Influence of Dietary Fatty Acids on Liver and Adipose Tissue Lipogenesis and on Liver Metabolites in Meal-Fed Rats[1]

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ABSTRACT Rats were trained to eat a fat-free high carbohydrate diet from 800 to 1100 hours each day. After adaptation to meal-eating, the fat-free diet was supplemented with 8% methyl stearate ($C_{18:0}$) or 3% methyl linoleate ($C_{18:2}$) for 7 days. Relative to the fat-free group, hepatic utilization of acetate unit equivalents (C_2 units) for fatty acid synthesis per mg soluble protein by the $C_{18:0}$ group was not significantly altered, whereas $C_{18:2}$ supplementation significantly depressed hepatic fatty acid synthesis. Supplemental $C_{18:2}$ also caused a significant decline in liver fatty acid synthetase and acetyl CoA carboxylase while fat-free and $C_{18:0}$ groups displayed similar enzyme activities. Within a treatment, C_2 unit utilization for in vivo fatty acid synthesis was identical to that of acetyl CoA carboxylase and fatty acid synthetase activities in vitro. Therefore, shortly after a meal, the hepatic activities of these two enzymes appear to be functioning at near capacity. $C_{18:2}$ supplementation to the fat-free diet for 7 days caused a 25% decline in glucokinase and pyruvate kinase activities, but only pyruvate kinase was significantly depressed. In contrast, citrate cleavage enzyme and fatty acid synthetase were both significantly reduced in activity by 50%. Plasma unesterified fatty acid levels in rats fed $C_{18:2}$ for 5 days were not significantly elevated prior to a meal, although dietary $C_{18:2}$ did cause a fourfold rise in plasma free linoleate. Quantitation of long chain acyl CoA esters in freeze-clamped liver tissue of rats fed fat-free or fat-free plus 3% $C_{18:2}$ or $C_{18:3}$ diets revealed no concentration differences between treatments either before or after a meal. Similarly, lactate and pyruvate concentrations as well as the lactate:pyruvate ratios were not significantly changed by dietary $C_{18:2}$ or $C_{18:3}$. The inhibitory effects of $C_{18:2}$ or $C_{18:3}$ appear not to be mediated through changes in total plasma free fatty acid levels, in total hepatic long chain acyl CoA concentration or in hepatic cytosolic redox state. J. Nutr. *107:* 1277–1287, 1977.

KEY INDEXING WORDS fatty acid synthesis · rat liver · linoleate · stearate · hepatic metabolites

Lipogenic enzymes and rates of fatty acid synthesis in rat and mouse liver appear to be regulated not only by the level of dietary fat (1, 2), but also by the type of dietary fat (1–9). Wiegand et al. (1) observed that the addition of 15% cocoa butter to a fat-free diet was necessary to achieve a degree of inhibition of rat liver fatty acid synthetase comparable to that

Received for publication August 2, 1976.

[1] Supported in part by NIH AM 18957 and by the Fats and Proteins Research Foundation. Michigan Agricultural Experiment Station Journal Article No. 7753.

obtained with 2.5% safflower oil supplementation. Because diets differing in fat level and lipid composition make it difficult to attribute specific regulatory mechanisms to specific lipid components, the addition of purified methyl esters of various fatty acids has been adopted to investigate the regulatory mode of action of dietary fatty acids (3–9). Methyl esters of long chain fatty acids are demethylated in the gut, but the absorbability of the fatty acid varies greatly with the degree of unsaturation (9).

The high activity of hepatic fatty acid synthetase in mice fed a fat-free diet could be rapidly lowered by the addition of 2% methyl linoleate, whereas 2% methyl palmitate supplementation had no inhibitory influence (4). Marked differences exist in absorbability between methyl linoleate and palmitate (3, 9), but even when the absorbed amount of both acids was similar, palmitate still did not reduce fatty acid synthetase activity or in vivo rates of fatty acid synthesis.[4] These observations with purified esters of fatty acids concur with the differential effects found with the natural fats, cocoa butter, and safflower oil (1).

In sharp contrast to in vivo studies with various dietary fats, stearate and palmitate consistently exert the greatest inhibitory influence on fatty acid synthesis of isolated cells (10–13). However, the little information available for stearate effects on in vivo lipogenesis is confounded by the poor absorbability of methyl stearate relative to unsaturated acids (9). The current report has compared the effects of methyl stearate and methyl linoleate on fatty acid synthesis when similar amounts of both acids had been absorbed. Frequently the differential effects of various dietary fats on adipose tissue lipogenesis are overlooked and yet in the rat, adipose tissue accounts for a very large portion of total body fatty acid synthesis (14). In the present report, the influence of dietary stearate and linoleate on lipogenesis has been investigated in both rat liver and adipose tissue.

