Fatty acid metabolism and lipid secretion by perfused livers from rats fed laboratory stock and sucrose-rich diets

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Abstract To assess the possible role of altered hepatic processing of free fatty acids in dietary sucrose-induced accumulation of triglyceride in the liver and blood plasma, livers from rats fed commercial laboratory stock and high sucrose diets were perfused both with and without oleic acid substrate. Consumption of the sucrose diet exerted a multiplicity of effects on oleic acid metabolism, characterized by decreased conversion to both ketone bodies and carbon dioxide, increased esterification into liver triglyceride, and increased secretion in triglyceride-rich lipoproteins. During the infusion of oleic acid, livers from sucrose-fed rats also exhibited decreased ketogenesis, and increased secretion of triglyceride from endogenous sources. Since oleic acid uptake from the perfusion medium was identical in both groups, the observed effects of sucrose feeding are ascribed to altered rates of intracellular metabolic processes. Mass and radiochemical analyses of perfusate ketone bodies and triglycerides were indicative of greater mobilization of triglycerides from hepatocellular lipid droplets in the livers from sucrose-fed rats. These livers contained more triglyceride and secreted more triglyceride even in the absence of infused oleic acid. In summary, the sucrose-rich diet increased the esterification:oxidation ratio of intracellular free fatty acids derived from both the circulation and endogenous sources within the hepatocyte. In response, secretion of triglyceride-rich lipoproteins by the liver and deposition of triglyceride within the liver were promoted. It is concluded that alterations in the processing of free fatty acids by the liver contribute significantly to the liver and plasma triglyceride accumulation following sucrose consumption.—**Yamamoto, M., I. Yamamoto, Y. Tanaka, and J. A. Ontko.** Fatty acid metabolism and lipid secretion by perfused livers from rats fed laboratory stock and sucrose-rich diets. *J. Lipid Res.* 1987. **28:** 1156–1165.

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The consumption of a diet rich in sucrose promotes the synthesis and accumulation of triglyceride, and the secretion of triglyceride-rich lipoproteins, by the liver and leads to elevations in the liver and plasma triglyceride concentrations (1–11). The accumulation of liver and plasma triglyceride in such animals may be, at least in part, ascribed to increased hepatic fatty acid synthesis (4, 12–15). In addition, it remains possible that altered subcellular utilization of free fatty acids via oxidation and esterification contributes significantly to the observed effects of dietary sucrose. However, despite the importance and long history of the metabolic consequences of sucrose consumption, a detailed study of the hepatic utilization of long chain fatty acids in animals fed a high sucrose diet has not been conducted.

As shown in the study of Windmueller and Spaeth (11), perfused livers from rats fed a purified diet rich in sucrose secrete more triglyceride than livers from rats fed an ordinary stock (chow) diet. In view of the magnitude of this (11) and other (1–10, 12–15) responses to dietary sucrose, and their relation to our previous studies on metabolic and genetic factors that influence the hepatic production of triglyceride-rich lipoproteins (16–21), the concurrent metabolic conversions of oleic acid were comprehensively examined in isolated perfused livers from rats fed such a diet. Livers from stock diet-fed rats of the same age, sex, and genetic origin were perfused under identical conditions for comparison. Both basal rates of activity and responses to the infusion of oleic acid were determined. These studies revealed that, indeed, altered hepatic disposition of long chain free fatty acids is a causative factor in the liver and plasma triglyceride accumulation produced by a sucrose-rich diet.

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EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats from the Kyudo Co., Kumamoto, were housed at constant temperature (22°C) with lights turned on at 7:00 AM and off at 7:00 PM. Animals were maintained on a commercial rat diet (Type CE-2,Nihon Clea, Osaka) and water ad libitum. This diet was a mixture of natural sources and contained 24.8% protein, 4.4% fat, 3.5% fiber, 51.6% carbohydrate, 7.0% ash, and 8.7% water. Rats, as indicated later, were fed a semipurified high-sucrose diet for 10 days and water ad libitum. This diet was also obtained from Nihon Clea, and contained vitamin-free casein, 18%; corn oil, 5%; DL-methionine, 0.23%; sucrose, 70.2%; salt mixture, 6.2%; and vitamin mixture, 0.37%. The salt mixture contained the following, all listed in mg/100 g diet: calcium carbonate, 1,355.4; calcium dihydrogen phosphate, 1,500; potassium phosphate, monobasic, 1,730; magnesium sulfate, 800; sodium chloride, 600; iron (III) citrate, 190; zinc carbonate, 6.0; copper (II) sulfate, 1.26; cobalt (II) chloride, 0.4; potassium iodate; 1.54; and manganese (II) sulfate, 15.4. The total salt mixture was 6,200 mg/100 g diet. The vitamin mixture contained the following, all listed in mg/100 g diet: vitamin A • D_3, 2.4; vitamin E, 20; vitamin K_3, 0.3; thiamine, 1.5; riboflavin, 1.56; pyridoxine, 1.02; biotin, 0.5; calcium pantothenate, 4.0; *p*-aminobenzoic acid, 10.15; nicotinic acid, 10.15; inositol, 15; folic acid, 0.2; choline chloride, 300; and vitamin B_{12}, 5.0. The total vitamin mixture was 371.8 mg/100 g diet.

