The Regulation of Hepatic Triglyceride Metabolism by Free Fatty Acids*

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SUMMARY

Livers from normal fed male rats were perfused in vitro with equimolar quantities of either caproic, caprylic, lauric, palmitic, stearic, oleic, linoleic, or linolenic acid. The concentration of free fatty acid in the medium was maintained at approximately 0.5 mM by constant infusion of the fatty acid during the experimental period of 4 hours. The rate of uptake of free fatty acid by the liver, about 13.2 μmoles per g of liver per hour, was similar for all fatty acids. The total output of triglyceride by the liver, however, was a function of the chemical structure of the specific fatty acid infused. The secretion of triglyceride into the medium and the accumulation of triglyceride by the liver was not stimulated by infusion of either caproic or caprylic acids. The output of triglyceride by the liver after 4 hours of perfusion was observed to be proportional to the number of carbon atoms in the saturated fatty acid; when long chain fatty acids containing 18 carbon atoms were infused, the output of triglyceride decreased as the number of double bonds in the fatty acid increased. These effects of the fatty acid substrate were not clearly apparent after perfusion for shorter periods of time; they may have been dependent on a critical intracellular concentration for that specific fatty acid (or metabolic derivative) which secondarily determined the reaction rates for esterification and secretion of the triglyceride. Accumulation of triglyceride in liver resulted from infusion of fatty acids containing 12 carbon atoms or more; more triglyceride seemed to accumulate when palmitic acid was infused than when other long chain fatty acids were infused. On examination of the triglyceride fatty acids of liver perfusate by gas-liquid chromatography, it was determined that the triglyceride had been enriched with the infused fatty acid, although a definite relationship between the percentage of enrichment of the fatty acid in the triglyceride and the stimulation of output of triglyceride was not discernible. The rates of ketogenesis, normally of small magnitude in livers from fed animals, were similar in all groups except the one receiving palmitic acid, in which case the output of ketone bodies was less than that of the other groups. Stimulation of output of glucose by fatty acids may vary with the ability of the specific acid to be esterified and secreted or stored as triglyceride. Medium and short chain fatty acids exerted a larger and longer lasting effect on stimulation of output of glucose than did any of the long chain fatty acids; the effect of palmitic acid was minimal. The differential effects of the various free fatty acids on secretion of triglyceride by the liver may, in part, be the mechanism by which dietary factors in the intact animal influence the concentration of triglyceride in the serum.

It has become patent from data obtained with intact animals (1-3) and in vitro (4-10) that the uptake of free fatty acids by the liver is proportional to the concentration of FFA in the serum perfusing the liver. If one extrapolates from data obtained with the isolated perfused rat liver (9, 10), uptake of FFA by the liver in vivo is proportional to all concentrations of FFA which occur in serum under normal or pathological conditions; the rate of uptake of FFA by perfused livers from normal or diabetic animals begins to approach saturation only at concentrations of FFA exceeding 3 mM (9, 10). The output of triglyceride by the liver has been observed by several investigators to be stimulated on addition of FFA to the medium (8-16). The secretion of triglyceride by the perfused liver from normal fed animals was proportional to the concentration of oleic acid in the medium, and the maximal rate of output of triglyceride was attained when the concentration of fatty acid in the perfusate was about 1.0 mM (10). The plasma triglycerides in the normal animal and in man in the postabsorptive state have been shown to be derived primarily from the metabolism of FFA by the liver (17-20), and must therefore be a consequence of the uptake of FFA by the liver, which in turn depends on the product of the concentration of FFA in the perfusing serum, the duration of perfusion, and the fractional extraction ratio of FFA by the liver. The extraction ratio for FFA by the perfused rat liver in a recycling system was about 1.