Both fasting and feeding of high fat diets are associated with low rates of liver and adipose tissue fatty acid synthesis, and are accompanied by increased plasma non-esterified fatty acid levels, elevated hepatic long chain acyl CoA concentrations and reduced liver cytosolic NAD:NADH ratios (15–18). During periods of fasting, polyunsaturated fatty acids purportedly undergo greater rates of mobilization from adipose tissue and oxidation by the liver than saturated fats (15, 16). If linoleate and linolenate turn over more rapidly, then changes in the previously mentioned parameters may contribute to reducing rates of hepatic fatty acid synthesis.

As an approach to explaining the efficient inhibitory mechanism of polyunsaturated fatty acids on rat liver fatty acid synthesis, the concentration and composition of plasma unesterified fatty acids, the level of hepatic long chain acyl CoA esters, and liver cytosoloic redox state were quantitated in rats fed fat-free or fat-free plus 3% linoleate or linolenate diets.

METHODS

General animal handling. Sprague-Dawley rats [5] were housed individually in stainless steel cages and had free access to water. Prior to the experimental phase, all rats were adapted to a 3 hour per day meal-eating regimen (access to food 0800–1100 hours). During this phase, all rats received the basal diet in table 1 except that 2% safflower oil replaced 2% carbohydrate. Following adaptation to meal-eating, the diet was switched to the fat-free basal diet (table 1) and the rats were fed for an additional 7 days. The basal diet contained 1 g butylated hydroxytoluene (BHT)/kg diet. On the eighth day, rats were allotted to the treatments as described for each experiment. Methyl esters [6] of stearate ($C_{18:0}$), linoleate ($C_{18:2}$) and linolenate ($C_{18:3}$) were supplemented to the fat-free diet.

Experiment 1. The effects of dietary methyl $C_{18:0}$ and $C_{18:2}$ on rat liver and adipose tissue fatty acid synthesis were examined. When low levels of fat are sup-

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[4] Clarke, S. D., Romsos, D. R. & Leveille, G. A. (1976) Specific control of hepatic lipogenesis exerted by dietary linoleate and linolenate. Am. Oil Chem. Soc. *53*, 146A (Abstr.).

[5] Spartan Research Animals, Haslett, Michigan.

[6] Stated to be 99+% pure by Sigma Chemical Company, St. Louis, Missouri.

plemented to a fat-free diet the effects are independent of carbohydrate intake (3, 7). Therefore, after the fat-free feeding period, 10 rats per treatment were randomly allotted to the following dietary treatments: a) fat-free diet (FF); b) FF + 8% $C_{18:0}$; or c) FF + 3% $C_{18:2}$. Because of the poor absorbability (9) of $C_{18:0}$, an 8% level of supplementation was necessary to achieve an amount absorbed equivalent to 3% $C_{18:2}$. On the first day of fat addition, rats were allotted 90% of their average daily intake during the fat-free feeding period. The 90% value was chosen to avoid a reduction in total food intake among the $C_{18:0}$ rats relative to the $C_{18:2}$ rats, because of the large amount of additional $C_{18:0}$ necessary for supplementation. Food allotments were increased by 0.5 g increments when all rats were able to finish the daily allotted food in 3 hours. The dietary fatty acids were added to eight meals as 8% $C_{18:0}$ or 3% $C_{18:2}$ of the daily fat-free diet allotted.

One hour after completion of the eighth meal, in vivo liver and adipose tissue lipogenic rates were quantitated by the amount of ^3H_2O [7] incorporated into fatty acids. Each rat was injected intraperitoneally with 1.5 mCi ^3H_2O in 0.5 ml physiological saline and killed 15 minutes post-injection. The rate of incorporation of ^3H_2O into hepatic fatty acids has been determined to be linear for 5 to 60 minutes after injection (19, 20). After killing the rats, the livers were removed and placed in cold saline. After weighing, a 1.0 g sample was homogenized for enzyme assay while the remainder was homogenized in an equal volume of water. Aliquots of the homogenate (0.5 ml) were removed for saponification in ethanolic KOH (30%). After extraction of the nonsaponifiable matter, the mixture was acidified and extracted with three 5.0 ml volumes of petroleum ether. The scintillation fluid contained 4.0 g scintillant [8] dissolved in 230 ml absolute ethanol and toluene to 1 liter. All visible, removable adipose tissue on the carcass was collected, weighed and homogenized in an equal volume of water. Extraction of fatty acids was handled in a manner similar to liver tissue. Plasma samples were collected at time of kill in order to determine the specific activity of the plasma. This was used as an index of body water specific activity which permitted calculating μmoles of ^3H incorporated into fatty acids per g tissue. C_2 unit incorporation into fatty acids by liver and adipose was calculated from ^3H_2O incorporation (21).

