltransferase, PUFA = polyunsaturated fatty acids, TG = triglyceride.

INTRODUCTION

Increasing nutrient demands of the fetus combined with depressed feed intake and dramatic endocrine changes results in NEFA mobilization from adipose tissue for most dairy cows beginning a few weeks prepartum (Grummer, 1995). The liver extracts NEFA proportional to their concentration in blood and is the major site of their metabolism (Bruss, 1993). Once in the liver, NEFA can be: 1) esterified and secreted as very-low density lipoproteins; 2) esterified and stored intracellularly; 3) oxidized completely to CO₂; or 4) oxidized partially to acetate or ketone bodies. The capacity of the ruminant liver to secrete triglycerides (TG) as very-low density lipoproteins is more limited than are other species (Kleppe et al., 1988; Pullen et al., 1990). Therefore, during times of increased NEFA mobilization and subsequent hepatic uptake, cows are susceptible to excess TG accumulation in the liver, also referred to as hepatic lipidosis. This syndrome has been linked to many metabolic disorders and decreased hepatic function (Grummer, 1993; Strang et al., 1998). Therefore, understanding the mechanisms involved in the etiology of hepatic lipidosis and identifying practices to reduce its severity will improve cow health and productivity and reduce the cascade of problems that commonly characterize the periparturient dairy cow.

In addition to increased blood NEFA concentrations, rates of gluconeogenesis are increased in early lactation in an attempt to meet glucose and energy requirements (Aiello et al., 1984). It has long been known that fatty acids stimulate gluconeogenesis in a variety of species (Williamson et al., 1966). In support of this, 2 mM oleic acid increased gluconeogenesis from propionate in goat hepatocytes in suspension compared with a control containing no fatty acids (Aiello and Armenatono, 1988). Rates of gluconeogenesis were not affected during short-term exposure (3 h) of monolayer cultures of bo-

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vine hepatocytes to media containing either oleic acid or a physiological mixture of fatty acids when no hormones were present (Cadorniga-Valino et al., 1997; Strang et al., 1998). However, to our knowledge, a direct comparison of the effects of different long-chain fatty acids on gluconeogenesis has not been tested in any species.

Because of the problems associated with hepatic lipodosis and suboptimal rates of gluconeogenesis, identification of ways to alleviate hepatic lipodosis and increase rates of gluconeogenesis is important. Most *in vivo* studies have focused on dietary manipulations to improve energy or nutrient balances in the periparturient dairy cow [for review, see Grummer (1995)]. A few studies have addressed feeding fat to periparturient dairy cows (Skaar et al., 1989; Grum et al., 1996; Douglas et al., 1998), and many *in vitro* studies address how hormones or different substrates affect hepatic metabolism. However, neither *in vivo* nor *in vitro* studies have compared the effects of different fatty acids on hepatic metabolism.

In the past several decades, research in rodents has shown that individual fatty acids may act to regulate cellular metabolism. Polyunsaturated fatty acids (PUFA), especially the *n*-3 PUFA, decrease lipogenesis and esterification and increase oxidation of fatty acids in the rodent liver. Therefore, the overall effect of PUFA is to partition fatty acids towards oxidation and away from production of TG and other esterified products. In support of this concept, both *in vivo* and *in vitro* studies show decreased cellular TG accumulation when exposing rodent hepatocytes to PUFA (Nicolosi et al., 1976; Lamb et al., 1977; Kabir and Ide, 1996; Ikeda et al., 1998; Kumamoto and Ide, 1998). However, the effects of different long-chain fatty acids on hepatic metabolism and the preference of different fatty acids as substrates for specific metabolic pathways in the ruminant liver is unknown. Therefore, the objectives of this study were to determine the hepatic metabolism of different long-chain fatty acids and their effects on palmitic acid metabolism and gluconeogenesis in ruminant hepatocytes.

