Utilization of exogenous free fatty acids for the production of very low density lipoprotein triglyceride by livers of carbohydrate-fed rats

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ABSTRACT High carbohydrate diets enhance the hepatic output of very low density lipoprotein triglycerides. The fatty acids of these triglycerides could come from exogenous sources (i.e., diet or adipose tissue) or from de novo fatty acid synthesis in the liver. The role of exogenous free fatty acids was evaluated in rats fed Purina Chow or diets containing 10% fructose for up to 14 wk. In carbohydrate-fed rats, serum triglycerides were twice normal, and VLDL accounted for about 60% of the increases. Pre-β-lipoprotein was increased and α- and β-lipoprotein were decreased. Phospholipid and cholesterol levels were unchanged.

Livers were perfused with glucose and free fatty acids. Perfusate free fatty acids rose from 180 to 1800 μeq/liter as the infused acids increased from 0 to 992 μeq/3 hr; simultaneously, net free fatty acid uptake rose from $<$ 1 to 18 μeq/g/hr and triglyceride output by the liver doubled. However, rates of secretion of triglyceride became constant, and triglyceride accumulated in liver at uptakes of free fatty acids $>$ 13 μeq/g/hr. More lauric and myristic acid appeared in the perfusate than was infused, suggesting the hepatic discharge of free fatty acids.

Livers of fructose-fed rats secreted twice as much oleate-^{14}C-labeled triglyceride as controls at all levels of free fatty acid uptake. The ratios of the specific activities of perfusate triglyceride to free oleate-^{14}C were unaffected by diet and were about 0.6 and 1.0 at low and high triglyceride secretion rates, respectively. Thus, carbohydrate feeding did not result in altered uptakes of free fatty acids or preferential secretion of triglycerides containing endogenously synthesized fatty acid. Instead, the increased secretion of triglyceride was accomplished by enhanced formation of VLDL triglyceride from exogenous free fatty acids.

SUPPLEMENTARY KEY WORDS VLDL triglyceride · fructose · liver perfusion

THE INCREASES in circulating VLDL levels seen in carbohydrate-induced hyperglyceridemia are associated with enhanced output of VLDL triglyceride by liver (1). The fatty acids of VLDL triglyceride may originate from circulating FFA derived from adipose tissue (recycling) (2), from the diet, or from de novo lipogenesis in liver. Thus, augmentation of triglyceride secretion rates in carbohydrate-fed animals could be accomplished by alterations either in the uptake and utilization of FFA by the liver or in the synthesis of fatty acids. We produced hyperglyceridemia in rats by fructose feeding and studied the hepatic uptake and utilization of FFA in isolated perfused livers. The utilization of exogenous FFA was altered in animals with carbohydrate-induced hyperglyceridemia.

METHODS

Animals and Diets

Male Sprague-Dawley rats were fed Purina Chow and tap water ad lib. A high carbohydrate diet, also fed ad lib., was obtained by substituting a 10% fructose solution for the tap water (3). Food was available to the animals until 2–3 hr before the start of the study. Blood for analysis was obtained from the inferior vena cava after the animals were anesthetized with pentobarbital (1 mg/100 g). The livers were removed, perfused free of blood with oxygenated Krebs-Ringer bicarbonate buffer, pH 7.4, and mounted for perfusion in a Miller-type apparatus (4) (J. R. Thebeau, Boston, Mass.).

Abbreviations: VLDL, very low density lipoprotein; FFA, free fatty acid; TGFA, triglyceride fatty acid.

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Perfusions were carried out at 37°C and were always started between 10 and 11 AM. All the livers perfused were functionally viable by gross appearance and by the rate of flow of bile and perfusate. Some of the livers were also examined at the end of perfusion by electron microscopy.[1] Perfusates consisted of 100 ml of Krebs-Ringer buffer, 3 g of bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo., lot 99B00450), 300 mg of glucose, and 90 mg of amino acids (the mixture was prepared from equal weights of 21 amino acids, Sigma Kit LAA-21).