Liver perfusion

Liver perfusions were at 37°C as described previously (16, 17, 21). The recirculating medium (17) contained 90 ml of Krebs-Henseleit buffer (pH 7.4), 25 mM glucose, 1.5% fatty acid-free bovine serum albumin (Fraction V fatty acid-free, Miles Laboratories), and 30 ml of washed bovine erythrocytes. A priming dose of [1-^{14}C]oleic acid (25 dpm/nmol) substrate was added (5 ml of 20 mM potassium oleate in 0.9% NaCl) at the beginning of the perfusion and the same solution was infused continuously (4.5 ml/hr) for 225 min. The total quantity of oleic acid infused was, therefore, 437.5 μmol and the total radioactivity infused was 10,937,500 dpm. The radioactive oleic acid was procured from New England Nuclear (53.8 mCi/nmol). Total $^{14}CO_2$ was measured as described previously (21), except that the samples were added to ACS II (Amersham) scintillation cocktail and counted in an Aloka LSC-1000 liquid scintillation counter. Appropriate corrections for quenching were made.

Analytical methods

The methods employed for the lipid and ketone body analyses in the rat livers and liver perfusates were as described previously (16–18, 21). Statistical comparisons were calculated using Student's *t*-test with a two-tailed measurement of *P* values.

RESULTS

The animals and perfused livers in the present experiments are described in **Table 1.** The measured parameters were uniform in both groups except for the slight increase in total liver weights observed in rats fed the high-sucrose diet. This difference was significant only in the livers perfused in the absence of oleic acid substrate. Expression of the data in units/g liver provides a correction for small differences in liver weight.

A glucose concentration of 25 mM was perfused in the present and previous (17–21) studies. This is based on

TABLE 1. Animals and perfused livers

	Substrate[a]	Chow Diet (4)[b]	Sucrose Diet (4)	*P*
Body weight (g)	–	311 ± 4[c]	323 ± 7	NS[d]
	+	320 ± 8	318 ± 8	NS
Liver weight (g)[e]	–	13.8 ± 0.4	15.8 ± 0.7	<0.05
	+	13.7 ± 0.5	14.9 ± 0.5	NS
Stomach weight (g)	–	9.2 ± 1.4	8.1 ± 0.8	NS
	+	9.3 ± 0.9	9.0 ± 0.4	NS
Bile production (μl)[f]	–	2173 ± 265	1538 ± 81	NS
	+	2127 ± 223	1833 ± 180	NS
Perfusate flow (ml/min)[g]	–	17.0 ± 0.1	17.2 ± 0.1	NS
	+	17.3 ± 0.2	17.6 ± 0.3	NS

[a] Indicates perfusion with or without oleic acid substrate.
[b] Indicates the number of animals and perfused livers in each group.
[c] Standard error of the mean.
[d] NS, not significant.
[e] Determined at the end of the perfusion period.
[f] Entire volume produced in 225 min of perfusion.
[g] The flow rate was measured at 45-min intervals in each perfusion. The average rate in each perfusion was used to calculate the mean value.

a) the elevated glucose concentration in portal blood of fed animals relative to that commonly measured in peripheral blood, and *b*) the observation of Brunengraber, Boutry, and Lowenstein (22) that high concentrations of glucose are required to support physiological rates of fatty acid synthesis by the perfused liver. If the portal blood concentration does not reach 15 mM [the initial level of the plateau range of 15–25 mM reported by Brunengraber et al. (22)], the reason(s) for such a requirement by the perfused liver are not immediately explicable. Such a requirement may be related to the high levels of hepatic metabolites achieved under these conditions (22), the absence of certain plasma constituents in the synthetic perfusion medium, and/or the absence of the hepatic arterial circulation in the organ perfusion circuit. As summarized by Gardemann, Strulik, and Jungermann (23), in the traditional perfused liver system (in which the entire hepatic circulation is supplied via the portal vein) glucose balance is generally unresponsive to insulin. This hormone was therefore not included in the perfusion medium. It was observed, however, that when a portal-arterial glucose concentration gradient was established, insulin-dependent glucose uptake by the perfused liver occurred (23). These observations suggest the possibility that a portal glucose concentration less than 15 mM may support physiological rates of hepatic metabolism only in the presence of both insulin and a portal-arterial glucose concentration gradient.