MATERIALS AND METHODS

Reagents

Sodium thiopental was purchased from Abbott Laboratories (North Chicago, IL), Beuthanasia-D Special from Schering-Plough (Union, NJ), [1-¹⁴C]palmitic, [1-¹⁴C]oleic, [1-¹⁴C]linoleic, [1-¹⁴C]linolenic, [1-¹⁴C]eicosapentaenoic, [1-¹⁴C]docosahexaenoic, and [2-¹⁴C]propionic acids from American Radiolabeled Chemicals, Inc. (St. Louis, MO); sodium salts of palmitic, oleic, linoleic, linolenic, eicosapentaenoic, docosahexaenoic, and propionic acid and Type IV collagenase from Sigma Chemi-

cal (St. Louis, MO); and bovine serum albumin from Interger (Purchase, NY). All other chemicals were cell culture grade and the highest available purity from Sigma Chemical. Perfusion and wash media were as previously described (Donkin and Armentano, 1993). The incubation medium was Dulbecco's modified Eagle's medium, containing: 10 mM HEPES, 2.5 mM propionic acid, 0.5 mM L-carnitine, 4 mM L-glutamine, and 46 mM NaHCO₃.

Hepatocytes and Treatments

Four Holstein bull calves were weaned 5 wk after birth and then fed a diet consisting of calf starter and alfalfa-grass mixed hay *ad libitum*. At approximately 10 wk of age, the calves were anesthetized with sodium thiopental (1.5 g), the caudate process of the liver was removed, and hepatocytes were isolated as previously described (Donkin and Armentano, 1993). After removal of the caudate process, the calves were euthanized with 10 ml of Beuthanasia-D Special. Approximately 8 to 12 mg dry weight of hepatocytes in suspension were placed in 25-ml Erlenmeyer flasks containing 2.5 ml of incubation medium and one of the following treatments: 1 mM palmitic acid (1C16), 2 mM palmitic acid (2C16), or 1 mM palmitic acid plus either 1 mM oleic (C18:1), 1 mM linoleic (C18:2), 1 mM linolenic (C18:3), 1 mM eicosapentaenoic (C20:5), or 1 mM docosahexaenoic acid (C22:6). All fatty acids were bound to albumin in a 4:1 molar ratio. For each of the above treatments, two sets of triplicate flasks contained [1-¹⁴C]palmitic acid and another two sets of triplicate flasks were labeled with the [1-¹⁴C]fatty acid other than palmitic acid. An additional set of triplicate flasks was labeled with [2-¹⁴C]propionate to measure the conversion of propionic acid to glucose in the presence of the fatty acid treatments. Flasks were incubated in water baths at 37°C for 3 h and shaken at 40 strokes/min.

Measurements and Analysis

In two sets of flasks containing the radiolabeled fatty acids or radiolabeled palmitic acid, 0.4 ml of perchloric acid was injected into the flask to terminate the incubation, and 0.2 ml of phenethylamine (Aldrich Chemical Company, Milwaukee, WI) was injected into a hanging centerwell containing filter paper to trap the CO₂. After 1 h, the centerwell was removed and radioactivity was measured. The media in the flasks were centrifuged at (2050 × *g*), and 2 ml of the supernatant was neutralized with 1.0 ml of 20% KOH. After centrifugation, the supernatant was tested for radioactivity as a measure of acid-soluble products (ASP). However, the ASP had falsely elevated counts in the treatments where the

PUFA contained the radiolabel. This was evident because flasks that were terminated immediately after addition of labeled PUFA had elevated counts in the ASP fraction that were similar to flasks terminated after the 3-h incubations. The erroneous values may have risen due to peroxidation of the radiolabeled PUFA or to impurities in the radiolabeled fatty acids. At most, 3% of the total radiolabel added to flasks could be accounted for in the ASP fraction. Therefore, the conversion of unsaturated fatty acids into ASP is not reported. The problems involving contamination of the ASP fraction with radiolabel were likely unique to the measurement of ASP and did not affect other measurements. The CO₂ values in the control flasks terminated immediately after addition of [1-¹⁴C]PUFA were also subtracted from the final CO₂ values. The control values for CO₂ were slightly elevated in the flasks containing [1-¹⁴C]PUFA compared with those containing [1-¹⁴C]palmitic acid, but these values were still less than 0.03% of the total radiolabel added and less than 10% of the final CO₂ values for all flasks. Control flasks for measurement of radiolabeled fatty acids incorporated into cellular lipids were similar to each other and at most comprised less than 1% of total incorporation into any cellular lipid measurement.