TABLE 1

Fat-free basal diet composition

Ingredient	Parts
Glucose	72.0
Casein	20.0
Fiber[1]	3.0
D, L-methionine	0.3
Choline chloride	0.3
Vitamin mix[2]	0.4
Mineral mix[3]	4.0
	100.00

[1] Solka-floc. Brown Company, Berlin, New Hampshire. [2] Vitamin mixture describe by Yeh & Leveille (1969) J. Nutr. *98*, 356–366. [3] Mineral mix described by Leveille & O'Hea (1967) J. Nutr. *93*, 541–545.

The activities of hepatic fatty acid synthetase and acetyl CoA carboxylase (EC 6.4.1.2) were quantitated from a 100,000 × *g* (40 minutes) supernatant of a homogenate containing 0.15 M KCl, 1.0 mM $MgCl_2$, and 10 mM n-acetyl-cysteine, pH 7.6 (22, 23). Protein content of the 100,000 × *g* supernatant was determined by the Lowry method (24). The data analyzed by an analysis of variance for a completely randomized design and treatment differences were determined by Tukey's "*t*" test procedure (25).

Experiment 2. Long chain acyl CoA esters were quantitated in the liver of rats before and after the sixth meal of either the FF-diet or a FF and unsaturated fatty acid diet. $C_{18:2}$ was supplemented in the before-meal trial and $C_{18:3}$ fed in the after-meal trial. Rats were paired for body weight and food intake and fed the FF diet or FF diet plus 3% $C_{18:2}$ or FF diet plus 3% $C_{18:3}$ for six meals. Before the sixth meal, rats were killed by decapitation and blood samples were collected rapidly for plasma nonesterified fatty acid composition and concentration analyses.

[7] New England Nuclear, 575 Albany Street, Boston, Massachusetts.
[8] Omniflor. New England Nuclear, Pilot Chemicals Division, 575 Albany Street, Boston, Massachusetts.

Immediately following the blood collection, the livers were exposed and tissue frozen in situ using the freeze-clamp technique (26). The time required for blood and tissue collection was less than 20 seconds. One hour after the sixth meal, rats were stunned by a blow to the head, livers rapidly exposed and frozen tissue samples collected in less than 10 seconds by the freeze-clamp method (26). In both trials the liver samples were processed as described by Romsos et al. (26). Long chain acyl CoA concentrations were quantitated by the recycling procedure of Allred and Guy (27).

Plasma samples were extracted for nonesterified fatty acids (28). The fatty acids were methylated using borotrifluoride and methanol (29), and analyzed by gas-liquid chromatography (29). Results were expressed as μg per ml plasma.

Treatment differences were determined by a paired "*t*" test analysis (25).

Experiment 3. Three trials were conducted to examine the effects of $C_{18:2}$ and $C_{18:3}$ supplementation on the concentration of liver lactate and pyruvate, and lactate:pyruvate ratio. The preliminary experimental protocol was the same as that previously described. In trials 1 and 2 eight pairs of rats, matched for body weight and food intake, were fed the FF-diet, FF diet plus 3% $C_{18:2}$ (trial 1) or FF diet plus 3% $C_{18:3}$ (trial 2) for six meals. Trial 3 involved 10 pairs of rats and fed the FF-diet or the FF-diet plus 3% $C_{18:2}$ for seven meals.

One hour after the last meal, the rats were stunned by a blow on the head, the liver was rapidly exposed and frozen in situ using the freeze-clamp technique (26). Liver samples were processed by the method of Romsos et al. (26), and lactate and pyruvate quantitated enzymatically with lactate dehydrogenase by the procedures of Bucher et al. (30) and Hohorst (31), respectively. From the concentrations of lactate and pyruvate, the ratios of lactate:pyruvate and liver cytosolic NAD:NADH were quantitated (17). Statistically significant differences were determined by a paired "*t*" test analysis (25).

Experiment 4. To obtain informtaion on the effect of $C_{18:2}$ on glycolytic enzyme activities relative to lipogenic enzyme activities in liver, 10 pairs of rats were fed the FF diet or the FF diet plus 3% $C_{18:2}$ for eight meals. The glycolytic enzymes chosen for analysis were glucokinase (EC 2.7.1.2) and pyruvate kinase (EC 2.7.1.40), while citrate cleavage enzyme (EC 1.1.1.40) and fatty acid synthetase were selected as lipogenic enzymes. One hour following completion of the eighth meal, the rats were killed by decapitation and the livers were rapidly removed and placed in cold homogenizing buffer. Approximately 1 g of liver was homogenized in 0.15 M KCl, 1.0 mM $MgCl_2$, 10 mM n-acetyl-cysteine, and 0.5 mM dithiothrietol (pH 7.6). After 40 minutes of centrifugation at 100,000 × *g*, the supernatant was utilized for enzymatic analysis. Glucokinase, pyruvate kinase, citrate cleavage enzyme, and fatty acid synthetase were assayed by the method of Pilkis (32), Bergmeyer (33), Srere (34) and Gibson and Hubbard (22), respectively. All enzymes were assayed at 37° and soluble protein was quantitated by the Lowry procedure (24). A paired "*t*" test analysis was utilized to determine treatment differences (25).