When oleic acid was infused, ketogenesis was suppressed in livers from the rats fed the sucrose diet **(Fig. 1)**, as compared to the rate exhibited by livers from rats fed chow. Endogenous ketogenesis (the square symbols in Fig. 1) was similar in both groups. Accordingly, addition of the fatty acid substrate increased hepatic ketone body accumulation only 25% in the sucrose-fed group as compared to an elevation of over 100% in the chow-fed group. The differences between the dotted lines in Fig. 1 and the corresponding solid lines indicate the quantities of ketone bodies produced from endogenous sources when oleate was infused. Ketogenesis from endogenous sources clearly exceeded that from added fatty acid substrate in both groups. Since the liver can utilize ketone bodies (24–26), as discussed later, the observed accumulation of ketone bodies in the perfusate indicates net production. The rates of accumulation were constant after 45 min (Fig. 1) indicative of steady state conditions after this initial period. The observed burst in ketogenic activity at the beginning of the perfusion is probably caused by the priming dose of oleic acid, as this was not observed when the fatty acid substrate was not infused (square symbols in Fig. 1). The average β-hydroxybutyrate:acetoacetate ratios of perfusate ketone bodies at 45, 90, 135, 180, and 225 min in the absence of oleic acid substrate were 0.44, 0.51, 0.52, 0.55, and 0.60 in the chow group and 0.64^{b}, 0.68^{a}, 0.82^{c}, 0.88^{c}, and 0.95^{b}, in the sucrose group respectively. In the presence of oleic acid infusion the ratios were 0.69, 0.72, 0.73, 0.79, and 0.86 in the chow group and 0.53^{a}, 0.58, 0.66, 0.72, and 0.77 in the sucrose group, respectively. The a, b, and c superscripts indicate significant differences in ratios between diet groups at $P < 0.05$, 0.01, and 0.005, respectively. When chow versus chow plus oleic acid were compared, the ratios at 45 and 225 min were increased with $P < 0.01$ and $P < 0.05$, respectively. When sucrose versus sucrose plus oleic acid were compared, the values at 45, 135, and 180 min were decreased with $P < 0.05$ at each of these intervals. All livers exhibited ratios indicative of a satisfactory mitochondrial pyridine nucleotide redox state and, accordingly, a normal adenine nucleotide phosphorylation state.

Conversely, hepatic triglyceride secretion was promoted by the high sucrose diet **(Fig. 2).** This increase in secretory activity was prominent in livers perfused both with and without oleic acid. Comparison of the dotted lines

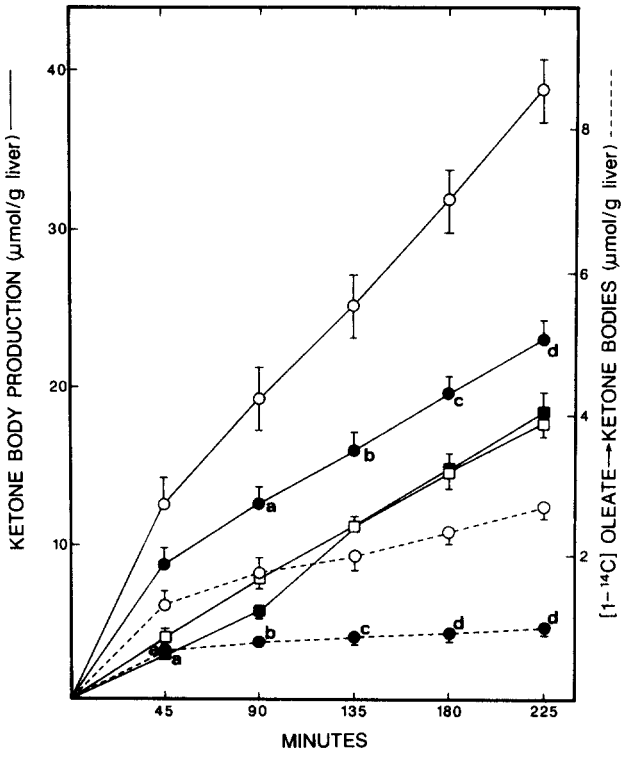

Fig. 1. Production of ketone bodies by the perfused rat livers. Livers from fed rats were perfused with Krebs-Henseleit buffer (pH 7.4) containing 25 mM glucose, 1.5% bovine serum albumin, with and without [1-^{14}C]oleic acid substrate (see Experimental Procedures), and 25% bovine erythrocytes in a recirculating system at a rate of 17 ml/min. The animals and livers are described in Table 1. Each group had four livers. Solid lines indicate total ketone body accumulation. Dotted lines indicate [1-^{14}C]oleic conversion to ketone bodies. Values designated a, b, c, and d are significantly different from the chow-fed rat liver values at $P < 0.05$, 0.01, 0.005, and 0.001, respectively. (□) Chow-fed, perfused without oleic acid substrate; (■) sucrose-fed, perfused without oleic acid substrate; (○) chow-fed, perfused with oleic acid substrate; (●) sucrose-fed, perfused with oleic acid substrate. Vertical bars indicate SEM.