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1 The abbreviations used are: FFA, free fatty acid; TG, triglyceride; VLD lipoprotein, very low density lipoprotein.
2 W. F. Woodside and M. Heimberg, unpublished observations.
90% over a wide range of concentration (10). The hepatic extraction ratio for splanchnic FFA in normolipemic man was calculated to be 42% (20). Although the release of lipid by the perfused liver has also been correlated positively with biosynthesis of fatty acid de novo (21, 22), quantitatively, plasma FFA appears to be the major determinant. The secretion of triglyceride by liver, moreover, can be stimulated by FFA when biosynthesis of fatty acid is negligible, as is the case in experimental diabetes. The accumulation of triglyceride in the liver is influenced, in part, by the same factors which stimulate output of triglyceride. Sustained elevation of plasma FFA in animals induces the accumulation of triglyceride in the liver (23, 24). The isolated liver from normal rats perfused with FFA accumulates lipid only after maximal rates of secretion of triglyceride have been attained (10). The rate of secretion and accumulation of triglyceride by the liver is influenced also by additional factors which may be hormonal, nutritional, toxic, or pathological. More triglyceride, for example, accumulates in livers poisoned with CCl₄ than in normal livers when both groups are exposed to the same concentration of FFA in the medium (25); this is probably due to inhibition of secretion of lipoproteins by CCl₄. Conversely, triglyceride can accumulate in livers from normal animals at concentrations of serum FFA lower than are required to induce accumulation of triglyceride in livers from diabetic rats (26); this difference may result from increased hepatic lipolytic activity in insulin deficiency (27, 28).

It is apparent, then, that secretion and accumulation of triglyceride by livers from normal animals are dictated, in part, by the quantity of FFA made available to the liver, and it is probable that these hepatic processes are regulated by hormonal mechanisms (10-14, 24, 26, 27). Whether hepatic secretion or storage of triglyceride is affected in any way by the structure of the FFA substrate is less clear. Nestel and Steinberg perfused isolated rat livers with either palmitic acid or linoleic acid, but did not distinguish any quantitative differences in output of triglyceride because of the FFA, even though the composition of perfusate and hepatic triglyceride fatty acids was altered (12). Their data, which were obtained from experiments which lasted only 90 min, are in agreement with ours, although it was not possible from their work to evaluate the common fatty acids in perspective as substrates for hepatic formation and secretion of triglyceride. It was the purpose of the study reported here to determine whether output or accumulation (or both) of triglyceride by the liver, and the composition of the triglyceride fatty acids, is affected by the chemical structure of the FFA substrate.

For reasons, livers from normal fed male rats were perfused in vitro with equimolar quantities of a homologous series of saturated fatty acids differing in the length of the carbon chains, and with a series of 18-carbon FFAs differing in the number of double bonds. It is clear from these results that output of triglyceride by the liver, accumulation of triglyceride in the liver, and composition of the triglyceride fatty acids are very much functions of the chemical structure of the FFA substrate. Preliminary reports of this work have appeared (29, 30).

**METHODS**

Livers from normal male rats weighing 210 to 300 g were perfused in vitro with equipment (7) and procedures (31) which were described previously. Blood for the perfusate was obtained from the abdominal aorta of normal male rats anesthetized with ether. All animals received a balanced laboratory ration and water ad libitum. Hepatectomy was performed as reported earlier (31), except that the liver was perfused with oxygenated 0.9% NaCl solution at room temperature after cannulation of the portal vein until the organ was positioned in the perfusion chamber. Heparin was not administered to the animal from which the liver was removed nor was it added to the medium. The liver was placed in the perfusion apparatus and perfused at 37° for 20 min with a medium consisting of 40 ml of defibrinated rat blood and 30 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 (32). The medium was gassed continuously with 95% O₂-5% CO₂. During the 20-min period of equilibration, maximal rates of flow of perfusate through the liver were attained, and these rates were maintained for the duration of the experiment. Following the period of equilibration, various free fatty acids in equimolar concentrations were infused as the complex with bovine serum albumin. Albumin was infused alone in the control group. The method of preparation of the fatty acid-albumin complex was reported earlier (9, 10). The fatty acid-albumin complex was filtered through a Millipore filter (pore size, 3 μm) before use. Complete containing 500 μmoles/50 ml of solution of either caproic acid (6:0), caprylic acid (8:0), lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), or linolenic acid (18:3) were prepared with 5.0 g of albumin for all fatty acids except stearic acid, in which case 6.0 g were used. The bovine serum albumin fatty acid complex was infused into the medium at the rate of 0.2 ml per min for the duration of the 4-hour experimental period.

