Metabolic factors underlying high serum triglycerides in the normal hamster

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Abstract Comparative lipid metabolism of rats and hamsters was investigated to determine the metabolic basis for the relatively high concentrations of serum triglycerides in the hamster. It was found that serum free fatty acids (FFA) in the hamster are higher than in the rat in the fed condition. In addition, a higher percentage of the fatty acids esterified in the liver of the hamster is utilized for triglyceride synthesis. These factors combine to elevate hepatic triglyceride synthesis in the hamster. However, triglyceride does not accumulate in the liver in these animals in the fed state. In fact, liver triglycerides are lower in the fed hamster than in the fed rat, and the hamster stores much less triglyceride in liver lipid droplets than does the rat in this nutritional state. Most of the liver trigylceride in fed hamsters is present in dense particles corresponding to vesicular lipoprotein triglyceride in the secretory pool. In isolated organ perfusion experiments hamster livers exhibited greater net triglyceride secretion than did rat livers. Serum triglycerides in the hamster remain elevated in the fasting state. In this condition the high proportion of free fatty acids utilized for liver triglyceride synthesis, relative to that incorporated into hepatic phospholipids, persists in the hamster and marked liver triglyceride accumulation occurs. Lipid droplets are extremely abundant in these livers. The present study implicates increased conversion of free fatty acids to triglyceride in the liver and increased hepatic production of very low density lipoproteins (VLDL) in the hamster in the genesis of the hyperglyceridemia characteristic of this species. —**Ontko, J. A., Q. Cheng, and M. Yamamoto.** Metabolic factors underlying high serum triglycerides in the normal hamster. *J. Lipid Res.* 1990. **31:** 1983-1992.

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The hamster has received increased attention in recent years as an animal model for studies in lipid metabolism. This may be attributed to the fact that cholesterol and bile acid metabolism in the hamster resembles that in the human more closely than does steroid metabolism in the rat (1-7).

The serum triglyceride concentrations in the hamster are considerably higher than those in the rat, based on several reports (8-15) in which the levels in hamsters ranged above those found in numerous studies with rats (16-23). While the comparative metabolism of cholesterol in rats and hamsters has been extensively examined (2-4, 6, 7), triglyceride metabolism in these species has not been systematically compared.

In the present study the elevated level of serum triglyceride in the hamster was verified and the metabolic basis for this difference was investigated. Results implicate alterations in the supply of plasma free fatty acids (FFA), differences in hepatic triglyceride synthesis and, consequently, increased production of triglyceride-rich lipoproteins by the liver in the development of the hyperglyceridemia exhibited by these animals.

MATERIALS AND METHODS

Animals

Male Holtzman rats and male Golden Syrian hamsters were obtained from Sasco, Inc. (Omaha, NE). These animals were given water and Purina Rodent Chow ad libitum for at least 1 week prior to the various experiments. The chow diet, (#5001, Purina Mills, Inc., St. Louis, MO) contained crude protein not less than 23.0%, crude fat not less than 4.5%, crude fiber not more than 6.0%, ash not more than 8.0%, and added minerals not more than 2.5%. All animals were given free access to water and were housed at constant temperature (22 °C with lights on at 6 AM and off at 6 PM). Fed animals were fed ad libitum until used for experiment. Fasted animals were fasted 24 h. At the time of killing (8 AM-10 AM) animals were given diethyl ether anesthesia. Blood was removed from the abdominal vena cava. The animals were then exsanguinated by severing the abdominal vena cava. Livers were immediately removed and plunged into ice-cold saline. After cooling, the nonhepatic tissue was removed, the livers were blotted dry, weighed, and wrapped in Saran wrap and frozen until analysis. Blood

Abbreviations: FFA, free fatty acids; VLDL, very low density lipoproteins.

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was allowed to clot for 30 min before centrifugation to obtain serum.

Lipid analyses

Serum was analyzed for triglyceride by the spectrophotometric analysis of glycerol generated via lipase-catalyzed hydrolysis (24). In this procedure H_2O_2 is produced via glycerol phosphate oxidase and a pink chromophore develops via reaction of H_2O_2 with 4-aminoantipyrene and *p*-chlorophenol. Total cholesterol was analyzed by an enzymatic cholesterol oxidase method after hydrolysis of cholesteryl esters with cholesterol esterase. In this kit assay procedure H_2O_2 is generated and reacts with 4-aminoantipyrene and phenol to produce a pink color (#65064/93, EM Diagnostic Systems, Gibbstown, NJ, procured from Scientific Products, McGaw Park, IL). Liver triglyceride was analyzed as previously described (25), except that a Zeolite mixture (#990-2, Sigma Chemical Co., St. Louis, MO) was used instead of silicic acid to remove phospholipids. Free fatty acids in serum were measured with ^{63}Ni according to Khoo, Miller, and Goldberg (26). In this procedure for triglyceride, samples that are not incubated with the lipase buffer may be directly analyzed for FFA.

In the experiments in which a labeled fatty acid was injected intraperitoneally into the animals, [9,10-^{3}H]palmitic acid or [9,10-^{3}H]oleic acid (New England Nuclear, Boston, MA) in albumin-bound form was administered into conscious rats and hamsters. The dose was 80 μCi/100 g body weight. The solution injected contained 160 μCi of the labeled fatty acid/ml of 1% bovine serum albumin (Fraction V fatty acid-free, ICN ImmunoBiologicals, Lisle, IL) in 0.9% NaCl. The specific radioactivity of the injected fatty acid was 2 μCi/nmol and the concentration of the fatty acid in the injected solution was 0.08 mM. When the fatty acid was injected intravenously, the dose was 30 μCi/100 g body wt. The solution injected contained 200 μCi of [9,10-^{3}H]palmitic acid/ml of 3% bovine serum albumin in 0.9% NaCl. The specific radioactivity of the injected fatty acid was 0.8 μCi/nmol and the concentration of the fatty acid in the injected solution was 0.25 mM. Intravenous injections were made into the hepatic portal vein of anesthetized animals immediately following abdominal incision under ether anesthesia. The livers were removed as described above, at the times indicated in each experiment, and homogenized in 4 volumes of cold saline. The 20% homogenates were then extracted in chloroform–methanol 2:1, analyzed for triglyceride content, as described above, and for radioactivity in phospholipids, diglycerides, free fatty acids, triglycerides, and cholesteryl esters, as previously described (25, 27, 28).