The other series of flasks containing each of the radiolabeled fatty acids was placed on ice to terminate the incubation. The medium was centrifuged at 2000 × *g*, and the supernatant was removed. The cell pellet was washed with Krebs buffer and frozen at -20°C until its analysis for cellular lipids. Lipids were extracted (Folch et al., 1957) and resuspended in 100 µl of chloroform:methanol (2:1). Twenty microliters was spotted on Adsorbosil Plus 1 silica gel plates (Alltech Associates Inc., Deerfield, IL), which were developed with petroleum ether:diethyl ether:glacial acetic acid (80:20:1). Spots corresponding to phospholipids, cholesterol, triglycerides, and cholesterol esters were visualized with 5% Rhodamine G in ethanol and scraped into scintillation vials for quantification of radiolabeled cellular lipids. For the flasks containing [2-¹⁴C]propionic acid, 0.5 ml of sulfuric acid was added to terminate the incubation, and the medium was frozen for later analysis of [¹⁴C]glucose as previously described (Mills et al., 1981). The method of LaBarca and Paigen (1980) was used to determine DNA concentrations.

Statistical Analysis

Data were analyzed by the Mixed Procedure of SAS (SAS, 1999). The model included fixed effects of treatment, random effects of calf, and the residual error term. For analysis of combined metabolism of palmitic acid and treatment fatty acids, means were calculated

for each treatment within the calf using the PROC MEANS procedure of SAS (1999). The means were then subjected to the mixed procedure of SAS. Any differences between treatments were determined using the PDIFF procedure of SAS. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Fatty acids are oxidized primarily in the mitochondria or the peroxisome. The process of β -oxidation in the mitochondria and peroxisome shortens fatty acids two carbons at a time starting with the carboxyl end of the fatty acid. Because the radiolabel on all fatty acids was on the carboxyl carbon, rates of oxidation or incorporation into cellular lipids, as measured in the current study, reflect the metabolism of the carboxyl carbon only and not the entire fatty acid.

Rates of oxidation to CO₂ were highest for C22:6 and intermediate for C20:5 (Table 1). Addition of C20:5 or C22:6 to the medium increased palmitic acid oxidation to CO₂ compared with the addition of other fatty acids with the exception of C18:1 (Table 2). Oxidation of palmitic acid to CO₂ was lowest when the media contained C18:2. Addition of C20:5 yielded the highest rates of palmitic acid oxidation to ASP and total oxidation (CO₂ + ASP) of palmitic acid. The combined total oxidation of palmitic acid and the respective treatment fatty acid to CO₂ was highest for treatments containing C22:6, C20:5, and C18:1 (Figure 1). Madsen et al. (1999) found C20:5, but not C22:6, increased oxidation of palmitic acid to ASP in cultured rat hepatocytes exposed to fatty acids for 4 h. With the same model, C20:5 yielded higher rates of oxidation to both CO₂ and ASP compared to C22:6 (Berge et al., 1999). Several studies have shown increased carnitine palmitoyltransferase-I (CPT-I) activity in rodents fed fish oil (Willumsen et al., 1993; Ikeda et al., 1998; Ide et al., 2000). However, these studies employed long-term feeding of fatty acids as opposed to the short-term incubations of the current study. It is unknown if fatty acid metabolism can be regulated significantly through changes in enzyme abundance within a 3-h period, but this possibility seems unlikely. Substrate competition for intracellular pathways involved in oxidation and esterification of fatty acids may have been responsible for the observed differences in metabolism of long-chain fatty acids.

As shown in Table 1, rates of label incorporation into cellular TG were highest for 2C16 and C18:1 with intermediate rates for 1C16. The combined metabolism of palmitic acid and treatment fatty acids into cellular TG was nearly double for the treatment containing C18:1 than for any other treatment. All PUFA were poor substrates for TG synthesis; PUFA were incorporated into

Table 1. Metabolism of [1-¹⁴C]fatty acids to CO₂ and cellular lipids.