Aliquots of the perfusate were removed for chemical analysis immediately preceding the infusion of the fatty acids and at hourly intervals thereafter. At the termination of the experiment, the liver was perfused with ice-cold 0.9% NaCl to remove medium from the hepatic sinuses, trimmed of extrahepatic tissue, blotted, and weighed, and an extract of total lipid was prepared with ethanol-diethyl ether (33). The perfusate was centrifuged to sediment the erythrocytes and the lipids were extracted from 1 to 2 ml of the cell-free perfusate with CHCl₃-CH₂OH, 2:1, v/v (27). The samples were filtered and the filtrates were washed with 0.5 volume of 0.02% MgCl₂. The CHCl₃ layer was evaporated to dryness in a vacuum at about 50°, dissolved in a minimal volume of CHCl₃, and passed through columns containing 3.0 g of silicic acid. Appropriate aliquots of the CHCl₃ eluates of the columns were analyzed colorimetrically for triglyceride (34) and for free fatty acids. When 12 to 18 carbon atom fatty acids were infused, the FFA present in the CHCl₃ extracts was measured by the Duncombe procedure (35) with palmitic acid as a standard. Caprylic acid was used as the standard for its own analysis; since caproic acid did not produce any color in the Duncombe procedure, it was estimated titrimetrically (30) with Nile blue as an acid-base indicator. One aliquot (usually 3 ml) of the total lipid extract of the liver (100 ml of petroleum ether) was evaporated to dryness, taken up in a small volume of CHCl₃, and passed through the silicic acid column. Aliquots of the CHCl₃ eluates were analyzed for triglyceride; the columns were also eluted with CH₂OH and fractions were analyzed for lipid-soluble phosphorus (37).

For analysis of the fatty acid composition of the triglyceride by gas-liquid chromatography, portions of the petroleum ether extracts containing hepatic lipids were evaporated under reduced
pressure and stored under N₂ in the freezer until analyzed; samples of cell-free perfusate were lyophilized and stored in the freezer. The freeze-dried perfusate was extracted later with CHCl₃-CH₃OH, and the extract was washed with MgCl₂ evaporated under reduced pressure, dissolved in a small volume of CHCl₃, and plated on thin layer plates coated with silica gel (0.5 mm thick) containing 0.01% rhodamine 6G. The plates were developed in petroleum ether-ethyl ether-glacial acetic acid, 85:15:1 (by volume). The hepatic lipids were treated similarly. The bands containing triglycerides were visualized with ultraviolet light, scraped from the plates, and extracted with two 20-ml portions of CHCl₃. The CHCl₃ extracts were evaporated at 40° under N₂ and dissolved in about 5 ml of petroleum ether; 1 ml of the petroleum ether extract was added to 3 ml of BF₃ in anhydrous CH₂OH (14%, w/v) in conical (13-ml) glass-stoppered centrifuge tubes and heated in a water bath to boiling for 5 min (38). Five milliliters of water and 5 ml of petroleum ether were added; the contents were mixed and centrifuged, and the petroleum ether layer was separated and evaporated under N₂ at 40° (at room temperature when 6:0 and 8:0 were used as substrates). The methyl esters of the fatty acids were dissolved in a small volume of petroleum ether and injected into an MT-220 Microtek gas-liquid chromatograph. The glass columns contained 15% diethylyglycol succinate as the liquid phase and Gas-chrom P (80 to 100 mesh) as the supporting phase. Column temperature was maintained at 190°. When 6:0 was the substrate, column temperature was maintained at 155° until 16:0 emerged; when 8:0 was the substrate, temperature was maintained at 165° until 16:0 emerged. A flame ionization detector was used to analyze the emerging peaks.

Samples (1-ml) of perfusate were hemolyzed by addition to 3 ml of water and were treated with 1 ml each of 0.15 X Ba(OH)₂ and 2.5% ZnSO₄·7H₂O to precipitate the proteins. Aliquots of the protein-free supernatant were then analyzed for glucose (39) and for total ketones (9, 40, 41).

The fatty acids used were chromatographically pure and were obtained from Applied Science Laboratories (6:0, 8:0) and Mann (12:0, 16:0, 18:0, 18:1, 18:2, 18:3). The bovine serum albumin (Fraction V powder) was purchased from Pentex and was extracted with isooctane-glacial acetic acid, dialyzed, and lyophilized before use (42). Chemicals were of reagent grade and solvents were redistilled before use. Gas-chrom 1', diethylyglycol succinate, and the CH₂OH-BF₃ were obtained from Applied Science Laboratories. Animals were purchased from the Holtzman Company, Madison, Wisconsin.