Separation of liver triglyceride pools

The subpopulations of lipid droplets and nascent VLDL particles in the rat and hamster livers were separated by a discontinuous sucrose density gradient centrifugation procedure (24). In this separation technique bands A–F and Zone G are harvested in order of increasing density. Band A is found at the upper surface of the water layer at the top of the gradient. Bands B–F are found at the interfaces of the water–10% sucrose, 10%–25% sucrose, 25%–35% sucrose, 35%–44% sucrose, and 44%–52% sucrose layers, respectively. Zone G is the entire 65% sucrose layer at the bottom of the gradient, and includes the interface between the 52% and 65% sucrose layers.

Liver perfusion experiments

Livers were perfused at 37°C as described previously (25, 29, 30). The rat livers were perfused with a recirculating medium that contained 90 ml of Krebs-Henseleit buffer (pH 7.4), 25 mM glucose, 1.5% fatty acid-free bovine albumin (Fraction V fatty acid-free, ICN ImmunoBiologicals, Lisle, IL), and 30 ml of washed bovine erythrocytes. A priming dose of oleic acid substrate was added (5 ml of 20 mM sodium oleate in 0.9% NaCl) at the beginning of the perfusion and the same solution was infused continuously (4.5 ml/h) for 180 min. The oleate concentration in the perfusion medium at 1, 2, and 3 h was 0.17–0.25 mM. The hamster livers, which were approximately one-half the size of the rat livers, were perfused under identical conditions with one-half of the volume of perfusion medium (45 ml of Krebs-Henseleit buffer, pH 7.4, 25 mM glucose, 1.5% fatty acid-free bovine albumin, and 15 ml of washed bovine erythrocytes) and one-half as much oleic acid substrate (2.5 ml of 20 mM sodium oleate in 0.9% NaCl as priming dose and a continuous infusion of 2.25 ml/h of the same solution) to maintain identical FFA concentrations.

Analysis of results

The significance of differences was evaluated by Student's *t* test with a two-tailed measurement of *P* values.

RESULTS

The fed and fasted rats and hamsters were maintained under equivalent nutritional and environmental conditions. The liver and serum lipid concentrations are shown in **Table 1**. Serum triglycerides in both fed and fasted hamsters greatly exceeded the corresponding levels in rats. Hamsters also exhibited much higher serum cholesterol, as previously documented (2–4, 6, 7). Fasting did not lower the serum triglycerides in hamsters as markedly as in rats. Liver triglycerides in fed hamsters were lower than in fed rats. In sharp contrast, liver triglycerides in fasted hamsters greatly exceeded the fasting rat liver triglycerides.

The metabolic basis for the relatively high concentrations of serum triglycerides in hamsters was then examined. Since circulating FFA are major precursors of triglyceride rich lipoproteins of hepatic origin, serum FFA were measured in the subsequent experiment (**Fig. 1**). The serum FFA were much higher in hamsters than in rats under ad libi-

TABLE 1. Serum and liver lipids in fed and fasted rats and hamsters

	Rats	Hamsters	P
Serum triglycerides (mg/dl)			
Fed	111.7 ± 4.8 (30)[a]	224.5 ± 7.9 (33)[b]	< 0.001
Fasted	50.7 ± 2.6 (31)[c]	181.2 ± 12.6 (31)[d]	< 0.001
P	< 0.001	< 0.01	
Serum cholesterol (mg/dl)			
Fed	45.6 ± 1.9 (31)[e]	157.5 ± 3.4 (30)[f]	< 0.001
Fasted	37.9 ± 1.4 (31)[g]	151.4 ± 6.8 (21)[h]	< 0.001
P	< 0.005	NS	
Liver triglycerides (μmol/g)			
Fed	6.63 ± 0.26 (30)[a]	3.60 ± 0.08 (33)[b]	< 0.001
Fasted	5.91 ± 0.53 (20)[i]	14.28 ± 1.72 (20)[j]	< 0.001
P	NS	< 0.001	

All values are mean ± SEM. Values in parentheses indicate the number of animals in each group.
[a] Body wt 301.5 ± 2.9 g; liver wt. 11.2 ± 0.2 g.
[b] Body wt 124.9 ± 0.9 g; liver wt. 5.6 ± 0.1 g.
[c] Body wt 265.7 ± 7.6 g.
[d] Body wt 122.8 ± 2.2 g.
[e] Body wt 339.7 ± 4.8 g.
[f] Body wt 123.8 ± 2.1 g.
[g] Body wt 257.3 ± 12.8 g.
[h] Body wt 120.2 ± 3.1 g.
[i] Body wt 266.9 ± 5.1 g; liver wt. 7.5 ± 0.2 g.
[j] Body wt 126.8 ± 3.2 g; liver wt. 4.3 ± 0.1 g.

tum feeding conditions. Fasting elevated the serum FFA to a greater extent in rats than in hamsters. In both species, after 24 h and 48 h without food, the serum FFA concentrations were similar.