Product	1C16	2C16	C16 + 18:1	C16 + 18:2	C16 + 18:3	C16 + 20:5	C16 + 22:6	SEM
	(pmol/μg DNA × 3 h)							
CO ₂	77.4 ^c	68.9 ^c	99.8 ^c	50.4 ^c	56.6 ^c	155.0 ^b	283.2 ^a	37.5
Triglycerides	202.0 ^b	307.8 ^a	287.5 ^a	19.4 ^c	60.1 ^c	21.7 ^c	46.9 ^c	39.0
Phospholipids	104.9 ^{bcd}	128.6 ^{ab}	144.6 ^a	81.2 ^d	117.9 ^{abc}	78.1 ^d	90.5 ^{cd}	16.9
Cholesterol	31.0 ^{bcd}	36.4 ^{bc}	84.5 ^a	42.9 ^b	40.8 ^b	24.6 ^{cd}	22.9 ^d	7.3
Cholesterol esters	36.2 ^b	58.2 ^a	74.4 ^a	20.2 ^b	36.5 ^b	25.4 ^b	29.5 ^b	17.1
Total cellular lipid ¹	373.9 ^b	530.6 ^a	565.4 ^a	158.7 ^{cd}	226.0 ^c	145.0 ^d	188.6 ^{cd}	41.5

^{a,b,c,d,e}Means within a row with unlike superscripts differ ($P < 0.05$).

¹Total cellular lipid = triglycerides + phospholipids + cholesterol + cholesterol esters.

cellular TG at 7 to 20% of the rates observed for 2C16 and C18:1. Similar results have been obtained from feeding PUFA to rodents. When compared to palm oil supplementation, liver TG concentrations in rodents decreased in response to supplemental PUFA (Fremont and Gozzelino, 1996; Kabir and Ide, 1996; Kumamoto and Ide, 1998). Although oils containing C18:2 and C18:3 decreased liver TG concentration in rodents compared with palm oil, the largest decrease resulted from feeding them fish oils, which contain high concentrations of C20:5 and C22:6 (Ide et al., 2000). The decrease in TG formation from PUFA in the studies cited is likely a result of PUFA regulation of enzymes involved in TG synthesis. Indeed, PUFA decrease the activity of enzymes involved in lipogenesis, including ATP-citrate lyase, acetyl CoA carboxylase, and fatty acid synthase (Toussant et al., 1981; Willumsen et al., 1993; Iritani et al., 1998). However, the ruminant liver only contributes about 5% to whole body lipogenesis (Ingle et al., 1972); thus the decrease in lipogenesis is probably a small contributor to the observed decrease in TG formation. A more plausible explanation may be changes in the enzymes responsible for esterification of free fatty acids to TG. Phosphatidate phosphohydrolase and diacylglycerol acyltransferase (**DGAT**) are responsible for the for-

mation of 1,2-diacylglycerol and TG, respectively. These enzymes are decreased by diets high in PUFA, especially C20:5 and C22:6 (Geelen et al., 1995; Fremont and Gozzelino, 1996; Cha et al., 1998). It is not known, however, whether acute exposure to PUFA, as in the current study, can affect enzyme activity.

Treatments C18:1, 2C16, and C18:3 yielded the highest rates of incorporation into cellular phospholipids, whereas C18:2, C20:5, and C22:6 were poor substrates for incorporation into cellular phospholipids (Table 1). Treatment 2C16 yielded the highest rates of palmitic acid metabolism to phospholipids, whereas addition of C18:2 yielded the lowest rates (Table 2). The combined metabolism of palmitic acid and treatment fatty acids to phospholipids was highest when the media contained C18:1 and lowest for the 1C16 treatment (Figure 1). In contrast to the current study, most studies involving laboratory animals show increased incorporation of C20:5 and C22:6 into phospholipids compared with saturated or monounsaturated fatty acids (Berge et al., 1999; Madsen et al., 1999). It could be speculated that the C20:5 and C22:6 decreased 1,2-diacylglycerol formation through their action on phosphatidate phosphohydrolase. Further regulation at DGAT would cause more 1,2 diacylglycerol to be shunted towards phospho-

Table 2. Effects of long chain fatty acids on metabolism of [1-¹⁴C]palmitic acid.