RESULTS

The rate of uptake of FFA by the perfused liver, as reported earlier for oleic acid (9, 10), was a linear function for the duration of the experiment. The rate of uptake, moreover, appeared to be identical for all FFAs regardless of chain length or degree of unsaturation (Fig. 1); no statistically significant differences between groups were observed. The concentration of FFA in the medium under these conditions was maintained at approximately 0.5 mM. The output of TG by the liver, in contrast to the uptake of FFA, was clearly influenced by the structure of the FFA substrate (Fig. 2). The quantity of TG secreted by the liver was identical whether 6:0, 8:0, or no exogenous FFAs at all were infused. The secretion of TG was increased above control levels (p < 0.01) when 12:0, 16:0, 18:0, or the unsaturated 18-carbon FFAs were infused. The total output of TG for the 4-hour period of perfusion was observed to increase linearly as the number of carbon atoms in the straight chain saturated FFA substrate increased, and to decrease linearly as the number of double bonds in the 18-carbon FFA increased. For example, the mean output of TG when 18:0 was the substrate was 1.65 greater than when 12:0 was the substrate and 1.49 greater than that when 18:2 was the substrate. These linear relationships were not evident at earlier periods of time, but, instead, the shape of the curve changed with time. In the experiments in which the homologous series of saturated fatty acids were infused, the shape of the curve changed gradually from sigmoid to linear as the duration of the infusion progressed. In the experiments in which the 18-carbon fatty acids were infused, linearity of response was evident after 4 hours of perfusion, but until then the best substrate for output of TG appeared to be oleic acid. Even after 4 hours of perfusion, the difference in output of TG, with either 18:0 or 18:1 as the substrate, was not significant (p < 0.10); the difference between 18:2 or 18:3 and 18:0, however, was significant (p < 0.005) and the regression coefficient of the best fit for these points suggested linearity. The linearity of the response after 4 hours of perfusion is emphasized further when one examines the ratio output of TG to uptake of FFA as a function of the chain length or the number of double bonds in the fatty acid substrate (Fig. 3).

The accumulation of TG in the liver was also dependent on the FFA substrate (Fig. 4). Triglyceride did not accumulate in the liver when 6:0, 8:0, or no FFAs at all were infused; the hepatic concentration of TG under these conditions was not different from the average concentration of TG observed in the liver prior to perfusion. When FFAs with chain lengths exceeding 12 carbon atoms were infused, the concentration of TG in the liver was elevated above control levels in all groups (p < 0.005). Surprisingly, the hepatic concentration of TG when 16:0 was
FIG. 2. Effect of chain length and degree of unsaturation of FFA substrate on the output of triglyceride by perfused rat liver. The data presented are mean values ± S.E.M. for the cumulative output of triglyceride after 1, 2, 3, and 4 hours of perfusion. Figures in parentheses indicate the number of experiments in each group. The numbers at the top of each panel show the output of triglyceride for each fatty acid at the 4th hour relative to that of the control. A, the output of TG is presented as a function of the number of carbon atoms in the homologous series of saturated fatty acids. B, the output of TG is presented as a function of the number of double bonds in the series of fatty acids containing 18 carbon atoms. After 4 hours of perfusion, the slope of the line in A was 0.339 and the linear correlation coefficient r = 0.866; the slope of the line in B was -0.744, and r = -0.603.

The triglyceride was isolated from liver and perfusate in representative experiments and the percentage of composition of the triglyceride fatty acids was estimated by gas-liquid chromatography. The results of these analyses are presented in Table I. It can be seen that the fatty acid composition of both perfusate and hepatic TG isolated at the termination of the experiment was similar when 6:0, 8:0, or no exogenous FFA was infused. It is probable that 6:0 and 8:0 were not incorporated into TG since fatty acids containing less than 12 carbon atoms were not detected. The fatty acid composition of the TG was altered dramatically by infusion of the long chain FFA. Approximately 64% of the perfusate TG fatty acids were 16:0 after infusion of this FFA; about 70% of perfusate TG fatty acids were 18:1 after infusion of 18:1. The percentage of palmitic acid and oleic acid in the TG was enriched by a factor of 2 above that obtained when FFA was not infused (Table II). The percentage of enrichment of 18:2 when 18:2 was infused was of a similar order of magnitude, even though the content of 18:2 when FFA was not infused was about 0.5 that of 16:0 or 18:1. The largest fractional enrichment was that of the 18:0 and 18:3 in the TG fatty acids, both of which were present in small quantities when FFA was not infused. The enrichment of 12:0 after infusion was infinite, since none was detected unless this FFA was infused. The enrichment of the hepatic TG fatty acids appeared to lag behind that of the perfusate TG fatty acids with all infused long chain FFAs. This observation suggests to us that substrate FFA equilibrated more rapidly with perfusate TG fatty acids than with hepatic TG fatty acids, which is in agreement with isotopic data reported in earlier publications (33).