RESULTS

$C_{18:0}$ versus $C_{18:2}$ effects on lipogenesis. The average daily consumption of the fat-free diet over the 7-day feeding period was not significantly different among treatments. The differences in diet consumption were a consequence of the random design (see methods) and not an effect attributable to dietary fatty acid supplementation. Because of the poor apparent absorbability (9) of methyl $C_{18:0}$, the level of daily supplementation had to be increased to 8% to reach an amount absorbed comparable to 3% $C_{18:2}$ (table 2). When enzyme activities were expressed as acetate units (C_2 units) utilized/min/mg soluble protein at 37°, fatty acid synthetase and acetyl CoA carboxylase displayed comparable rates. Supplementing 3% $C_{18:2}$ to the fat-free diet significantly inhibited the activities of fatty acid synthetase and acetyl CoA carboxylase, whereas comparable amounts of absorbed $C_{18:0}$ exerted no inhibitory action on the activity of either enzyme (table 2).

From the incorporation of ^3H into fatty acids, the amount of C_2 units utilized for

TABLE 2

Fatty acid synthesis in rats fed a fat-free diet plus 8% $C_{18:0}$ of 3% $C_{18:2}$ (experiment 1)

Parameter[1]	Dietary fatty acid		
	Fat-free (FF)	FF + 8% $C_{18:0}$	FF + 3% $C_{18:2}$
Final body wt., g	193 ±8[6,a]	188 ± 7[a]	192 ± 5[a]
Total wt. gain, g[2]	24 ±2[a]	26 ± 2[a]	27 ± 1[a]
Food intake, g/day[3]	14.0±0.7[a]	14.1± 0.9[a]	13.7± 0.4[a]
Fatty acid absorbed, mg/day[4]	—	395 ±24[a]	366 ±22[a]
Liver:			
Wt., g	9.2±0.4[a]	9.8± 0.4[a]	9.7± 0.4[a]
Soluble protein, mg/g liver	90 ±3	90 ± 6	92 ± 4
Fatty acid synthetase[5]	10 ±0.6[a]	12 ± 0.8[a]	6 ± 0.8[b]
Acetyl CoA carboxylase[5]	13 ±1.5[a]	10 ± 1.5[a]	5 ± 1.5[b]
In vivo FA synthesis:			
nmoles C_2 units/min/mg protein	9.1±0.7[a]	11.4± 0.7[a]	6.1± 0.7[b]
μmoles C_2 units, total liver/min	7.4±0.5[a]	10.2± 0.5[b]	5.2± 0.5[c]
Adipose:			
Total wt., g	6.8±0.8[a]	5.4± 0.8[a]	6.1± 0.8[a]
In vivo FA synthesis:			
μmoles C_2 units/min/g	2.7±0.3[a]	2.6± 0.3[a]	2.0± 0.3[a]
μmoles C_2 units/min/total	15.2±2.3[a]	11.6± 2.3[a]	13.6± 2.3[a]
Total FA synthesis distribution:			
% Liver	31 ±3[a]	49 ± 5[b]	32 ± 4[a]
% Adipose	69 ±3[a]	51 ± 4[b]	68 ± 3[a]

[1] Mean±SEM; n = 10 average initial body weight was 165±7 g. [2] Weight gain during 7-day period of ester supplementation. [3] Fat-free basal diet, does not include ester. [4] Calculated from absorbability values determined by Clarke et al. (9). [5] Acetate unit equivalents utilized nanomoles/min/mg protein at 37°. [6] Those values with different superscripts are significantly different ($P < 0.05$) using Tukey's "t" test (23).

fatty acid synthesis was calculated (21) and was expressed as nanomoles/min/mg protein for comparison to fatty acid synthetase and acetyl CoA carboxylase activities. $C_{18:2}$ supplementation resulted in a significant depression in fatty acid synthesis rates expressed on the basis of total liver, or per mg protein. In contrast, $C_{18:0}$ feeding was associated with a significant increase over fat-free controls in total liver fatty acid synthesis, but per mg protein the rates were statistically equivalent to the rates in the fat-free group. Most notable was the similarity within each treatment between fatty acid synthetase and acetyl CoA carboxylase activities when both were assayed at 37° (table 2), and in particular the extremely close relationship between the in vivo rates of acetyl units incorporated into fatty acids and the activities of carboxylase and synthetase (table 2).