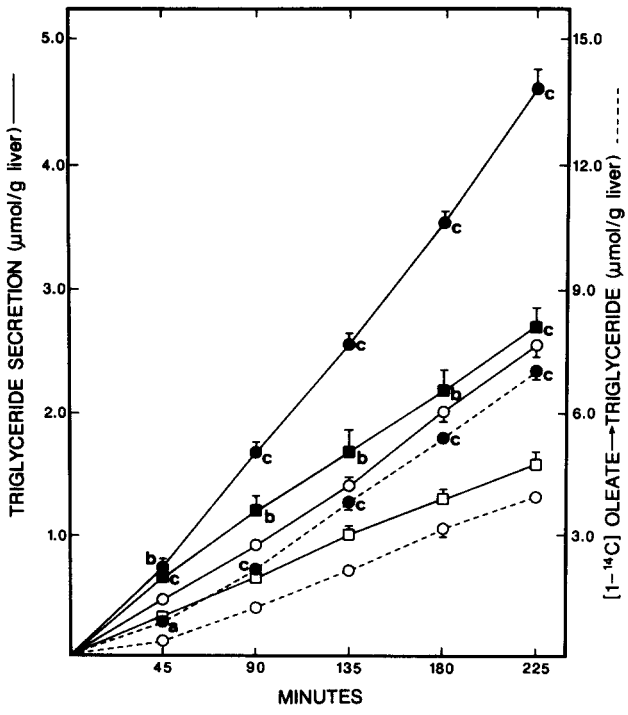

Fig. 2. Secretion of triglyceride by the perfused rat livers. The experiments are the same as those described in the legend to Fig. 1 and in Table 1. Solid lines indicate triglyceride accumulation in the perfusate. Dotted lines indicate [1-^{14}C]oleic conversion to perfusate triglyceride. The values designated a, b, and c are significantly different from the chow-fed rat liver values at $P < 0.05$, 0.005, and 0.001. (□) Chow-fed, perfused without oleic acid substrate; (■) sucrose-fed, perfused without oleic acid substrate; (○) chow-fed, perfused with oleic acid substrate; (●) sucrose-fed, perfused with oleic acid substrate. Vertical bars indicate SEM.

(radioactive triglyceride) in Fig. 2 with the corresponding solid lines (total triglyceride) demonstrates that 40–50% of the secreted triglyceride in both groups was derived from endogenous sources. Except for the expected lag period in the appearance of radioactive triglyceride in the perfusate, caused by the time required for the synthesis, assembly and intracellular transport of very low density lipoproteins, triglyceride secretion remained constant indicative of steady state conditions. Since the liver continually removes some lipids from the perfusate during the recirculation, the observed accumulation of triglyceride throughout the perfusion represents net secretion.

Cholesterol secretion by the perfused rat livers was not influenced by the dietary regimen **(Fig. 3)**. Cholesterol secretion was augmented by the infusion of oleic acid as expected, since the triglyceride-rich lipoproteins induced by fatty acid substrate (Fig. 2) contain cholesterol. Cholesterol secretion did not parallel triglyceride secretion exactly, however. Livers from rats fed the sucrose diet secreted lipoproteins which were relatively enriched with triglyceride in relation to cholesterol. Rates of cholesterol secretion were uniform throughout the entire 225 min of perfusion (Fig. 3).

The livers of rats fed the sucrose diet contained more triglyceride **(Table 2)**. The observed increase was substantial. These concentrations were measured at the end of the liver perfusion. Differences in the diet were without effect on the liver cholesterol content. The amounts of triglyceride in the livers exceeded net triglyceride secretion three- to fourfold in the various rat groups. In contrast, the amounts of cholesterol in the livers were 10–15 times greater than that which accumulated in the perfusion media. This may be attributed to differences in the intracellular compartmentation and transport of triglyceride, esterified cholesterol, and free cholesterol.

Uptake of the infused oleic acid by the perfused livers was identical in the two diet groups **(Table 3)**. Alterations in the utilization of the [1-^{14}C]oleate were therefore caused by differences in the rates of intracellular metabolic processes. More of the infused [1-^{14}C]oleic acid, namely 51.7 μmol more, was found in liver lipids in the sucrose-fed group at the end of the perfusion (Table 3). Other liver lipid esters were unaltered.