The output of total ketone bodies by the fed liver, which is small in any case, was increased as expected by infusion of fatty acids. Surprisingly, the production of ketone bodies was similar, except for 16:0, regardless of which fatty acid was infused (Table III). The output of ketones when 16:0 was the sub-
Table I

Composition of triglyceride fatty acids of liver and perfusate after infusion of various free fatty acids

The samples of the triglycerides of liver and perfusate were obtained for analysis after 4 hours of perfusion. All figures are percentage of composition. The areas of the peaks in the gas-liquid chromatograms were calculated as area = height × width (at 0.5 height). Fatty acids with less than 12 carbon atoms were not detected under several conditions. P, perfusate; L, liver.

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<th>14:1</th>
<th>16:0</th>
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<td>22.1</td>
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<td>19.1</td>
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<td>Trace</td>
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<td>0.8</td>
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<td></td>
<td>100 P</td>
<td>0.7</td>
<td>Trace</td>
<td>15.1</td>
<td>4.1</td>
<td>1.2</td>
<td>69.4</td>
<td>9.4</td>
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<td></td>
<td>74 L</td>
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<td>0.3</td>
<td>18.2</td>
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<td>2.5</td>
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<tr>
<td></td>
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<td>0.2</td>
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<td>1.5</td>
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<td>0.2</td>
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<td>5.0</td>
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<td>20.1</td>
<td>4.9</td>
<td></td>
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<tr>
<td></td>
<td>112 P</td>
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<td>0.3</td>
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<td>1.2</td>
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<td>6.1</td>
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<td>0.7</td>
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<td>1.9</td>
<td>19.2</td>
<td>41.3</td>
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<td></td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td>18:3 linolenic</td>
<td>113 P</td>
<td>0.6</td>
<td>0.4</td>
<td>13.7</td>
<td>3.8</td>
<td>1.1</td>
<td>18.9</td>
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<td></td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>141 P</td>
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<td>0.6</td>
<td>16.4</td>
<td>3.9</td>
<td>2.5</td>
<td>25.8</td>
<td>14.6</td>
<td></td>
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<tr>
<td></td>
<td>113 L</td>
<td>0.6</td>
<td>0.4</td>
<td>18.7</td>
<td>2.6</td>
<td>2.3</td>
<td>23.1</td>
<td>17.6</td>
<td></td>
<td></td>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>144 L</td>
<td>0.9</td>
<td>0.4</td>
<td>13.7</td>
<td>3.8</td>
<td>1.1</td>
<td>18.9</td>
<td>10.6</td>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
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</table>

The output of glucose by the liver was increased on addition of FFA to the medium (Table IV). The effect of the FFA, however, varied with duration of perfusion and the specific fatty acid. We reported previously that oleic acid stimulated the output of glucose when infused in experiments terminating after 2 hours (10); this observation is in agreement with the data reported here. During the 1st hour of perfusion, the output of glucose was stimulated by all fatty acids except palmitic acid. During the succeeding hours, the output of glucose by the control group increased and the relative effect of added FFA diminished. At the 3rd hour, only the medium chain fatty acids, 8:0 and 12:0, were stimulatory of output of glucose. By the 4th hour, 12:0 alone was effective in stimulating glucose output. The mechanism by which these FFAs increase hepatic output of glucose remains obscure. In all probability, the release of glucose by these livers from fed rats is the result of...
glycogenolysis. Fatty acids, by providing an oxidizable substrate (acetyl-CoA), perhaps may spare the oxidation of glucose required for hepatic function, and allow glucose to be released by the liver. Since equimolar quantities of fatty acid were infused, acetyl-CoA, which probably is oxidized primarily to CO₂ under these conditions, would be produced in the order 12:0 > 8:0 > 6:0. The stimulatory effect of these fatty acids on release of glucose was in the same order. The long chain fatty acids, however, may be preferentially esterified and secreted on release of glucose as a precursor of glycerol. The stimulatory effects of the long chain FFA on release of glucose are lost rapidly as the quantity of FFA in the liver increases and is esterified. Clearly, much more work is required to evaluate the variable effects of FFA on release of glucose.