Various amounts of FFA were infused as the sodium salt of oleic acid (>99% pure, Sigma) (5, 6) complexed to bovine serum albumin. The infusate consisted of 20 ml of saline, 2 g of bovine serum albumin, and the appropriate amounts of oleate. Oleate-1-^{14}C (11 μCi as the sodium salt, 99% pure, 40 mCi/mmole; New England Nuclear, Boston, Mass.) was also added to the infusate in some experiments. All solutions were brought to pH 7.4 before use. Samples of perfusates were removed at 5 min, and every 60 min thereafter. At the end of perfusion, livers were perfused with ice-cold saline, minced, and homogenized in the cold (VirTis homogenizer, Fisher Scientific Co., St. Louis, Mo.). An aliquot was then sonified (Sonifier, model LS-75, Bronson Instruments Inc., Ultransonic Power Division, Stamford, Conn.) and extracted (7).

In an attempt to increase the yield of triglyceride, some perfusions were carried out with albumin which had been purified by charcoal treatment (8); this resulted in a decrease in FFA from 5 to 0.5 μeq/g. It should be noted that the livers perfused with the treated and untreated albumins were exposed to 2.5 and 25 μeq of FFA, respectively, in addition to the FFA that was added to the infusate.

Analyses

Sera, perfusates, and liver homogenates were extracted with chloroform–methanol by the method of Carlson (7). Appropriate samples were analyzed for triglyceride (7), cholesterol (9), and lipid phosphorus (10). FFA was determined by the method of Duncombe (11) on extracts of sera and perfusates (12). Lipoprotein electrophoreses were done by the disc method of Narayan, Creinin, and Kummerow (13).

VLDL of sera and perfusates were isolated as previously described (14) and analyzed for triglyceride (7). Prior to radioactivity assays and gas–liquid chromatography, aliquots of the chloroform–methanol extracts of perfusates and liver were separated by thin-layer chromatography (15). Neutral lipids and FFA were eluted with chloroform, and aliquots of the eluates were counted (16); other aliquots were methylated (17) for gas–liquid chromatography (Beckman Model G-2 gas chromatograph; 15% DEGS on Chromosorb W). The procedures are described in a previous publication (18).

RESULTS

In Vivo Studies

The time course of carbohydrate-induced hyperglyceridemia in fructose-fed rats was followed for up to 14 wk. Weights of fructose-fed (n = 12) and control animals (n = 10) were indistinguishable (Fig. 1). Caloric intakes were similar for both groups (93 ± 11 and 98 ± 16 kcal/day in controls and fructose-fed animals, respectively). In the carbohydrate-fed group, fructose contributed 35–50% of total calories. Tail vein blood (0.1 ml) was taken every 2 wk for triglyceride analysis and lipoprotein electrophoresis. Triglyceride levels were significantly elevated above those of controls (paired t test) at 4 and 6 wk; they declined at 8 wk and rose again during the final 4 wk (Fig. 1). Concomitant with the rises in triglyceride levels, there were increases in pre-β- and decreases in β- and α-lipoproteins (Fig. 2).

In two other groups of animals, one fed the regular diet and the other the fructose diet for 5 wk, total triglyceride levels were 79 ± 22 and 142 ± 31 mg/100 ml and VLDL TG were 29 ± 5 and 72 ± 18 mg/100 ml, respectively (mean ± SD, n = 5 per group). Lipid phosphorus was 4.5 ± 0.5 and 4.0 ± 0.5 mg/100 ml and cholesterol was 66 ± 7 and 65 ± 12 mg/100 ml in fructose-fed and control rats, respectively.

Animals that were used as liver donors were fed fructose for 30–56 and 103–110 days; triglyceride levels (mg/100 ml) were 145 ± 30 (n = 48) and 136 ± 21 (n = 20), respectively. The concentrations were 68 ± 27 (n = 46) and 72 ± 18 mg/100 ml (n = 17) for the parallel controls.

Perfusions

At all levels of FFA infusion (from 124 to 496 μeq/3 hr), perfusate FFA concentrations rose during the first hour and tended to stabilize after 2 and 3 hr. As the amounts of infused FFA were increased, levels of FFA in the perfusate after 2 and 3 hr rose from approximately 400 to 1800 μeq/liter; simultaneously, uptake of FFA by the liver rose from 3 to 18 μeq/g/hr. Livers of control and fructose-fed rats took up similar amounts of FFA at comparable FFA concentrations (Table 1).