A balance of the utilization of [1-^{14}C]oleic acid by the

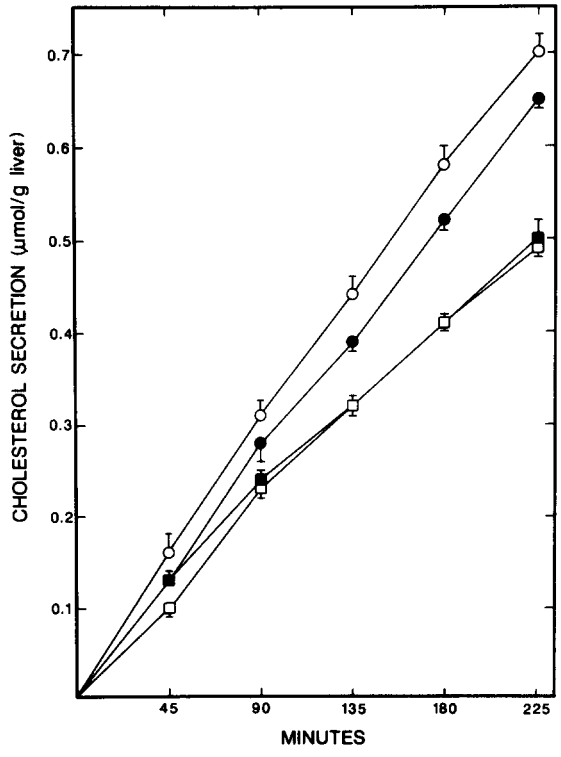

Fig. 3. Secretion of total cholesterol by the perfused rat livers. The experiments are the same as described in the legend to Fig. 1 and in Table 1. (□) Chow-fed, perfused without oleic acid substrate; (■) sucrose-fed, perfused without oleic acid substrate; (○) chow-fed, perfused with oleic substrate; (●) sucrose-fed, perfused with oleic acid substrate. Vertical bars indicate SEM.

TABLE 2. Lipid contents of the perfused rat livers

Diet	Substrate[a]	Triglyceride[b] (4)[c]	Cholesterol[b] (4)[c]
		μmol/g liver	*μmol/g liver*
Chow	–	5.88 ± 0.16[d]	7.04 ± 0.33
Sucrose	–	10.87 ± 0.54	6.99 ± 0.15
P		< 0.001	NS
Chow	+	9.21 ± 0.23	7.69 ± 0.18
Sucrose	+	13.01 ± 0.39	6.98 ± 0.27
P		< 0.001	NS

[a] Indicates perfusion with or without oleic acid substrate.
[b] Analyzed at the end of the 225-min perfusion period.
[c] Number of perfused livers in each group.
[d] Mean ± standard error of the mean.

perfused livers in all major pathways is presented in **Table 4**. Data are expressed in percentage of total utilization for comparison. The marked increase in conversion of infused oleate into secretory triglyceride was matched almost precisely by the increased conversion into liver triglyceride. Oxidation to both ketone bodies and carbon dioxide was depressed in similar increments. The undetermined products were largely water-soluble intermediates in the pathway of oxidation (17, 18, 21). The addition of ketone bodies, carbon dioxide, and undetermined products totaled 44.1% and 21.3% in the chow and sucrose groups, respectively. The esterification:oxidation ratios are therefore estimated to be 55.6:44.1 = 1.3 and 78.5:21.3 = 3.7 in these respective groups. In the livers from chow-fed rats, oleate esterification only slightly exceeded oleate oxidation. In the livers from sucrose-fed rats, oleate esterification exceeded oxidation by almost fourfold.

The effects of oleic acid substrate on hepatic ketogenesis, triglyceride storage, and lipid secretion under the two nutritional conditions are compared in **Table 5**. The β-hydroxybutyrate:acetoacetate ratios were increased by oleic acid in the chow group, as expected, since fatty acid oxidation generated reducing equivalents in the mitochondrial compartment. In contrast, oleic acid consistently lowered this ratio in the sucrose group (footnote *b* in Table 5). Although this decrease was undoubtedly related in part to the depression of fatty acid oxidation in these livers, other factors may be involved since ratios at all time intervals were less than the corresponding values observed in livers perfused in the absence of oleic acid. While oleic acid strongly promoted ketogenesis by the livers from chow-fed rats, the fatty acid had little effect on ketogenesis in the sucrose group. Thus, even though ketone body production by the livers from sucrose-fed rats was slightly stimulated by oleic acid (18.29 vs. 22.86 μmol/g per 225 min), inspection of the linear phases of the curves (Fig. 1, solid line, closed circles vs. solid line, closed squares) indicates that oleic acid was without significant effect in these livers following establishment of the steady state. In contrast, the provision of oleic acid clearly enhanced triglyceride secretion, cholesterol secretion, and triglyceride storage by the livers from both dietary groups (Table 5).

DISCUSSION

Consumption of the sucrose-rich diet produced pronounced alterations in the partition of long chain free fatty acids between the pathways of oxidation and esterification in the liver. Oxidation of oleic acid to both ketone bodies and carbon dioxide was depressed while esterification of the fatty acid to triglyceride was concurrently elevated. These metabolic changes promoted the secretion of triglyceride by the liver.