Product	1C16	2C16	C16 + 18:1	C16 + 18:2	C16 + 18:3	C16 + 20:5	C16 + 22:6	SEM
	(pmol/μg DNA × 3 h)							
CO ₂	74.9 ^{bc}	66.4 ^{cd}	87.3 ^{ab}	56.8 ^d	73.8 ^{bc}	96.2 ^a	94.2 ^a	14.7
Acid-soluble products	253.8 ^{bc}	203.7 ^{cd}	314.4 ^b	174.1 ^d	262.3 ^{bc}	417.0 ^a	289.2 ^b	50.0
Total oxidation ¹	328.6 ^{cd}	270.0 ^{de}	401.7 ^b	230.9 ^e	336.3 ^c	513.2 ^a	388.4 ^{bc}	62.7
Triglycerides	192.7 ^b	299.4 ^a	289.5 ^a	146.2 ^c	178.6 ^{bc}	219.6 ^b	195.0 ^b	48.5
Phospholipids	102.8 ^{bc}	126.5 ^a	108.8 ^b	81.0 ^d	89.8 ^{cd}	108.3 ^b	102.9 ^{bc}	15.1
Cholesterol	29.3 ^{bc}	34.7 ^b	34.6 ^b	28.9 ^{bc}	24.9 ^c	42.1 ^a	47.5 ^a	8.1
Cholesterol esters	36.2 ^b	47.6 ^a	41.0 ^{ab}	39.2 ^{ab}	38.8 ^b	37.5 ^b	37.6 ^b	10.4
Total cellular lipid ²	362.2 ^{bc}	507.2 ^a	474.1 ^a	295.0 ^d	330.9 ^{cd}	379.7 ^{bc}	392.2 ^b	70.5
Total metabolism ³	690.8 ^d	787.8 ^{bc}	875.8 ^{ab}	525.9 ^e	667.1 ^d	892.8 ^a	780.6 ^c	130.6

^{a,b,c,d,e}Means within a row with unlike superscripts differ ($P < 0.05$).

¹Total oxidation = CO₂ + acid-soluble products.

²Total cellular lipid = triglycerides + phospholipids + cholesterol + cholesterol esters.

³Total metabolism = total oxidation + total cellular lipid.

Figure 1. Combined metabolism of $[1-^{14}\text{C}]$ palmitic acid plus $[1-^{14}\text{C}]$ treatment fatty acids into metabolic products. Within a metabolic product, treatments with unlike letters differ ($P < 0.05$).

lipids rather than TG. The resultant scenario would yield decreased radiolabel incorporation into TG and small changes in radiolabel incorporation into phospholipids as was seen in the current study. Another explanation for alterations in fatty acid metabolism to phospholipids and TG may be the influence of diacylglycerol structure on its subsequent metabolism. Varying the fatty acid profile in the sn-1 or -2 positions alters the conformation of the diacylglycerol molecule (Applegate and Glomset, 1991) and could alter the channeling of diacylglycerol to phospholipids or TG. Changing the fatty acid moieties of diacylglycerol elicited large changes in DGAT activity in bovine subcutaneous adipose tissue (Lozeman et al., 2001). Similarly, different acyl-CoA substrates affected DGAT activity with C18:1 yielding a threefold increase among the various fatty acids tested (Lozeman et al., 2001).

In order for the radiolabeled carbon of the fatty acids to be incorporated into cellular cholesterol, the carbon must first be oxidized to acetyl CoA. The radiolabeled acetyl CoA can then leave the mitochondria as one of the TCA cycle intermediates or can escape after conversion to acetoacetate. Once in the cytosol, acetoacetate or acetyl CoA can be used as substrates for cholesterol synthesis. The rate of incorporation of C18:1 into cholesterol was nearly double the rate of any other treatment (Table 1). Although C22:6 and C20:5 were the poorest substrates for metabolism to cholesterol, addition of these two fatty acids resulted in the highest rates of palmitic acid metabolism to cholesterol (Table 2). Total fatty acid incorporation into cholesterol was highest when C18:1 was present in the media and lowest for the 1C16 treatment. Fatty acids can be incorporated into cholesterol esters through their metabolism to cholesterol or through direct esterification of the fatty acids to cholesterol. As shown in Table 1, the highest rates of metabolism to cholesterol esters were for C18:1 and

Figure 2. Effects of long-chain fatty acids on metabolism of $[2-^{14}\text{C}]$ propionic acid to glucose. Treatments with unlike letters differ ($P < 0.05$).