The FFA compositions of perfusates varied little over the hours of perfusion (Table 2). However, all perfusates

[1] Kindly performed by Dr. M. Kostianovsky of the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

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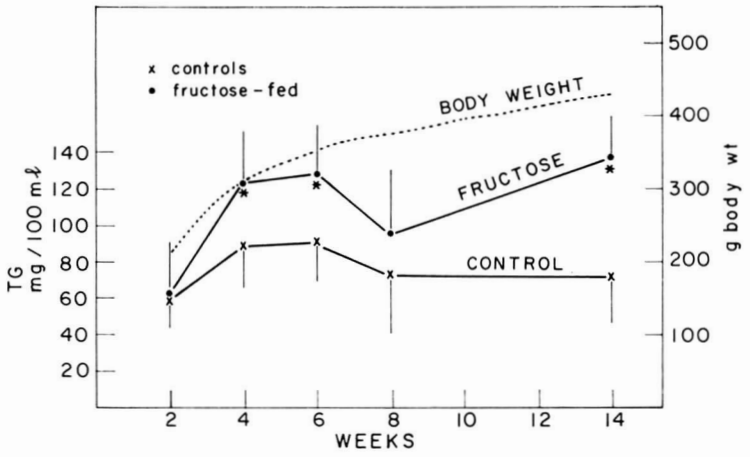

FIG. 1. Course of carbohydrate-induced hyperglyceridemia in Sprague-Dawley rats. Animals consumed similar quantities of either Purina Chow plus water, or chow plus 10% fructose. Bars represent one standard deviation (n = 10 and 12 for control and fructose-fed animals, respectively); asterisks indicate significant differences between the means of control and fructose-fed rats ($P < 0.025$, paired t test). TG, triglyceride.

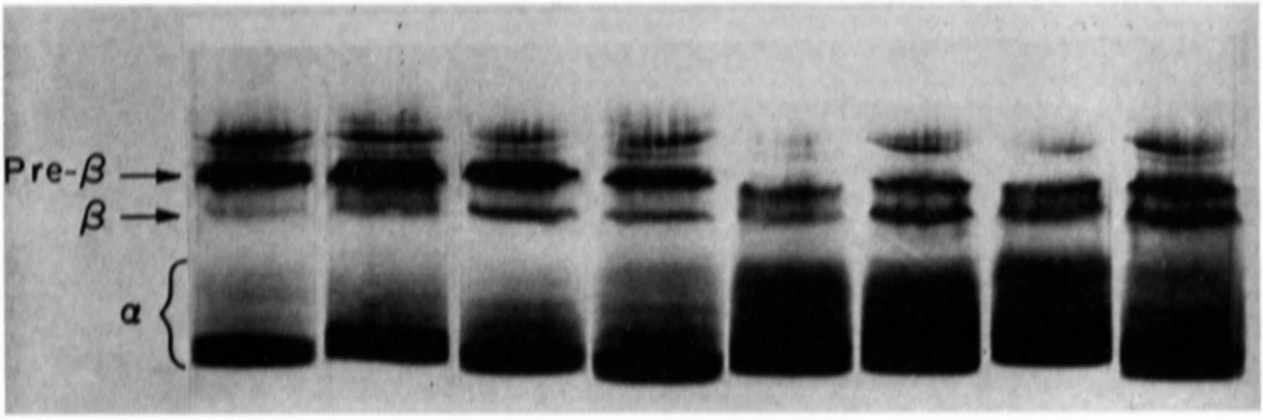

FIG. 2. Changes in the serum lipoproteins as a result of fructose feeding. Lipoprotein electrophoresis was performed in 3.75% polyacrylamide gel by the method of Narayan et al. (13). From left to right, the first four electropherograms are for fructose-fed rats; the last four are for control rats.

contained more myristic and lauric acids than were in the original infusion. In the absence of added FFA, the fatty acids on the albumin contained 1.4% of 12:0 plus 14:0, and perfusate FFA contained 35% of 12:0 plus 14:0. The albumin carrying the 496 μeq of oleate contained no detectable 12:0 or 14:0, yet these acids comprised up to 18% of the perfusate FFA.

The output of triglyceride by both kinds of livers was linear over 3 hr (Fig. 3). As FFA uptake increased 4- to 5-fold (from 3 to 12 or 15 μeq/g/hr), the rate of triglyceride output doubled (Table 3). However, triglyceride output remained constant at high rates of FFA uptake, and intrahepatic triglyceride levels rose (Table 3). The livers of animals fed fructose for either 29–56 or 103–110 days secreted about twice as much triglyceride as did control livers at all rates of infusion of FFA (Fig. 3 and Table 3). 75–100% of the triglycerides secreted by both kinds of livers were in VLDL.