The utilization of free fatty acids in the hepatic synthesis of triglycerides and other lipids, notably phospholipids, was then examined. Labeled palmitic acid was injected intraperitoneally into fed, conscious animals. After periods of 5, 10, 15, 20, and 30 min, the livers were removed from the different groups of animals for analyses of lipid content and radioactivity. The relative amounts of the labeled FFA that were esterified into triglycerides and phospholipids are shown in **Fig. 2**. The labeled FFA conversion to triglyceride in the livers of hamsters was 2–4 times greater than that utilized for phospholipid synthesis. At all times this preferential conversion into triglycerides was greater than that observed in rats. This partition of palmitic acid esterification between the various liver lipids was also measured in fasted rats and hamsters in vivo. The ratio of [9, 10-^{3}H]palmitic acid incorporation into triglycerides and phospholipids, 20 min after intraperitoneal injection of the labeled fatty acid, was 1.91 ± 0.13 in fasted rats (body wt 287.8 ± 4.6 g; n = 6) and 5.21 ± 0.30 in fasted hamsters (body wt 127.9 ± 1.3 g; n = 6). The difference in the triglyceride:phospholipid incorporation ratio was significant ($P < 0.001$).

To determine whether this difference in the esterification of FFA in the livers of rats and hamsters was peculiar for palmitic acid, similar experiments were conducted with [9, 10-^{3}H]oleic acid. A marked difference in the esterification of oleic acid by rats and hamsters was also observed (**Table 2**). The utilization of oleic acid for triglyceride synthesis relative to its conversion into phospholipids was much greater than that of palmitic acid (Fig. 2) in both species.

Hepatic fatty acid esterification was also compared in fed rats and hamsters when the fatty acid was administered intravenously. The same enhancement of fatty acid incorporation into liver triglycerides was again observed in the hamster (**Table 3**). Recovery of the injected fatty acid in liver lipids was, of course, much higher in this experiment, in which the labeled fatty acid was injected directly into the portal vein, than when the fatty acid was administered intraperitoneally.

The triglycerides in liver cells are present in several metabolically distinct pools (24, 31–34). The two major pools are lipid droplets and nascent VLDL particles. The triglyceride pools in rat and hamster livers were examined with a gradient centrifugation procedure developed to separate subpopulations of lipid droplets (24). Marked differences were observed (**Table 4**). The most prominent differences were the greater quantities of triglyceride in lipid droplet bands A–E in the rat and the greater relative abundance of triglyceride in the dense fractions F and G in the hamster, as exhibited by the high percentage of total liver triglyceride in these fractions. Thus, 18% and 65% of the total triglyceride in the hamster livers were recovered in fractions F and G versus only 7% and 28%, respectively, in the rat livers.

Since fasting caused a marked increase in liver triglyceride in the hamster, while plasma triglycerides remained ele-

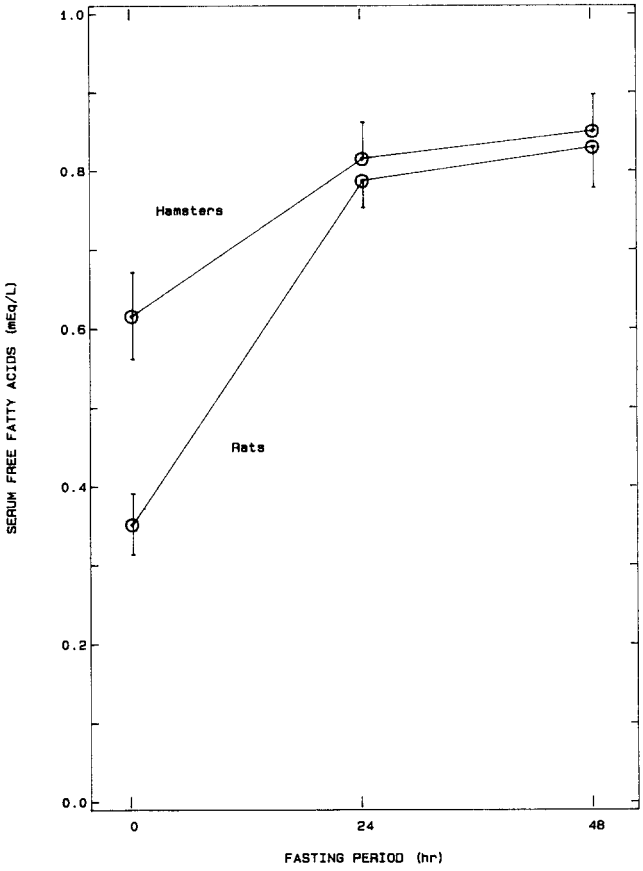

Fig. 1. Serum free fatty acids in fed and fasted rats and hamsters. Food was removed from fasted animals betwen 8:00 AM and 9:00 AM. Blood was also drawn at this time for FFA analysis with ^{63}Ni. The body weights of the animals at the time of blood sampling were 336.5 ± 6.4 g, 299.5 ± 9.1 g, and 277.3 ± 7.0 g for rats fed, fasted 24 h, and fasted 48 h, respectively, and 122.8 ± 7.1 g, 120.2 ± 3.1 g, and 110.4 ± 2.6 g for hamsters fed, fasted 24 h, and fasted 48 h, respectively. There were 21 animals in each group, except the fed hamsters, which contained 20. The vertical bars indicate ± SE. Fed hamsters exhibited significantly higher serum FFA than fed rats ($P < 0.001$).