Neither $C_{18:0}$ nor $C_{18:2}$ supplementation exerted a significant inhibitory action on adipose tissue fatty acid synthesis rates either on a per g adipose or on a total removable adipose tissue basis (table 2). Because of the significant increase in total liver fatty acid synthesis and the lower absolute rate of total adipose fatty acid synthesis, dietary $C_{18:0}$ was associated with a marked increase in the contribution of liver to total body fatty acid synthesis (table 2).

Metabolites. Because $C_{18:0}$ had no inhibitory effect on hepatic fatty acid synthesis, more detailed experiments with $C_{18:0}$ were not conducted. Supplementing a fat-free diet with 3% $C_{18:2}$ for 5 days did not significantly elevate plasma nonesterified fatty acid concentration or the amount of circulating free palmitate, stearate or oleate when the samples were collected prior to the sixth meal. However, dietary $C_{18:2}$ was associated with over a fourfold increase in the amount of plasma free linoleate (table 3). In these same rats, hepatic long chain acyl CoA concentrations before the meal were not significantly different from rats fed a fat-free diet. Similarly, rats fed a 3% $C_{18:3}$ supplemented

TABLE 3

Liver long chain acyl CoA concentration and plasma FFA composition in rats fed a fat free diet plus 3% $C_{18:2}$ or $C_{18:3}$ (experiment 2)

Parameter[1]	Dietary fatty acid			
	Fat-free (FF)	FF + 3% $C_{18:2}$	FF	FF + 3% $C_{18:3}$
Initial body weight	166± 6[a,4]	170± 7[a]	160±6	163±5
Final body weight	189± 8[a]	200± 8[a]	ND[1]	ND
Plasma FFA[2] (μg/ml)				
Palmitate	100±20[a]	119±15[a]	ND	ND
Stearate	45± 6[a]	56±10[a]	ND	ND
Oleate	78± 8[a]	66± 6[a]	ND	ND
Linoleate	7± 2[a]	32± 3[b]	ND	ND
Total	226±27[a]	273±18[a]	ND	ND
Long chain acyl-CoA:[3]				
Before 6th meal	133±19[a]	127±16[a]	ND	ND
After 6th meal	ND	ND	35±5[a]	30±4[a]

[1] Mean±SEM; n = 8. ND = not determined. [2] Samples collected prior to sixth meal. [3] Nanomoles per g liver. [4] Values with different superscripts are significantly different ($P < 0.05$) as determined by paired "*t*" test.

diet for 5 days displayed no significant change relative to fat-free controls in hepatic long chain acyl CoA content after the sixth meal. Although examined in two separate experiments, the effect of refeeding was a dramatic decline in hepatic long chain acyl CoA levels within 4 hours after initiation of eating (table 3).

The concentrations of lactate and pyruvate in the liver of rats fed a fat-free diet with 3% $C_{18:2}$ or $C_{18:3}$ supplementation were quantitated and used as an index of cytosolic redox state of liver (table 4). Since the response of rat liver lipogenesis to dietary $C_{18:2}$ or $C_{18:3}$ is similar (3, 5, 7), and because the lactate and pyruvate con-

TABLE 4

Estimates of liver cytosolic redox state in rats fed a fat-free diet plus 3% $C_{18:2}$ or $C_{18:3}$ (experiment 3)

Diet treatment	Parameter[1]			
	Lactate	Pyruvate[2]	Lactate:pyruvate	NAD:NADH
		Trial 1 (n = 8)[4]		
Fat-free (FF)	1,302± 96[a,3]	280±33[a]	4.74±0.68[a]	1,937
FF + 3% $C_{18:2}$	1,665±182[a]	217±13[a]	6.42±0.62[a]	1,174
		Trial 2 (n = 8)[4]		
Fat-free	1,596± 87[a]	185±18[a]	8.01±0.60[a]	1,113
FF + 3% $C_{18:3}$	1,384± 61[a]	171±15[a]	8.50±0.24[a]	1,044
		Trial 3 (n = 10)[5]		
Fat-free	1,424± 73[a]	396±18[a]	3.63±0.20[a]	2,505
FF + 3% $C_{18:2}$	1,253± 94[a]	319±10[b]	3.89±0.27[a]	2,293
		Mean of Trials 1–3		
Fat-free	1,441± 85[a]	287±61[a]	5.46±1.31[a]	1,852
FF + 3% PUFA	1,434±121[a]	236±44[a]	6.27±1.33[a]	1,504