In agreement with the work of Heimberg, Weinstein, and Kohout (5), purification of the albumin resulted in increased rates of secretion of triglyceride (Table 3). Moreover, livers of fructose-fed rats had greater rates of secretion of triglyceride than did control livers at both levels of FFA (0 and 496 μeq).

Fructose feeding increased the relative content of oleate in liver triglycerides (19) (Table 4). When no additional FFA was infused, hepatic and perfusate TGFA resembled each other closely in both control and carbohydrate-fed rats. When 496 μeq of oleate was infused, oleic acid in hepatic and perfusate triglyceride increased; however, in each case tested, there was more oleic acid in perfusate triglyceride than in liver triglyceride.

In the experiments using ^{14}C-labeled oleate, the incorporation of radioactivity into perfusate triglyceride in perfusions of liver from control and fructose-fed rats

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TABLE 1 EFFECT OF PERFUSATE FFA CONCENTRATION ON FFA UPTAKE BY PERFUSED LIVERS

FFA Infused	Perfusate [FFA]		FFA Uptake	
	Control	Fructose-fed	Control	Fructose-fed
μmoles/3 hr	*μmoles/liter*		*μmoles/g/hr*	
0	198 ± 63	253 ± 21	<1	<1
124	441 ± 54	409 ± 70	3.4 ± 0.8	3.3 ± 0.5
248	595 ± 67	679 ± 189	5.9 ± 0.7	6.2 ± 0.6
496	1104 ± 119	1009 ± 98	15.5 ± 2.8	12.3 ± 2.2
992	1837 ± 184	1473 ± 422	18.5 ± 1.5	18.6 ± 2.0

Perfusates consisted of 100 ml of Krebs-Ringer buffer, pH 7.4, bovine serum albumin (BSA) (3 g), glucose (300 mg), and amino acids (90 mg). The indicated amounts of FFA were infused as the sodium salt of oleate in 20 ml of saline with 2 g of BSA. (The BSA as obtained contained 5 μeq/g of FFA.) Control animals were fed Purina Chow and water; the fructose-fed animals were given chow and 10% fructose for 30–56 days. Each point represents the mean ± one standard deviation of 5–18 experiments. FFA uptake = (initial FFA content of perfusate + FFA infused) − (FFA removed for samples + FFA remaining at end of perfusion)/liver weight × time.

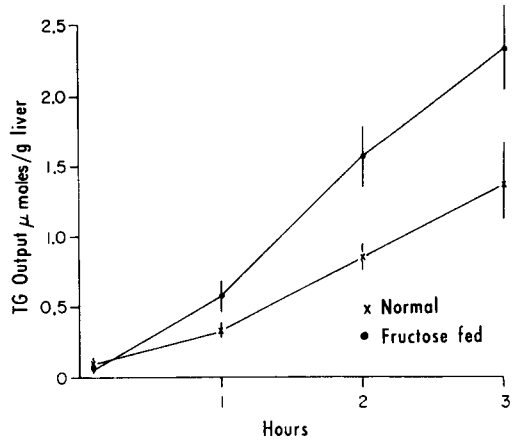

FIG. 3. Triglyceride (TG) output by isolated perfused livers of control and fructose-fed rats. Perfusate consisted of 100 ml of Krebs-Ringer bicarbonate buffer, 3 g of bovine serum albumin (BSA), 300 mg of glucose, and 90 mg of amino acids. 496 μeq of oleate and 2 g of BSA contained in 20 ml of saline were infused over a 3-hr period.

was linear. Specific activities increased over the first 1 and 2 hr of perfusion and remained constant thereafter (Table 5). Specific activities of perfusate free oleic acid increased initially and, in some cases, later declined.

There was a greater amount of ^{14}C incorporated into perfusate triglycerides in perfusions of livers from fructose-fed rats over a 3-hr period than in perfusions of control livers (Table 6). Although the use of purified albumin resulted in a shift of label from liver lipids to perfusate triglyceride, the relationship of control values to those for the fructose-fed animals was not altered. 82–102% of the infused radioactivity was recovered.