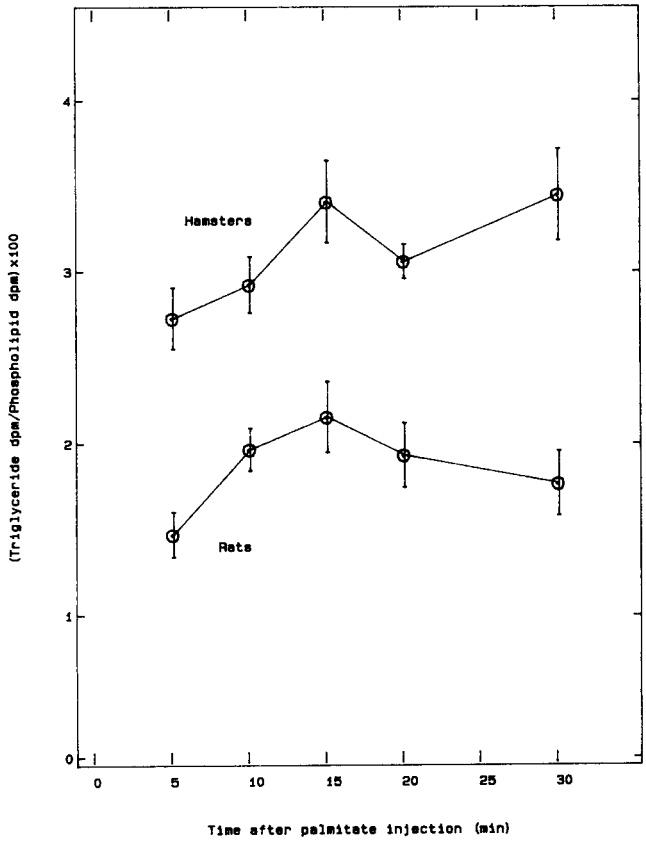

Fig. 2. Ratio of palmitic acid incorporated into triglycerides to that incorporated into phospholipids in livers of rats and hamsters in vivo. Fed conscious animals were injected intraperitoneally with [9, 10-^{3}H]palmitic acid bound to bovine albumin (See Materials and Methods). Livers were removed after injection at the times shown for lipid analysis. Each group contained five to seven animals. Vertical bars indicate ± SE. The incorporation ratios were significantly higher in hamsters than in rats at all times (at 5, 10, 15, 20, and 30 min, P values were <0.001, <0.001, <0.005, <0.001, and <0.001, respectively). The average dpm of tritium recovered in total rat liver triglycerides was 8,032 × 10^3, 14,070 × 10^3, 18,322 × 10^3, 27,529 × 10^3, and 16,260 × 10^3 (equal to 717 ± 197, 1272 ± 193, 1670 ± 369, 2307 ± 443, and 1511 ± 351 × 10^3 dpm/g liver, respectively) at 5, 10, 15, 20, and 30 min, respectively, and in the hamsters the corresponding values were 4,235 × 10^3, 8,051 × 10^3, 12,548 × 10^3, 11,734 × 10^3 and 11,473 × 10^3 dpm (equal to 687 ± 184, 1533 ± 321, 2261 ± 452, 2119 ± 479, and 2115 ± 492 × 10^3 dpm/g liver, respectively). These amounts represent 49.1 ± 2.9%, 58.6 ± 2.0%, 62.3 ± 1.7%, 61.0 ± 2.2%, and 58.4 ± 2.0% of the total liver lipid radioactivity in the five consecutive rat groups and 66.7 ± 1.5%, 69.6 ± 1.1%, 73.0 ± 1.3%, 71.7 ± 0.8%, and 74.2 ± 1.5% of the total liver lipid radioactivity in the corresponding hamster groups. The dpm of tritium recovered in total liver phospholipids in the five consecutive rat groups averaged 5,355 × 10^3, 7,130 × 10^3, 9,016 × 10^3, 14,342 × 10^3, and 10,401 × 10^3 (equal to 482 ± 142, 650 ± 91, 817 ± 189, 1198 ± 203, and 967 ± 295 × 10^3 dpm/g liver, respectively) and in the hamsters the corresponding values were 1,607 × 10^3, 2,872 × 10^3, 3,775 × 10^3, 3,907 × 10^3, and 3,292 × 10^3 dpm (equal to 261 ± 66, 551 ± 131, 688 ± 142, 698 ± 149, 610 ± 125 × 10^3 dpm/g liver, respectively). These amounts represent 34.0 ± 1.7%, 30.2 ± 1.2%, 29.6 ± 1.9%, 32.6 ± 2.0%, and 34.1 ± 2.5% of the total liver lipid radioactivity in the five consecutive rat groups and 24.9 ± 1.2%, 24.1 ± 1.0%, 21.9 ± 1.2%, 23.6 ± 0.7%, and 22.1 ± 1.3% of the total liver lipid radioactivity in the corresponding hamster groups. The rat body weights were 310.0 ± 7.2, 300.8 ± 6.7, 296.4 ± 8.4, 290.5 ± 4.5, and 298.4 ± 2.9 g, respectively. The hamster body weights were 129.1 ± 1.9, 124.0 ± 1.4, 123.0 ± 1.3, 124.7 ± 2.9, and 124.1 ± 1.6 g, respectively. The corresponding rat liver weights were 11.2 ± 0.5, 10.9 ± 0.5, 11.1 ± 0.4, 12.0 ± 0.2, and 10.6 ± 0.5 g and the hamster liver weights were 6.2 ± 0.2, 5.5 ± 0.2, 5.7 ± 0.3, 5.4 ± 0.4, and 5.5 ± 0.2 g, respectively. All values are the mean ± SE.

vated in these animals (Table 1), the liver triglyceride pools in fasting rats and hamsters were also examined (**Table 5**). Sucrose density gradient centrifugation of homogenates of livers from 24 h fasted hamsters demonstrated a massive (16-fold) increase in triglyceride in band A [335 nmol/g (Table 3) vs 5498 nmol/g (Table 5)], demonstrating a proliferation of lipid droplets. Much more triglyceride was found in all liver triglyceride subpopulations (gradient fractions A–G) in fasted hamsters than in fasted rats (Table 5), indicative of greater concentrations of both storage and secretory triglycerides in these hamsters. Comparison of the values in Table 4 with those in Table 5 indicate that while fasting altered only band E in the rat ($P < 0.005$), fasting increased all triglyceride fractions in the hamster (P values of < 0.005 for bands A, B and D, < 0.001 for bands C and F, Zone G and the total, and < 0.02 for band E).