[1] Mean±SEM. Mean initial and final body weights were comparable for both groups and the overall averages for initial were: Trial 1 = 178±7, Trial 2 = 163±5 and Trial 3 = 140±4; final were: Trial 1 = 194±8, Trial 2 = ND, and Trial 3 = 167±5. [2] Nanomoles per g liver. [3] Values with different superscripts are significantly different ($P < 0.05$) according to paired "*t*" test analysis. [4] Diet was fed to rats for six meals (one 3-hour meal/day). [5] Diet was fed to rats for seven meals (one 3-hour meal/day).

centrations of $C_{18:3}$ are as close to $C_{18:2}$ (trial 1) as the concentrations between the two $C_{18:2}$ trials, the data of all three experiments were combined (table 4). In no trial was the hepatic lactate:pyruvate ratio significantly altered by dietary $C_{18:2}$ or $C_{18:3}$. In addition, no consistent change in either lactate or pyruvate concentration occurred among the experiments. The mean values of all three trials indicate that the addition of 3% $C_{18:2}$ or $C_{18:3}$ does not significantly affect the cytosolic redox state of the liver.

Hepatic glycolytic and lipogenic enzyme activities. Clearly supplementing a fat-free diet with only 3% $C_{18:2}$ for 7 days will significantly depress hepatic rates of fatty acid synthesis and lipogenic enzyme activities whereas comparable amounts of absorbed saturated fatty acids are without inhibitory influence (table 2). The mechanism of inhibition of $C_{18:2}$ on lipogenesis remains unclear. One conclusion may be an effect on lipogenesis which is secondary to an inhibition of glycolytic activity. Of two key regulatory enzymes of glycolysis, glucokinase and pyruvate kinase, only pyruvate kinase was significantly depressed by 7 days of $C_{18:2}$ supplementation (table 5). In contrast, $C_{18:2}$ resulted in a significant depression in both hepatic fatty acid synthetase and citrate cleavage enzyme activities. In fact, the percent decline was approximately twice that of either glucokinase or pyruvate kinase.

TABLE 5

Changes in rat liver glycolytic and lipogenic enzyme activities after seven days of $C_{18:2}$ supplementation (experiment 5)

Parameter[1]	Diet	
	Fat-free (FF)	FF + 3% $C_{18:2}$
Final body wt., g	165 ± 4[7,a]	172 ± 6[a]
Total wt. gain, g[2]	13 ± 2[a]	20 ± 3[b]
Food intake, g/day[3]	12.2	12.2
Liver:		
Wt. g	7.7 ± 0.3[a]	8.1 ± 0.3[a]
Glucokinase[4]	17 ± 3[a]	13 ± 1[a]
Pyruvate kinase[5]	496 ±31[a]	360 ±34[b]
Citrate cleavage enzyme[5]	47 ± 4[a]	21 ± 3[b]
Fatty acid synthetase	19 ± 1[a]	10 ± 1[b]

[1] Mean ±SEM; n = 10; average initial body weight 152±5 g. [2] Gain during 7 day $C_{18:2}$ supplementation period. [3] Fat-free basal diet. [4] Glucokinase activity expressed as nanomoles glucose utilized/min/mg protein, 37°. [5] Pyruvate kinase and citrate cleavage enzyme activities expressed as nanomoles NADH oxidized/min/mg protein, 37°. [6] Fatty acid synthetase activities expressed as nanomoles NADPH oxidized/min/mg protein, 37°. [7] Values with different superscripts are significantly different ($P < 0.05$) according to paired "t" test.

DISCUSSION

In an earlier study (9), the addition of 3% methyl $C_{18:0}$ to a fat-free diet caused no reduction in the rate of fatty acid synthesis. However, the very poor absorbability of methyl $C_{18:0}$ made interpretation of these observations difficult. In the experiment presented in table 2, the level of dietary $C_{18:0}$ was increased to 8% in order to achieve an amount of absorbed $C_{18:0}$ equivalent to 3% $C_{18:2}$. Even when comparable quantities of $C_{18:0}$ and $C_{18:2}$ were absorbed, $C_{18:0}$ had no depressive action on lipogenic enzyme activities and was actually associated with a significant increase in total liver fatty acid synthesis. These in vivo effects of $C_{18:0}$ are in direct contrast to in vitro data with chick and rat hepatocytes and human skin fibroblasts in which stearic acid depressed rates of fatty acid synthesis with greater efficacy than unsaturated acids (10–13). The marked inhibitory effect of stearic acid in hepatocytes has been partially attributed to the low K_i of purified rat liver acetyl CoA carboxylase for stearoyl CoA (11). However, dietary $C_{18:0}$ caused no depression in acetyl CoA carboxylase activity in rat liver (table 2), while $C_{18:2}$ lowered the activity by more than 50%.