DISCUSSION

When diets high in carbohydrates are fed to rats, there are increases in concentration of serum triglyceride (3) and pre-β-lipoproteins (20). This phenomenon was also observed in the present study. The rise in VLDL triglyceride accounts for most of the increase in total serum triglycerides. With continued carbohydrate feeding to humans, there is a return to normal lipid levels in some studies (21, 22), but not in others (23). We observed that after 8 wk of fructose feeding there was a decrease in serum triglycerides to a concentration not significantly different from that of the control values. However, there was an increase after this time, and at 14 wk the concentration was significantly higher than in the control animals. Moreover, rates of output of liver triglycerides were equally elevated after 24 and 110 days of fructose feeding. Thus, in the rat there was no return to base line values with prolonged carbohydrate feeding.

During the perfusions, net hepatic FFA uptakes were directly related to perfusate FFA levels; the latter in turn were determined by FFA infusion rates (Table 1) (5). Although the net movement of FFA was clearly from perfusate to liver, the finding of a relatively large proportion of laurate and myristate in the perfusate and the observed fall of free oleic acid specific activities (in the "purified albumin" perfusions) suggest that movement of FFA from liver to perfusate was occurring as well. Similar findings have been reported for the Ehrlich ascites tumor cell (24). Possibly, myristate and laurate represent some of the products of partial oxidation of longer-chain fatty acids derived from hepatic stores of triglyceride, but this is not known.

Livers of control and fructose-fed rats took up similar amounts of FFA at similar perfusate FFA concentrations. This was not unexpected, since FFA uptake appears to be a "physical" process dependent solely upon the FFA/albumin molar ratio (18, 25). Thus, the increases in the hepatic secretion of triglyceride by carbohydrate-fed rats must have been due to factors other than the differences in FFA uptake.

On both diets the rate of secretion of triglyceride by the livers was directly related to uptake of FFA (5) until the triglyceride export system became saturated. The accumulation of further triglyceride in liver rules out incorporation of fatty acid into triglyceride as being the rate-limiting step. Exogenous FFA has been shown to represent the majority of exported hepatic TGFA in fasting dog and man (26, 27). This also appears to have

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TABLE 2 FFA Compositions of the Perfusates of Isolated Rat Livers

FFA Infused	Animal*	12:0	14:0	16:0	16:1	18:0	18:1	18:2
μmoles/3 hr				*% of total FFA*				
0	1C		4.9	28.6 ± 3.2	2.1	23.3	38.2 ± 4.6	4.9
0	2C	13.2	17.5	25.8 ± 1.2	1.2	14.9	22.7 ± 5.0	4.7
0	3C	16.9	18.0	24.8 ± 1.3	1.8	15.2	19.2 ± 2.4	3.9
0	1F	8.1	15.1	23.6 ± 2.1	1.8	15.9	31.5 ± 7.4	3.5
0	2F		6.7	27.0 ± 2.5	3.8	14.8	40.3 ± 7.3	7.7
0	3F	11.1	17.1	28.9 ± 2.0	5.6	13.4	22.5 ± 3.2	1.8
496	1C	1.1	4.3	9.8 ± 1.7	1.0	6.1	74.1 ± 2.8	3.2
496	2C	10.1	8.1	10.4 ± 1.2	0.4	5.9	61.5 ± 4.4	1.2
496	3C	0.4	5.0	12.2 ± 1.7	1.1	6.8	69.7 ± 2.2	4.9
496	1F	3.9	3.8	12.5 ± 2.2	0.9	7.7	71.2 ± 6.9	2.8
496	2F	1.3	4.0	13.3 ± 1.0	2.1	8.5	68.5 ± 5.6	2.2
496	3F	4.4	5.8	11.3 ± 1.3	1.0	6.3	67.1 ± 8.7	4.3
Infusate FFA composition								
0			1.4	12.1	5.4	18.9	45.1	21.6
496				2.0		1.0	97.0	

Mean ± SD of 1-, 2-, and 3-hr samples. Perfusates were extracted, and FFA were separated by thin-layer chromatography and analyzed by gas–liquid chromatography as the methyl esters.

* C, control; F, fructose-fed.