**Table II**

<table>
<thead>
<tr>
<th>Fatty acid infused and measured</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perfusate</td>
</tr>
<tr>
<td>16:0</td>
<td>2.0</td>
</tr>
<tr>
<td>18:0</td>
<td>10.6</td>
</tr>
<tr>
<td>18:1</td>
<td>1.9</td>
</tr>
<tr>
<td>18:2</td>
<td>2.5</td>
</tr>
<tr>
<td>18:3</td>
<td>&lt; 50%</td>
</tr>
</tbody>
</table>

* Percentage of a specific fatty acid in perfusate or hepatic triglyceride after infusion of that FFA + percentage observed without infusion of exogenous FFA.

b Assume same percentage of composition for 18:3 in perfusate and measured hepatic TG as in hepatic TG when no exogenous FFA was infused.

**Table III**

Effect of FFA substrate on ketogenesis

The data presented are mean values ± S.E.M. for the cumulative production of ketone bodies by liver. The figures in parentheses indicate the number of observations.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Hour</th>
<th>mg acetone/g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (4) (no FFA)</td>
<td>1</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>6:0 caproic (6)</td>
<td>2</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>8:0 caprylic (7)</td>
<td>3</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>12:0 lauric (8)</td>
<td>4</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>16:0 palmitic (8)</td>
<td>1</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>18:0 stearic (11)</td>
<td>2</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>18:1 oleic (9)</td>
<td>3</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>18:2 linoleic (9)</td>
<td>4</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>18:3 linolenic (11)</td>
<td>1</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>18:2 linoleic (9)</td>
<td>2</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>18:3 linolenic (11)</td>
<td>3</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>18:2 linoleic (9)</td>
<td>4</td>
<td>0.24 ± 0.02</td>
</tr>
</tbody>
</table>

**Table IV**

Effect of FFA substrate on glucose output

The data presented are mean values ± S.E.M. for the cumulative output of glucose by liver. Output under Column A is that observed during the 20-min period of equilibration; this amount has been subtracted from the totals reported for each hour. These values marked with an asterisk are significantly different from the control (p < 0.05 or less). At the 4th hour, 12:0 was significantly different from all groups except 8:0. The figures in parentheses indicate the number of observations.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Hour</th>
<th>mg glucose/g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (4) (no FFA)</td>
<td>1</td>
<td>7.76 ± 0.65</td>
</tr>
<tr>
<td>6:0 caproic (6)</td>
<td>2</td>
<td>8.23 ± 0.82</td>
</tr>
<tr>
<td>8:0 caprylic (7)</td>
<td>3</td>
<td>7.91 ± 0.71</td>
</tr>
<tr>
<td>12:0 lauric (8)</td>
<td>4</td>
<td>10.22 ± 0.66</td>
</tr>
<tr>
<td>16:0 palmitic (8)</td>
<td>1</td>
<td>8.61 ± 0.97</td>
</tr>
<tr>
<td>18:0 stearic (11)</td>
<td>2</td>
<td>7.99 ± 0.90</td>
</tr>
<tr>
<td>18:1 oleic (9)</td>
<td>3</td>
<td>9.39 ± 0.95</td>
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<tr>
<td>18:2 linoleic (9)</td>
<td>4</td>
<td>8.47 ± 0.94</td>
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<tr>
<td>18:3 linolenic (11)</td>
<td>1</td>
<td>8.51 ± 0.90</td>
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<tr>
<td>18:2 linoleic (9)</td>
<td>2</td>
<td>1.99* ± 0.49</td>
</tr>
<tr>
<td>18:3 linolenic (11)</td>
<td>3</td>
<td>4.81* ± 0.61</td>
</tr>
<tr>
<td>18:2 linoleic (9)</td>
<td>4</td>
<td>4.81* ± 0.61</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It has been known for many years that the concentration, constitution, and properties of blood and tissue lipids can be altered by dietary manipulation (43), and that the fatty acid composition of plasma triglycerides and phospholipids will reflect changes in the dietary intake of fats and carbohydrate (44–48). There is convincing evidence that isocaloric replacement of diets containing saturated fats with oils containing polyunsaturated fatty acids (44, 48–52) or with medium chain triglycerides (53, 54) can lower the levels of triglyceride, phospholipid, and cholesterol in the plasma of man and animals. The amount and kind of fatty acid coming to the liver directly in dietary triglyceride fatty acids, or indirectly in FFA released by adipose tissue will vary with the diet and must secondarily influence the hepatic secretion of triglycerides. It should be evident also that the hyperlipemia seen in vivo in man and animals on a high carbohydrate diet (48, 55–57) results from endogenous biosynthesis of fatty acids in liver and adipose tissue, and the subsequent effect of these fatty acids on hepatic secretion of triglyceride.