Although in vitro assays do not necessarily reflect in vivo enzyme activity or rate of substrate flux (35, 36), theoretically the assays for acetyl CoA carboxylase and fatty acid synthetase do represent total tissue enzyme protein (37, 38). Under our conditions, the in vivo rate of acetate unit utilization for hepatic fatty acid synthesis agreed very closely with the in vitro activities of fatty acid synthetase and acetyl CoA carboxylase. This indicates that at the time de novo fatty acid synthesis was quantitated (4 hours after meal initiation), synthetase and carboxylase enzymes were functioning at near maximal rates and that enzyme activity was indicative of substrate flux. Thus, the level of these enzymes could dictate the maximum in vivo rate of fatty acid synthesis (39). The accuracy of the calculations in table 2 is substan-

tiated by the close similarity between our data and in vivo fatty acid synthesis rates reported by others for meal-eating rats (19, 20) and isolated hepatocytes (40).

A very significant portion (50–70%) of the total body de novo fatty acid synthesis of rats is contributed by adipose tissue (table 2). However, the differential response of adipose tissue to the addition of various fatty acids to a fat-free diet, particularly in vivo studies, has largely been ignored. The total amount of removable adipose tissue did not significantly vary among treatments. This quantity of adipose tissue was only about 3% of the body weight. Although Sprague-Dawley rats of comparable body size have been shown to contain about 12% solvent extractable body fat, much of this is not associated with dissectable fat depots. Our values for removable fat are very close to those reported for Osborne-Mendel rats of similar size (41, 42).

Adipose tissue lipogenesis in vivo remained unchanged by the addition of $C_{18:0}$ or $C_{18:2}$ which was consistent with our earlier data (3). In contrast, Allmann and Gibson (4) reported that mouse adipose tissue fatty acid synthesis was rapidly depressed by adding 2% $C_{18:2}$ to a low fat diet devoid of essential fatty acids, but the addition of 2% $C_{16:0}$ had no inhibitory effect. This apparent discrepancy in response of mouse and rat adipose tissue to dietary fat may be attributed to: 1) the poor digestibility of $C_{16:0}$ (3); 2) species differences; and/or 3) the meal-eating regimen of our rats (14).

The increased rate of total liver fatty acid synthesis and greater contribution of liver to total body fatty acid synthesis associated with dietary $C_{18:0}$ is in accordance with the apparent site shift observations of Waterman et al. (2). In their comparison of diets high in polyunsaturated fat versus saturated fat, rats fed a tallow diet were found to possess greater rates of liver fatty acid synthesis whereas the rates of adipose tissue fatty acid synthesis were higher in rats fed a safflower oil containing diet.

The addition of either $C_{18:2}$ (table 2) or $C_{18:3}$ (3, 4, 9) to the fat-free diet causes a significant reduction in rates of hepatic lipogenesis of meal-fed rats. Furthermore, both $C_{18:2}$ and $C_{18:3}$ are equivalent in precipitating this depression (3, 43). For this reason both of these acids were used in the investigation of changes in hepatic and metabolite concentrations. Supplementation was not associated with an elevation in pre-meal plasma free fatty acids or pre-meal hepatic long chain acyl CoA concentrations (table 3). Like $C_{18:2}$ supplementation, the addition of $C_{18:3}$ to the fat-free diet caused no change in post-meal hepatic long chain acyl CoA concentration relative to the fat-free control group. $C_{18:2}$ supplementation did cause a fourfold increase in plasma free linoleate, but had no significant effect on the composition of the remaining fatty acids studied (table 3). If the inhibition of liver fatty acid synthesis associated with dietary polyunsaturated fatty acids is mediated in some way via their CoA derivatives, then the effects of these derivatives on mitochondrial citrate efflux (44) and acetyl CoA carboxylase (45, 46) may be more dependent on composition of acyl CoA than on concentration. The liver of rats and humans has been shown to remove non-esterified fatty acids from plasma at differential rates and the rate of uptake was greater for polyunsaturated fatty acids (42, 47). Furthermore, the feeding of safflower oil to rats greatly elevated the concentration of unesterified $C_{18:2}$ in liver tissue (6). Therefore, the proportion of hepatic linoleoyl CoA may be expected to increase. However, the CoA ester of linoleic acid prevented citrate efflux from isolated mitochondria with far less effectiveness than did added CoA derivatives of saturated acids (37). In addition, the K_i of acetyl CoA carboxylase for unsaturated fatty acids is higher than for the saturated acids (45). Therefore, an inhibition of in vivo fatty acid synthesis by an increased proportion of linoleoyl-CoA is not consistent with observations in isolated systems. Interestingly the observations with isolated enzyme and transport systems are consistent with the greater inhibitory effect of $C_{18:0}$ in isolated hepatocytes (9–13).