TABLE 3 Effect of FFA Uptake on Triglyceride Output by Perfused Livers of Control and Fructose-fed Rats

FFA Uptake*		TG Output†		Liver TG Contents	
Control	Fructose-fed	Control	Fructose-fed	Control	Fructose-fed
μmoles/g/hr		*μmoles/g/hr*		*μmoles/g*	
<1	<1	0.14 (0.37) ± 0.03 (0.07)	0.40 (0.71) ± 0.11 (0.12)	8.9 ± 2.9	9.9 ± 2.9
3.4 ± 0.8	3.3 ± 0.5	0.26 ± 0.06	0.47 ± 0.23	8.9 ± 2.0	11.6 ± 3.8
5.9 ± 0.7	6.2 ± 0.6	0.29 ± 0.07	0.53 ± 0.11	10.4 ± 3.5	11.5 ± 4.2
15.5 ± 2.8	12.3 ± 2.2	0.43 (0.63) ± 0.10 (0.08)	0.77 (1.07) ± 0.09 (0.17)	12.9 ± 2.5	14.4 ± 2.0
18.5 ± 1.5	18.6 ± 2.0	0.39 ± 0.09	0.82 ± 0.22	19.3 ± 3.4	18.9 ± 2.2

Perfusates consisted of 100 ml of Krebs-Ringer buffer, pH 7.4, bovine serum albumin (BSA) (3 g), glucose (300 mg), and amino acids (90 mg). The indicated amounts of FFA were infused as the sodium salt of oleate in 20 ml of saline with 2 g of BSA. (The BSA as obtained contained 5 μeq/g of FFA.)

* Data from Table 1.

† Mean ± SD (n = 5–13). Calculated as follows: (TG at end perfusion + TG removed for samples)/liver weight × time. TG, triglyceride. Values in parentheses are for perfusions performed with charcoal-purified albumin.

been true of livers taken from our rats fed either diet even at the relatively low infusion rates of 124 μeq/3 hr. It is not clear whether this export of triglyceride was accompanied by suppression of endogenous synthesis of fatty acid (28, 29) or by preferential shunting of exogenous FFA to VLDL triglyceride even in the face of continued endogenous fatty acid synthesis. In any case, the findings suggest that in carbohydrate-induced hyperglyceridemia there is not a disproportionately greater export of endogenously synthesized fatty acid.

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TABLE 4 EFFECT OF OLEIC ACID INFUSION AND DIET ON PERFUSATE AND HEPATIC TRIGLYCERIDE FATTY ACID COMPOSITIONS

FFA Infused*	Animal†		14:0	16:0	16:1	18:0	18:1	18:2
μmoles/3 hr					*% of total fatty acids*			
0	1C	P	3.0	18.0	2.6	17.2	40.2	18.9
0	2C	P	1.9	26.5	3.7	7.3	32.8	28.1
		L	1.5	28.4	2.3	4.6	30.4	32.9
0	3C	P	2.5	32.4	4.3	4.8	30.4	25.7
		*L	0.7	35.8	4.1	3.0	31.2	25.4
0	1F	P	0.8	20.6	6.5	3.9	53.6	14.6
		L	1.5	22.3	8.3	6.0	47.6	14.3
0	2F	*P	1.6	32.3	17.7	1.4	42.9	4.0
		L	1.0	28.9	14.9	2.6	45.9	6.7
0	3F	*P	5.3	37.4	20.0	6.1	45.1	4.1
		L	1.0	33.6	15.0	3.0	42.6	4.7
496	1C	P		7.7	2.5	2.2	74.8	7.8
		L	0.3	10.5	2.0	4.3	61.5	22.0
496	2C	*P	0.7	10.4	2.1	2.2	74.4	10.2
		L	1.0	22.2	2.3	3.3	53.1	18.1
496	3C	*P	0.6	12.2	3.0	1.0	75.0	8.1
		L		18.8	2.3	2.9	60.0	16.0
496	1F	*P	1.2	15.0	5.9	1.9	74.1	1.9
		L		17.9	6.4	1.5	63.0	11.2
496	2F	*P	1.0	15.7	5.0	1.3	75.1	1.8
		L		16.4	2.9	2.6	69.9	8.2
496	3F	*P	1.2	13.8	3.0	1.4	75.5	5.1
		L		12.8	2.4	2.7	69.5	12.5

* Charcoal-purified albumin was used in these perfusions. At the end of perfusion, final perfusates and liver were extracted, triglycerides were separated by thin-layer chromatography, and the fatty acids were methylated and analyzed by gas–liquid chromatography.
†C, control; F, fructose-fed; P, perfusate; L, liver.