2C16. Similarly, the highest rates of palmitic acid metabolism to cholesterol esters, in addition to the 2C16 treatment, were when palmitic acid was incubated with C18:1 and to a lesser extent C18:2 (Table 2). The differences in metabolism of palmitic acid and various treatment fatty acids to cholesterol esters did not follow the same pattern as metabolism of fatty acids to cholesterol. Therefore, it is plausible that a large portion of the radiolabel in cholesterol esters resides in the fatty acid portion of the molecule. There were no differences in combined fatty acid metabolism to cholesterol esters.

Total palmitic acid metabolism was highest when incubated with C20:5 or C18:1 and lowest when incubated with C18:2 (Table 2). Differences in total metabolism of palmitic acid suggest that the uptake of palmitic acid into hepatocytes may have differed between treatments. Previous research in perfused ovine livers showed differences among uptakes of palmitic, oleic, and stearic acids (Thompson and Darling, 1975). However, no studies in ruminants have compared PUFA uptake to saturated fatty acids or tested if competition exists between fatty acids for transport into hepatocytes.

The effects of long-chain fatty acids on gluconeogenesis from propionic acid are shown in Figure 2. The only significant effect observed was for an increase in propionic acid metabolism to glucose for the treatment containing C18:1. Oleic acid increased gluconeogenesis from propionic acid in goat hepatocytes in suspension cultures during 1-h incubations containing 2 mM oleic acid compared with a control containing no fatty acids (Aiello and Armentano, 1988). However, the same study showed that increasing the concentration of oleic acid from 0 to 1 mM did not have an effect on gluconeogenesis from propionic acid in calf hepatocytes. The effects

of 2 mM of oleic acid on gluconeogenesis in calf hepatocytes was not tested. Cadorniga-Valino et al. (1997) observed decreased gluconeogenesis when bovine hepatocytes were exposed to oleic acid from 45 to 48 h after initial plating. The effects of fatty acids on gluconeogenesis were pooled across treatments containing additions of insulin or glucagon to the media, however. No statistical comparisons were made between incubations with or without fatty acids in the absence of hormones. In control incubations without hormone additions, conversion of propionic acid to glucose appeared to be similar between incubations containing 0 or 2 mM oleic acid. Strang et al. (1998) did not observe a change in gluconeogenesis when hepatocytes were exposed to a physiological mixture of fatty acids from 48 to 51 h after initial plating. Considering all of the data, the effect of oleic acid on gluconeogenesis from propionic acid in ruminant hepatocytes appears to be modest, and differences between 1- to 3-h incubations in freshly isolated hepatocytes and 48-h monolayer cultures may have contributed to some of the discrepancies between studies.

Oleic acid may act to increase gluconeogenesis through its own catabolism. Chow and Jesse (1992) showed that inhibition of CPT-I leads to decreased gluconeogenesis from propionic acid in sheep hepatocytes. However, the means by which the inhibition of mitochondrial β -oxidation decreases gluconeogenesis is not known. Furthermore, it is not known if β -oxidation in the peroxisome has a similar role in modulating gluconeogenesis. Previous studies have shown a difference in peroxisomal substrate preference between fatty acids varying in chain length and number of double bonds (Alexson and Cannon, 1984). In the current study, total fatty acid oxidation was highest for treatments containing C20:5, C22:6, and C18:1. If rates of oxidation were linked to gluconeogenesis, we would expect increased rates of gluconeogenesis for all three treatments; the current findings contradict this. It could be speculated, however, that C18:1 may have higher rates of mitochondrial oxidation, and subsequently higher rates of gluconeogenesis when compared to C20:5 and C22:6, which are preferred substrates for peroxisomal oxidation (Christensen et al., 1986). Since gluconeogenesis is an important metabolic process of ruminants, future research should identify the regulatory mechanisms of different long-chain fatty acids on carbohydrate metabolism.

CONCLUSIONS

Overall, the results of the current study demonstrate significant differences in hepatic metabolism of individual long chain fatty acids and in their effects on other fatty acids such as palmitic acid. Specifically, fatty acids

found in high concentrations in fish oils increased oxidation, and PUFA decreased the incorporation of fatty acids into cellular TG. These findings show a potential for PUFA to moderate the development of hepatic lipodosis. Because individual fatty acids influenced lipid and carbohydrate metabolism, the profile of fatty acids reaching the liver may be important modulators of hepatic energy metabolism.

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