As calculated from the data in Fig. 1, specific radioactivities of ketone bodies produced by the livers from sucrose-fed rats were lower. Thus, 68.4% and 81.2% of the ketone bodies were derived from endogenous (unlabeled) sources in the chow-fed and sucrose-fed groups, respectively. This indicates greater mobilization of fatty acids from lipids present in the livers from sucrose-fed rats

TABLE 3. Uptake and incorporation of [1-^{14}C]oleate into hepatic lipid fractions

	Chow Diet (4)	Sucrose Diet (4)	*P*
	μmol/liver	*μmol/liver*	
Uptake of [1-^{14}C]oleate[a]	405.0 ± 1.3[b]	407.6 ± 1.7	NS
Incorporation of [1-^{14}C]oleate into hepatic lipids[c]			
Cholesterol ester	2.1 ± 0.1	2.2 ± 0.1	NS
Triglyceride	115.0 ± 2.2	166.7 ± 7.2	< 0.001
Free fatty acid	2.2 ± 0.3	1.5 ± 0.3	NS
Diglyceride	8.4 ± 1.2	6.7 ± 0.6	NS
Phospholipid	41.5 ± 1.3	36.2 ± 2.3	NS

[a] Calculated from the total amount of oleate infused (100 μmol of priming dose plus 90 μmol/hr) less the amount remaining in the perfusate at 225 min.
[b] All values are mean ± SEM with four livers in each group.
[c] As found in the livers at the end of the 225-min perfusion.

TABLE 4. Summary of conversions of infused oleic acid substrate by the perfused livers

	Oleate Utilization (% of total)		
	Chow Diet (4)	Sucrose Diet (4)	*P*
		%	
Products of esterification in perfusate			
Triglyceride	13.4 ± 0.7	25.6 ± 0.9	< 0.001
Diglyceride	0.2 ± 0.0	0.2 ± 0.0	NS
Phospholipid	0.5 ± 0.0	0.5 ± 0.1	NS
Cholesteryl ester	0.2 ± 0.0	0.2 ± 0.0	NS
Total	14.3 ± 0.9	26.5 ± 0.9	< 0.001
Products of esterification in liver			
Triglyceride	28.4 ± 0.6	40.9 ± 1.8	< 0.001
Diglyceride	2.1 ± 0.3	1.7 ± 0.1	NS
Phospholipids	10.3 ± 0.4	8.8 ± 0.5	NS
Cholesteryl ester	0.5 ± 0.0	0.6 ± 0.0	NS
Total	41.3 ± 0.8	52.0 ± 1.4	< 0.001
Products of oxidation			
Ketone bodies	9.6 ± 0.3	3.8 ± 0.5	< 0.001
Carbon dioxide	15.5 ± 0.6	9.2 ± 0.3	< 0.001
Total	25.1 ± 0.8	13.0 ± 0.7	< 0.001
Undetermined[a]	19.0 ± 1.3	8.3 ± 1.4	< 0.005

These values were calculated from the data in Fig. 1, Fig. 2, Table 2, thin-layer chromatographic analysis of perfusate lipids, and $^{14}CO_2$ collection. Results are expressed as percentages of the total [1-^{14}C]oleate utilized ± SEM; total utilization represents total [1-^{14}C]oleic acid infused less that recovered in the free fatty acid fraction at the end of the perfusion.

[a]These values refer to total utilization less that recovered in the various fractions above. The total recoveries of radioactivity were, therefore, 81.0% and 91.7% in the chow and purified diet groups, respectively.

which contain more triglyceride (Table 2). Triglyceride storage occurs in the form of cytoplasmic lipid droplets (27–33). The present observations, therefore, suggest greater mobilization of triglyceride from hepatocellular lipid droplets in the sucrose group. Although oxidation of [1-^{14}C]oleic acid to ketone bodies was depressed in the sucrose group, ketogenesis in the absence of added oleate was similar in both groups (Fig. 1, square symbols). This may also reflect elevated intracellular production of free fatty acids from the enlarged pool of lipid droplet triglyceride, and possibly also from increased de novo fatty acid synthesis, in the sucrose group. Thus, the increased esterification:oxidation ratio and increased supply of intracellular free fatty acids from endogenous sources affected ketogenesis in the sucrose group in an equal and opposite manner, resulting in similar rates of endogenous ketogenesis in both animal groups. From the data in Fig. 2, it can be calculated that the sucrose-fed rat liver perfusate triglycerides contained about twice as many endogenous fatty acids, relative to the chow-fed group, providing further evidence for increased mobilization of triglyceride fatty acids from the expanded population of liver cellular lipid droplets in the sucrose group.