TABLE 5 ^{14}C SPECIFIC ACTIVITIES OF HEPATIC AND PERFUSATE TRIGLYCERIDE OLEATE AND PERFUSATE FREE OLEIC ACID

		4F*		5F		4C		5C	
FA Infused	Time	FFA	TG	FFA	TG	FFA	TG	FFA	TG
μmoles/3 hr	*min*				*dpm/nmole*				
124	5	76	7	97	7	100	5	72	11
	60	80	30	87	41	159	52	72	30
	120	80	42	91	46	183	96	93	55
	180	72	57	111	54 (32)	163	97	111	62
		6F		7F		6C		7C	
496†	5	67	20	64	6	63	6	66	7
	60	21	20	33	30	17	34	31	26
	120	18	22	26	29	24	20	28	27
	180	21	24 (17)	26	25 (19)	29	26 (18)	25	25 (15)
		8F		9F		8C		9C	
496‡	5	78	0.7	55	4	48	2.0	77	2.0
	60	77	33	55	34	53	32	63	30
	120	52	35	49	40	48	40	65	46
	180	43	48 (27)	33	46 (26)	44	42 (27)	63	48 (31)

Lipids were extracted and separated by thin-layer chromatography prior to liquid scintillation counting.
* Indicates animal numbers. C, controls; F, fructose fed; TG, triglyceride. Values in parentheses are for liver TG ^{14}C specific activity.
† 2 μCi of oleate-^{14}C and 40 μeq of oleate-^{13}C were added to the perfusate at the start of the perfusion ("loading dose"); then 9 μCi (496 μeq) of oleate-^{14}C were infused over 3 hr.
‡ Charcoal-purified albumin was used in these perfusions.

The capacity of livers of carbohydrate-fed rats to secrete triglyceride was greater at every level of FFA uptake, i.e., greater amounts of exogenous FFA were exported as VLDL TGFA. The in vivo correlate of these findings would be that in the carbohydrate-induced state hepatic FFA uptake would not be altered, but a greater fraction of the plasma FFA taken up by liver would be exported as VLDL triglyceride at every level of circulating FFA. In this sense carbohydrate feeding has effects opposite to those observed in starvation (30)

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TABLE 6 DISTRIBUTION OF ^{14}C AT END OF PERFUSION

FFA Infused	Animal	Perfusate TG		Perfusate FFA		Liver Lipid		Total*	
μeq/3 hr		*dpm*†	%‡	*dpm*†	%	*dpm*	%	*dpm*	%
124	4F	4.96	20.3	2.88	11.8				
	5F	5.14	21.1	2.79	11.4				
	4C	1.16	4.8	2.71	11.1				
	5C	1.82	7.5	4.69	19.2				
496	6F	2.03	8.3	3.98	16.3	17.40	74.3	23.41	96
	7F	2.23	9.1	3.80	15.6	15.23	62.4	21.26	87
	6C	1.46	6.0	4.62	18.9	17.91	73.4	23.99	98
	7C	0.80	3.3	4.05	16.6	16.02	65.6	20.87	86
496§	1F	6.31	25.9	2.97	12.2	15.70	64.3	24.98	102
	2F	7.00	28.7	2.80	11.5	14.42	59.1	24.22	99
	3F	4.96	20.3	3.42	14.0	12.20	50.0	20.58	84
	1C	2.83	11.6	5.12	21.0	11.91	48.8	19.86	81
	2C	4.18	17.1	3.26	13.4	14.80	60.7	22.24	91
	3C	3.29	13.5	4.99	20.5	12.12	49.7	20.40	84

TG, triglyceride.
* Total, liver lipid plus perfusate FFA and TG.
† dpm $\times 10^6$, calculated as follows: dpm in total perfusate at end + dpm removed during samplings.
‡ % = % of total ^{14}C dpm (11 μCi) infused, e.g., $4.96 \times 10^6 \times 100/24.4 \times 10^6$.
§ Perfusions with purified albumin.

and diabetes (31), in which the liver shunts FFA towards ketogenesis and away from export as VLDL triglyceride.

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