Three metabolic characteristics observed in the fed hamster, namely *i*) elevated serum FFA (Fig. 1), *ii*) increased

TABLE 2. Relative incorporation of [9, 10-^{3}H]oleic acid into liver triglycerides and phospholipids in rats and hamsters in vivo

	Rats (6)[a]	Hamsters (6)[a]	*P*
Body wt, g, fed	284.0 ± 3.8[b]	126.2 ± 0.8	< 0.001
Body wt, g, fasted	271.3 ± 6.8	128.9 ± 1.6	< 0.001
Serum TG, mg/dl, fed	124.7 ± 8.2	262.0 ± 15.0	< 0.001
Serum TG, mg/dl, fasted	49.0 ± 4.5	144.4 ± 3.3	< 0.001
	< 0.001	< 0.001	
Liver TG, μmol/g, fed	6.87 ± 0.86[c]	3.41 ± 0.27[c]	< 0.005
Liver TG, μmol/g, fasted	5.39 ± 0.46[c]	20.14 ± 2.30[c]	< 0.001
	NS	< 0.001	
TG dpm ÷ PL dpm, fed	5.52 ± 0.46[d]	10.53 ± 0.89[d]	< 0.001
TG dpm ÷ PL dmp, fasted	5.46 ± 0.45[d]	16.54 ± 1.31[d]	< 0.001
	NS	< 0.005	
TG dpm/g liver, fed	1018 ± 168[e]	1509 ± 220[e]	NS
TG dpm/g liver, fasted	675 ± 90[e]	1231 ± 248[e]	NS
PL dpm/g liver, fed	188 ± 30[e]	148 ± 20[e]	NS
PL dpm/g liver, fasted	131 ± 23[e]	78 ± 18[e]	NS

The [9, 10-^{3}H]oleic acid was administered intraperitoneally into conscious animals (See Materials and Methods). Animals were anesthetized with ether 18 min later. Blood and livers were removed 20 min after isotope injection. The average amounts of tritium recovered in total liver triglycerides in the fed rats, fasted rats, fed hamsters and fasted hamsters were 11,825 × 10^3 dpm, 5,183 × 10^3 dpm, 8,845 × 10^3 dpm, and 5,201 × 10^3 dpm, respectively. These amounts represent 76.4 ± 1.4%, 71.2 ± 1.7%, 87.6 ± 0.6%, and 90.0 ± 0.3% of the total liver lipid radioactivity in the fed rats, fasted rats, fed hamsters, and fasted hamsters, respectively. The corresponding values for liver phospholipids were 2,174 × 10^3 dpm, 1,008 × 10^3 dpm, 842 × 10^3 dpm, and 328 × 10^3 dpm, which represent 14.3 ± 1.1%, 13.3 ± 0.6%, 8.6 ± 0.6% and 5.6 ± 0.4%, respectively, of the total liver lipid radioactivity in these four animal groups.

[a] Number of animals in each group.

[b] All values are the mean ± SEM.

[c] The liver weights were: fed rats 11.7 ± 0.2 g; fasted rats 7.6 ± 0.2 g; fed hamsters 5.9 ± 0.2 g; fasted hamsters 4.2 ± 0.2 g (mean ± SEM).

[d] TG dpm/g liver ÷ PL dpm/g liver.

[e] All values have been multiplied by 10^{-3}.

proportion of FFA utilized in the liver for triglyceride synthesis (Fig. 2, Tables 2, 3), and *iii*) lack of triglyceride accumulation in these livers (Tables 1-4), all favor increased hepatic production of VLDL in these animals. Direct examination of triglyceride secretion by perfused livers isolated from rats and hamsters was therefore conducted. When these livers were perfused under the same conditions, triglyceride accumulated at a greater rate in the hamster liver perfusates (**Fig. 3**), demonstrating greater net secretion of triglyceride-rich lipoproteins by these livers. The hamster

TABLE 3. Relative incorporation of intravenous [9, 10-^{3}H]palmitic acid into liver triglycerides and phospholipids in fed rats and hamsters in vivo

	Rats (8)[a]	Hamsters (8)[a]	*P*
Body wt, g	269.0 ± 3.2[b]	104.3 ± 2.9[b]	< 0.001
Liver wt, g	11.1 ± 0.4	5.0 ± 0.3	< 0.001
Liver TG, μmol/g	5.19 ± 0.52	2.60 ± 0.37	< 0.005
Liver TG dpm ÷ Liver PL dpm	2.26 ± 0.16	3.24 ± 0.20	< 0.005
Liver TG, dpm × 10^{-3}/g liver	4,585 ± 427	5,932 ± 396	< 0.05
Liver PL, dpm × 10^{-3}/g liver	2,039 ± 132	1,840 ± 84	NS

[9, 10-^{3}H]Palmitic acid (30 μCi/100 g body wt) was injected into the hepatic portal vein exposed by abdominal incision in animals under diethyl ether anesthesia. Livers were removed 5 min later for analysis. The average amounts of tritium recovered in total liver triglycerides were 51,286 × 10^3 dpm ± 5449 × 10^3 dpm and 29,037 × 10^3 dpm ± 1871 × 10^3 dpm in the rats and hamsters, respectively, which were equivalent to 62.63 ± 2.17% and 67.55 ± 2.49%, respectively, of the total liver lipid radioactivities in these animals. The corresponding values for phospholipids were 22,811 × 10^3 dpm ± 1836 × 10^3 dpm and 9003 × 10^3 dpm ± 349 × 10^3 dpm, which were equivalent to 28.47 ± 1.56% and 21.16 ± 0.95% respectively, of the total liver lipid radioactivities in these animals.