Considering the total uptake of circulating free fatty acids by the liver, diversion of a rather small percentage of this total into the secretory pathway can produce a substantial increase in lipoprotein triglyceride secretion. Uptake of infused oleic acid was identical in both groups. Virtually all of the oleic acid removed by the livers was immediately metabolized since only 2% of that removed was recovered as free fatty acid (Table 3). It is evident that consumption of the sucrose diet caused about 12% more of the total oleic acid metabolized to be secreted in lipoprotein triglycerides (Table 4). A corresponding increase in oleic acid conversion to liver triglyceride was also found (Table 4). Therefore, the additional triglyceride molecules synthesized in the liver in response to the sucrose diet were transferred in approximately equal portions to intracellular lipid droplet storage reservoirs and to nascent very low density lipoproteins en route to secretion. Conversion of [1-^{14}C]oleic acid to secretory triglyceride was consequently increased 100%. The mass secretion data of Fig. 2 are in agreement. Thus, diversion of only 12% of the incoming free fatty acids in the hepatocyte to the process of lipoprotein assembly may double total lipoprotein triglyceride secretion.

Increased hepatic triglyceride synthesis, deposition, and secretion consequent to sucrose ingestion is clearly related to rapid hydrolysis of the disaccharide and fast absorption of the monomeric products. The literature is well documented with effects of diets high in glucose and fructose on plasma triglycerides (2–6, 8). It is therefore suggested that whenever high concentrations of readily metabolized monosaccharide sugars are present in the portal venous circulation of normal animals, multiple alterations in the metabolism of free fatty acids in the directions observed in the present study may be anticipated. The magnitude of the increase in triglyceride

TABLE 5. Effects of oleic acid substrate on ketogenesis, lipid secretion, and liver triglyceride content of perfused livers from rats fed chow and sucrose diets

	Chow Diet			Sucrose Diet		
	Oleate Substrate			Oleate Substrate		
	−	+	*P*	−	+	*P*
Ketone body production (μmol/g per 225 min)	17.43 ± 0.94	38.50 ± 1.86	< 0.001	18.29 ± 1.08	22.86 ± 1.26	< 0.05
β-hydroxybutyrate: acetoacetate ratio	0.52 ± 0.03[a]	0.76 ± 0.03[a]	< 0.001[b]	0.79 ± 0.06[a]	0.65 ± 0.04[a]	NS[b]
Triglyceride secretion (μmol/g per 225 min)	1.58 ± 0.09	2.55 ± 0.09	< 0.001	2.17 ± 0.15	4.60 ± 0.14	< 0.001
Cholesterol secretion (μmol/g per 225 min)	0.49 ± 0.01	0.70 ± 0.02	< 0.001	0.50 ± 0.02	0.65 ± 0.01	< 0.001
Liver triglyceride (μmol/g)	5.88 ± 0.16	9.21 ± 0.23	< 0.001	10.87 ± 0.54	13.01 ± 0.39	< 0.02

[a]These ratios were determined at each of the five 45-min intervals during each liver perfusion (see Results). Each value is the mean of these five values in each group.

[b]When statistically analyzed as paired values at each of the five 45-min intervals, oleic acid increased the ratio in the chow group ($P < 0.001$) and decreased the ratio in the sucrose group ($P < 0.001$).

production by the liver is influenced by several factors, including the type of monosaccharide carbohydrate, the insulin response, the dietary fat content, and the nutritional state of the animal prior to feeding. Several studies have shown fructose to be more effective than glucose (4, 8, 13, 34–38). While glucose is metabolized by all tissues, fructose is actively metabolized only in the liver (37), which potentiates the effects of fructose in this organ. The stimulatory effect of fructose on the hepatic production of very low density lipoprotein triglyceride was demonstrated by Schonfeld and Pfleger (39). The plasma insulin concentration affects both hepatic metabolism and release of free fatty acids from adipose tissue (4, 40, 41). The dietary fat content is an important modulator of hepatic fatty acid synthesis (4, 42–44). When the fat intake is low, fatty acid synthesis may increase markedly. Finally, in animals that are fasted prior to the consumption of a high-carbohydrate diet, the triglyceride synthetic response is accentuated (4, 7, 11, 45–47).

In addition to the increased fatty acid esterification:oxidation ratio, enhanced flow of monosaccharide sugars to the liver accelerates de novo fatty acid synthesis. In a hypercaloric state, when carbohydrate influx exceeds that which can be stored as glycogen and utilized for energy, excess substrate is converted to triglyceride. Induction of fatty acid synthesis by dietary carbohydrate is well known (48–50). Quantitative comparisons became possible with the advent of tritiated water incorporation into fatty acids for the measurement of mass flux. Thus, conversion of acetyl units into fatty acids by the livers from glucose-fed rats of Brunengraber et al. (22) was threefold greater than that observed in their chow-fed group.