The physiological significance of long chain fatty acid CoA esters in controlling lipogenic rates has been criticized by Shafrir and Ruderman (48) who suggested that other intracellular modifiers such as NADH:NAD and acetyl CoA:CoA

ratios, may be more significant factors. If polyunsaturated fatty acids are degraded in the liver more rapidly than saturated fatty acids (15), a greater turnover rate of CoA esters in the liver of rats fed $C_{18:2}$ or $C_{18:3}$ could occur without a significant change in total tissue long chain acyl CoA content. The potential net effect would be increased acetyl CoA and NADH levels which would negatively affect pyruvate dehydrogenase activity and pyruvate utilization for fatty acid synthesis (18, 49). Using lactate:pyruvate ratios as an index of cytosol redox potential (17), we found no indication that dietary $C_{18:2}$ or $C_{18:3}$ caused a more reduced state in liver (table 4). In addition, the concentrations of lactate and pyruvate were not consistently altered by dietary $C_{18:2}$ or $C_{18:3}$. The average of all three experiments demonstrates no significant change in lactate or pyruvate concentrations, the lactate:pyruvate or NAD:NADH ratios (table 4). A reduction in NAD:NADH ratio caused by fasting, diabetes or high-fat diets has been related to lower rates of lipogenesis and glycolysis (17). The inability to demonstrate such a relationship with 3% added $C_{18:2}$ or $C_{18:3}$ may be due to the high intake of dietary carbohydrate and the insensitivity of the lactate:pyruvate ratio to less dramatic nutritional manipulations. Nevertheless the redox state of hepatic cytosol does not appear to be a major regulatory mechanism to explain the inhibitory effects of polyunsaturated fatty acids on liver fatty acid synthesis.

Fatty acid synthesis from dietary glucose requires an active flow of glucose through glycolysis in order to produce substrate for de novo fat synthesis. All earlier work (3–9) designed to study the effects of polyunsaturated fatty acids on lipogenesis have focused primarily on changes in lipogenic enzyme activities in liver. However, the depression in the activities of fatty acid synthetase, acetyl CoA carboxylase and citrate cleavage enzyme (tables 2 and 5), and in rates of fatty acid synthesis (table 2, ref. 3–9) characteristic of $C_{18:2}$ supplementation of a fat-free diet, could be the consequence of a specific inhibition of glycolysis. The data in table 5 indicate that dietary $C_{18:2}$ had a minimal negative effect on liver glucokinase and pyruvate kinase activities. Only the decline in pyruvate kinase activity was significant and the percent decline in both glucokinase and pyruvate kinase activities was about half that of citrate cleavage enzyme or fatty acid synthetase (table 5). However, glucose flux through glycolysis has not been examined under our experimental conditions. Therefore, a specific or more potent inhibition of glycolytic flow associated with dietary $C_{18:2}$ cannot be totally eliminated at this time. However, glucokinase activity has been noted not to change after three meals of $C_{18:2}$ or $C_{18:3}$ supplementation, whereas fatty acid synthetase activity and hepatic fatty acid synthesis were significantly lowered.[4] This suggests the inhibitory effect of polyunsaturated fatty acids is primarily on the lipogenic machinery and only secondary on the glycolytic machinery.

In summary, dietary $C_{18:2}$ very effectively depressed rat liver fatty acid synthesis whereas comparable amounts of absorbed $C_{18:0}$ had no inhibitory action. This inhibition by $C_{18:2}$ was not the result of increased plasma free fatty acid or hepatic fatty acid CoA ester levels or changes in redox state of the liver. The only detectable metabolite change was more than a fourfold increase in plasma free linoleate concentration. The absolute decline in the activity of two key glycolytic enzymes, glucokinase and pyruvate kinase, was only half that of the lipogenic enzymes citrate cleavage enzyme and fatty acid synthetase. However, without measurements on glycolytic flux rates one cannot ascertain if the effect of polyunsaturated fatty acids is primarily on lipogenesis or glycolysis. The less dramatic decline in activity of glucokinase and pyruvate kinase than in fatty acid synthetase and citrate cleavage enzyme, and the very close relationship between in vivo rates of fatty acid synthesis and activities of acetyl CoA carboxylase and fatty acid synthetase might indicate that polyunsaturated fatty acids exert their rate regulating effect on hepatic fatty acid synthesis in meal-fed rats by controlling the level of carboxylase, synthetase or both (39).

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