[a] Number of animals in each group.

[b] All values are the mean ± SEM.

TABLE 4. Density gradient separation of liver triglyceride pools in rats and hamsters fed ad libitum

Band[a]	Triglyceride			
	Rats (6)[b]		Hamsters (6)[b]	
	nmol/g[c]	%	*nmol/g*[c]	%
A	3310 ± 393[d]	53.3 ± 2.7[d]	335 ± 51[e]	11.9 ± 1.6[e]
B	144 ± 25	2.3 ± 0.3	34 ± 8[f]	1.1 ± 0.2[g]
C	152 ± 32	2.4 ± 0.3	17 ± 6[f]	0.5 ± 0.2[e]
D	162 ± 23	2.6 ± 0.2	26 ± 8[e]	0.9 ± 0.2[e]
E	254 ± 40	4.1 ± 0.4	94 ± 19[f]	2.8 ± 0.5
F	428 ± 44	7.1 ± 0.6	513 ± 44	18.4 ± 1.2[e]
Zone G	1755 ± 278	28.4 ± 2.6	1885 ± 175	64.3 ± 2.2[e]
Total	6207 ± 682		2900 ± 239[f]	

[a] Sucrose gradients were constructed above 20% liver homogenates in 65% sucrose and centrifuged at 12,500 rpm at 4°C for 30 min. Band A is at the top. Bands B–F are at interfaces of the water–10% sucrose, 10%–25% sucrose, 25%–35% sucrose, 35%–44% sucrose, and 44%–52% sucrose layers, respectively. Zone G is the 65% sucrose layer at the bottom of the tube.

[b] Parentheses indicate the number of animals in each group. The rats and hamsters weighed 287 ± 12.7 g and 131 ± 7.9 g, respectively.

[c] Nmol of triglyceride/g liver fresh weight. The rat and hamster livers weighed 12.3 ± 0.7 g and 6.0 ± 0.7 g, respectively.

[d] All values are the mean ± SEM.

[e] $P < 0.001$.

[f] $P < 0.005$.

[g] $P < 0.025$.

livers also produced more ketone bodies than did the rat livers (Fig. 3, legend). Because of the large variation, this difference was found to be of statistical significance only at the 1 h point. The β-hydroxybutyrate:acetoacetate ratios in hamster liver were much higher than in rat liver. The observed values in the liver perfusates were 2.63, 2.30, and 2.28 at 1, 2, and 3 h, respectively, in the hamster system, while the corresponding ratios in the rat liver perfusates were 0.83, 0.81, and 1.02. Rat versus hamster P values were < 0.001, < 0.001, and < 0.005 at 1, 2, and 3 h, respectively. The net secretion of cholesterol by the rat and hamster livers was similar. The accumulation of total cholesterol in the liver perfusates was 0.54 ± 0.07, 0.82 ± 0.08, and 1.19 ± 0.13 μmol/g liver in the rat and 0.42 ± 0.02, 0.68 ± 0.01, and 1.08 ± 0.04 μmol/g liver in the hamster at 1, 2, and 3 h, respectively.

TABLE 5. Density gradient separation of liver triglyceride pools in rats and hamsters fasted 24 h

Band[a]	Triglyceride			
	Rats (6)[b]		Hamsters (8)[b]	
	nmol/g[c]	%	*nmol/g*[c]	%
A	2644 ± 757[d]	41.1 ± 4.9	5498 ± 1300	35.5 ± 2.3
B	130 ± 34	2.5 ± 0.9	281 ± 61[e]	1.8 ± 0.2
C	141 ± 34	2.8 ± 0.8	288 ± 53[e]	2.0 ± 0.3
D	164 ± 31	3.2 ± 0.8	445 ± 98[f]	2.9 ± 0.3
E	367 ± 93	5.8 ± 0.5	1306 ± 403[e]	8.2 ± 1.1
F	1147 ± 193	20.0 ± 1.7	1980 ± 259[e]	15.0 ± 1.9
Zone G	1290 ± 115	24.6 ± 3.1	4758 ± 577[g]	34.7 ± 2.2[f]
Total	5882 ± 1062		14558 ± 2578[h]	

[a] Sucrose gradient centrifugation is described in Table 4.

[b] Parentheses indicate the number of animals in each group. The rats and hamsters weighed 244 ± 7.6 g and 124 ± 8.1 g, respectively.

[c] Nmol of triglyceride/g liver fresh weight. The rat and hamster livers weighed 7.0 ± 0.4 g and 4.4 ± 0.2 g, respectively.

[d] All values are the mean ± SEM.

[e] $P < 0.05$.

[f] $P < 0.02$.

[g] $P < 0.001$.

[h] $P < 0.01$.