The relative contributions of increased conversion of plasma free fatty acids to triglyceride, via *a*) elevated esterification:oxidation ratio and *b*) increased de novo fatty acid synthesis, to increased liver and plasma triglyceride in the sucrose-fed animal have not been quantified. Based on observations in livers of lean and obese Zucker rats (20), the contribution of fatty acid synthesis to construction of triglyceride-rich lipoproteins is estimated to be about 10% in the normal chow-fed rat. In the hypercaloric, genetically obese Zucker rat, the contribution of fatty acid synthesis is approximately 45% (20). Rats fed diets high in rapidly absorbed sugars may approach this level and even exceed it when the fat content of the diet is sufficiently low to allow fatty acid synthesis to proceed at an unrestricted rate. The stage of diurnal feeding and the presence or absence of a prefasting period, as mentioned above, influence this rate. Of interest in this regard is the extremely rapid rate of triglyceride synthesis apparently achieved in the animals of Kempen, Solterik, and de Lange (47), when fasted 48 hr and then refed a high glucose fat-free diet.

Acetoacetate can be utilized in the liver for fatty acid synthesis and cholesterogenesis, the latter of which appears to be of greater significance (24–26). The specific radioactivity of ketone bodies in the present study was somewhat less than that found in perfusate triglyceride. Conversion of acetoacetate to fatty acids would therefore not cause isotope enrichment. Conversion of 5 μmol of acetyl units/g of dry liver wt per 90 min in perfused livers from fed rats has been reported (25). When converted totally to triglyceride, this value is 0.16 μmol of triglyceride/g of wet liver per 225 min. In fact, a considerable proportion is converted to phospholipid (18, 20). This value may be compared with the observed overall rate of triglyceride synthesis. Triglyceride secretion was 2.55 μmol/g of liver per 225 min (Fig. 2). Considerably greater amounts of newly synthesized triglyceride were recovered in the liver. Therefore, acetoacetate conversion to fatty acids could account for only a minute fraction of the liver triglyceride synthesized in the present experiments.

In view of the elevated fatty acid esterification:oxidation ratio and the enhanced net secretion of triglyceride-rich lipoprotein by livers from the sucrose-fed rats, these livers provide a more sensitive model system

for studies on substrates, hormones, and drugs which *a*) increase fatty acid oxidation, *b*) decrease triglyceride synthesis, *c*) decrease the formation and/or secretion of very low-density lipoproteins, and/or *d*) promote the uptake of circulating triglyceride-containing lipoprotein species by the liver. Thus, agents under examination may exert greater effects in such livers than in livers from animals fed a standard chow, or stock, diet.

The natural chow and high sucrose purified diets fed to rats in the current study differ in several respects. The major difference is in the type of carbohydrate, starch in the chow and sucrose in the purified diet. Other differences include sources of protein, fiber content, and levels of some micronutrients. The chow diet also contains natural products not added to the purified diet. The literature cited earlier (1–15) strongly indicates that the observed alterations in fatty acid and triglyceride metabolism were caused by the high level of sucrose in the purified diet in contrast to the high starch content of the natural diet. Nevertheless, it remains possible that some other difference in these diets was contributory. The present study was not designed to provide a strict comparison of dietary starch and sucrose, but to compare a natural chow diet and a purified diet high in the rapidly digestible carbohydrate sucrose. These two types of diets are most commonly employed in metabolic studies. Sucrose has long been chosen as the carbohydrate source in a purified diet because of its high purity and low cost. Both of these rations contain an excess of essential nutrients for the rat and support normal growth, reproduction, and lactation in this animal species.

The following reconstruction of metabolic events in the hepatocyte, in response to consumption of the high sucrose diet, is suggested. *1*) Increased glycolytic conversion of glucose and fructose to pyruvate, and subsequently to citrate, with conversion of excess citrate to long chain fatty acids. *2*) Increased energy production from the flux of carbohydrate to pyruvate and citrate, and greater oxidation of acetyl-CoA of carbohydrate origin in the Krebs cycle. *3*) Increased formation and concentration of malonyl-CoA as a result of increased flux of citrate to fatty acids. *4*) Suppression of fatty acid oxidation via increased ATP synthesis from carbohydrate combustion (36, 51) and inhibition of carnitine palmitoyltransferase by malonyl-CoA (52, 53). *5*) With continuation of fatty acid uptake unabated, stimulation of triglyceride synthesis via increased availability of fatty acid substrate. *6*) Greater transfer of triglyceride to cytoplasmic lipid droplets and very low density lipoprotein particles in the endoplasmic reticulum, resulting in *7*) greater deposition of triglyceride in the liver and increased secretion of triglyceride-rich lipoproteins.

The present results indicate that an altered partition of free fatty acids in the liver between oxidation and esterification significantly contributes to the increased triglyceride synthesis, accumulation, and secretion in animals consuming large amounts of sucrose. It is anticipated that high levels of dietary glucose and fructose produce the same alterations. Although the relative contributions of the increased hepatic free fatty acid esterification:oxidation ratio and increased hepatic fatty acid synthesis are not established, these two processes are probably the major factors responsible for the accelerated liver triglyceride synthesis and secretion and the elevated plasma triglyceride concentrations observed in such animals.

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