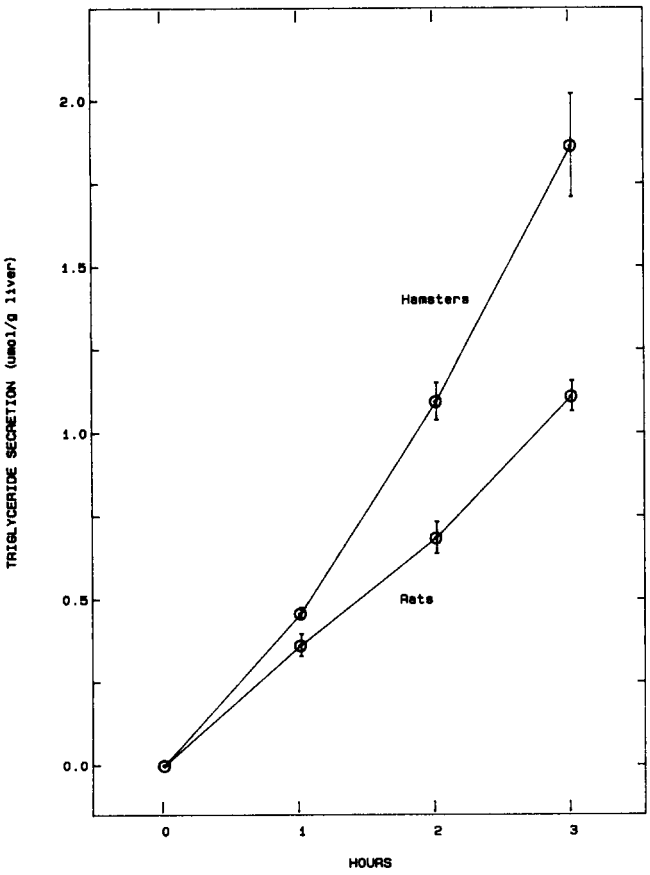

Fig. 3. Triglyceride secretion by perfused rat and hamster livers. Isolated livers from fed animals were perfused with oleic acid in a recirculating system. There were four livers in each group. Donor rats and hamsters weighed 318.0 ± 14.3 g and 120.5 ± 2.9 g, respectively. Rat and hamster livers weighed 11.3 ± 0.4 g and 5.0 ± 0.1 g, respectively. Perfusate flow rates were 1.7-2.3 ml/min per g liver in all perfusions. Vertical bars indicate ± SE. Hamster livers demonstrated greater net triglyceride secretion than rat livers. *P* values at 1, 2, and 3 h were <0.05, <0.005, and <0.005, respectively. Net ketone body production by rat livers was 16.2 ± 1.6, 26.7 ± 2.7, and 33.8 ± 3.8 μmol/g at 1, 2, and 3 h, respectively. Corresponding values for hamster livers were 27.7 ± 4.2, 40.9 ± 5.9, and 52.8 ± 7.4 μmol/g, with $P < 0.05$ at 1 h. All values are the mean ± SE.

DISCUSSION

The hamster exhibits considerably higher levels of serum triglyceride in both the fed and fasting state than does the rat (Table 1). In fed animals the metabolic basis for this difference has been found to relate to increased hepatic production of VLDL in the hamster (Fig. 3) in response to greater serum FFA and altered metabolism of FFA within the liver (Figs. 1, 2). The source of the serum FFA is mainly adipose tissue, with a certain portion also liberated during the intravascular hydrolysis of lipoprotein triglyceride catalyzed by lipoprotein lipase. Since the former is the major contributor, it is likely that the hormone-sensitive lipase in the adipose tissues normally exists in a more activated state than present in the rat. This may be attributed to an altered hormonal milieu or differences in the regulatory properties of this complex lipolytic system. The elevated level of triglyceride-rich lipoproteins circulating in the hamster may also contribute to the increased FFA in these animals, since these lipoproteins are substrates for the intravascular generation of FFA via lipoprotein lipase. Some of these FFA escape uptake at endothelial sites of lipoprotein lipase-catalyzed hydrolysis and enter the general circulation. The specific metabolic factors responsible for the elevated FFA in the fed hamster remain to be clarified.

A greater proportion of FFA were utilized for triglyceride synthesis in the liver in the hamster than in the rat (Fig. 2, Tables 2, 3). Such a difference could be caused by several factors, including greater activity of the enzyme diacylglycerol acyltransferase which catalyzes the final step in triglyceride synthesis, a higher concentration of liver diacylglycerol, higher levels of long chain acyl-CoA, and decreased utilization of diacylglycerol for phospholipid synthesis. The elevated concentration of serum FFA in the hamster may be a key factor in promoting the partition of FFA between triglyceride and phospholipid synthesis in favor of the former. Thus, several years ago it was found (35) that *i*) in isolated hepatocytes at low levels of FFA in the incubation medium, FFA was preferentially utilized for phospholipid synthesis; *ii*) as the level of FFA was increased, phospholipid synthesis approached saturation while triglyceride synthesis increased sharply; and *iii*) at intermediate levels of FFA triglyceride synthesis surpassed phospholipid synthesis. Thus, the concentration of FFA influences the partition of FFA between triglyceride and phospholipid synthesis in the liver. The elevated serum FFA in the hamster may shift this partition toward triglyceride synthesis in the liver in such a manner as that observed in the isolated hepatocytes (35).

The serum triglyceride concentration is a function of influx and efflux rates. In the fed state the serum triglyceride level represents the balance between influx from fat absorption and hepatic VLDL production versus removal via lipase-catalyzed and receptor-mediated processes. The hamsters exhibited serum triglycerides two-fold greater than rats in this nutritional state (Table 1). Since the diets were identical and contained less than 4.5% fat, differences in fat absorption are probably not involved in the higher level of serum triglycerides in the hamster. Net triglyceride secretion by the perfused hamster livers was 68% greater than displayed by the perfused rat livers (Fig. 3), under conditions of the same concentrations of FFA (oleic acid substrate) in the perfusion medium. Since the perfused liver secretes triglyceride in proportion to the FFA concentration (36), the 75% elevation of serum FFA in the fed hamster (Fig. 1) could easily promote hepatic VLDL production to a rate that could totally account for the higher level of serum triglyceride in this species. While it remains possible that differences in fat absorption and/or feeding behavior in rats and hamsters contribute to the difference in serum triglycerides in the fed animals, the importance of these factors is diminished by retention of the observed

difference in the fasting state. Serum triglycerides in fed hamsters were twice that observed in rats and in fasted hamsters they were three-fold greater (Table 1). The present study has focused on the role of hepatic fatty acid metabolism in these differences. Clearly, the hamster and rat livers partition fatty acids between triglyceride and phospholipids in a different manner. Further study will be needed to define the specific enzyme-catalyzed reactions responsible and their genetic basis, and to determine whether these are in part influenced by gastrointestinal activity. Feeding behavior could be more closely examined by introduction of a controlled feeding schedule and measurement of food consumption. It also remains possible that the activities of triglyceride removal mechanisms in the two species differ.

Triglycerides are synthesized in the outer leaflet of the endoplasmic reticulum (37). These hydrophobic lipids are rapidly transferred from sites of synthesis to cytoplasmic lipid droplets and to developing VLDL particles in the cisternae of the endoplasmic reticulum in hepatocytes. These processes have received considerable study in rat liver (31–34). Results in the present study indicate that hamsters and rats exhibit striking differences in this partition of newly synthesized triglyceride between lipid droplets and VLDL in both fed and fasting states (Tables 4, 5). The fed hamster stores much less triglyceride in lipid droplets than does the rat, as evidenced by the 10-fold difference in triglyceride in band A of the sucrose gradient separation of liver lipid droplet subpopulations (Table 4). Most of the triglyceride present in hamster hepatocytes is located in the secretory pool, located predominantly in Zone G of the sucrose gradient (Table 4). In rat liver most of the triglyceride is located in lipid droplets (Table 4).

In this study the labeled fatty acids were provided in pulse form. The plasma FFA specific radioactivities were therefore not constant and these specific radioactivities were not known. Thus, the absolute quantities of plasma FFA converted to liver triglycerides were not established. The injected dose was the same in rats and hamsters, per 100 g body wt, and since the plasma FFA in the fed hamsters were elevated (Fig. 1), it is reasonable to assume that the plasma FFA specific radioactivities in these animals were lower. Thus, the observation of equal or greater radioactivity in liver triglycerides in the fed hamsters (Fig. 2, Tables 2, 3) indicates that greater quantities of plasma FFA were converted to liver triglycerides in these animals, in support of the conclusion that hepatic triglyceride synthesis was greater in hamsters than in rats.

In the fasting state serum triglycerides in the hamster remain much greater than in the rat (Table 1), even though serum FFA were similar (Fig. 1). This may be attributed to marked elevation in the utilization of plasma FFA for liver triglyceride synthesis in the fasting hamster. Thus, the ratio of incorporation of palmitic acid into triglyceride to phospholipid increased in hamsters from about 3 in the fed state (Fig. 2) to 5 (text) in the fasting state, while in the rat this ratio was approximately 2 in both nutritional states (Fig. 2, text). Fasting also increased the relative incorporation of oleic acid into liver triglycerides in the hamster (Table 2). The molecular basis for such an increase remains to be defined. However, accelerated triglyceride synthesis and transfer to lipid droplets may account for the marked liver triglyceride accumulation in these animals (Tables 1, 2, 5). It is also possible that in the fasting hamster extrahepatic removal of plasma triglyceride-rich lipoproteins is depressed. Recycling of these lipoprotein particles to the liver would lead to lysosomal generation of free fatty acid substrates for resynthesis and storage of triglyceride. The excess liver triglyceride in the fasting hamster reflects a proliferation of both lipid droplets (predominantly band A in Table 5) and more dense triglyceride-rich particles (fractions E, F, and G in Table 5). The latter are probably a combination of newly assembled VLDL in the secretory pathway and lipoprotein species removed from the circulation. Both types of particles are encapsulated within vesicular structures and accordingly of relatively high density. Despite the increased hepatic triglyceride in these secretory fractions (Table 5), fasting decreased the hamster serum triglyceride. This suggests that in the fasting hamster the secretion of triglyceride-rich lipoproteins by the liver is partially restricted at some latter stage of the intracellular secretory pathway. The large accumulation of liver triglyceride in these animals is consistent with such a restriction.

Plasma FFA and de novo fatty acid synthesis are both important carbon sources of VLDL triglycerides in the fed rat (38). The relative contributions of these sources in the fed hamster have not been determined. Accordingly, it remains possible that enhanced fatty acid synthesis contributes to the elevated plasma triglyceride in these animals. However, FFA are known to inhibit fatty acid synthesis (39, 40). The elevated level of plasma FFA in the fed hamster (Fig. 1) lessens the possibility that enhanced fatty acid synthesis is a major participant in the elevated plasma triglyceride in this species. Fatty acid synthesis is almost abolished by fasting (39, 40) and it is therefore not likely that this process is involved in the higher plasma triglyceride in the hamster in this nutritional state.

The present studies identify metabolic factors underlying the relatively high concentrations of serum triglycerides in the hamster. While factors responsible for the higher levels of serum cholesterol in the hamster (Table 1) remain to be determined, the present results suggest that greater hepatic production of VLDL is contributory. Thus, Goh and Heimberg (41) observed that perfused livers secreted more cholesterol when the FFA concentration was increased. It is therefore likely that elevated serum FFA in the hamster (Fig. 1) promotes hepatic secretion of lipoprotein cholesterol. The higher level of serum cholesterol in the hamster than in the rat is probably not a consequence of decreased removal of low density lipoproteins (LDL) from the circulation, since Spady, Meddings, and Dietschy (42) found that

various aspects of LDL transport were quantitatively very similar in these two species.

While the rat is resistant to atherosclerosis, hamsters readily develop atherosclerotic lesions as a consequence of moderate hyperlipidemia (43). This species is therefore a valuable model for the study of lipid metabolism in relation to atherogenesis.

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