The conclusions derived from work reported here may help us to understand how FFA influences the level of plasma triglyceride. Since it appears from our data that the various FFA were taken up by the liver at identical rates, the differences in output of TG must result from the specific metabolic disposition for the individual fatty acid. Although it has been reported that individual fatty acids are taken up by liver of intact dogs at
It is curious that the output of TG is a linear function of the number of carbon atoms or of the number of double bonds in the FFA only after 4 hours of perfusion under our experimental conditions. It is conceivable that linearity would have been attained earlier had the liver been perfused with higher concentrations of FFA. Since the rate of uptake of FFA per hour was constant, the changing shape of the curves (Fig. 2) with time may mean that maximal rates of output of TG for a specific FFA will not occur until the intracellular pool for that FFA (or more probably, a metabolic derivative of the FFA) has reached a critical concentration. The order 18:1 > 16:0 > 18:2 best describes the suitability of the common long chain FFAs as substrates for biosynthesis and release of TG in this system. Stearic acid (18:0) resulted in the maximal output of TG only after long term perfusion. It is possible that the apparent Michaelis constant ($K_m$) for 18:0 for the over-all sequence of reactions leading to secretion of TG is larger than for 16:0 or 18:1, and that maximal rates with 18:0 occur only as the metabolic pool reaches appropriate concentrations. Although the total output of triglyceride was largest when 18:0 was infused, the percentage of 18:0 in perfusate and hepatic TG was less than that of 18:1 or 16:0 when these fatty acids were infused. However, the enrichment above control levels of 18:0 in TG was larger than the enrichment of 18:1 or 16:0, since oleic and palmitic acids are more common constituents of rat plasma and hepatic TG than 18:0. It is probable that 18:1 and 16:0 are incorporated into TG more rapidly than is 18:0, but this supposition would be answered best by the use of FFA labeled with radioactive isotopes. Clearly then, as for any other enzymatic reaction, the structure and the chemical and physical properties of the FFA must in part determine the rate at which they are incorporated into TG and secreted in the VLD-lipoprotein. A novel suggestion for the hypolipemic action of unsaturated fatty acids in accord with the above statement is that since unsaturated fatty acids occupy more space than the saturated fatty acids, fewer lipid molecules can be accommodated at the appropriate site of attachment to the lipoprotein apoprotein, and less lipid, therefore, is secreted (44). Competitive reactions for the fatty acid will also determine the rate of secretion of TG. Octanoic acid, for example, is oxidized to CO$_2$ by rat liver slices more rapidly and is incorporated into lipids to a much smaller extent than is palmitic acid (60). Even though it has been studied extensively as a model fatty acid, octanoic acid should not be considered so physiologically, since it probably is not incorporated as an intact molecule into serum or hepatic TG; 12:0 is incorporated intact only to a modest extent. It was observed in experiments with octanoate-1-¹⁴C that 82.5% of the total radioactivity incorporated into hepatic lipids was present in fatty acids having chain lengths of 10 carbon atoms or more (60). In intestinal mucosa, however, little if any 8:0 is converted to long chain triglyceride fatty acids (61). Clearly, in our experiments, infusion of 8:0 or of 6:0 did not result in the accumulation of triglyceride in the liver. It has also been demonstrated that the rate of CO$_2$ production by rat liver mitochondria was greater from short chain fatty acids than from long chain fatty acids (62); furthermore, for three long chain fatty acids, the $V_{max}$ was higher and the $K_m$ was lower in the order 18:0, 18:1, 18:2. Thus, the rate of oxidation of fatty acids to CO$_2$ by liver may be inversely related to chain length for the saturated fatty acids, and directly related to the number of double bonds in the molecule. The order for CO$_2$ production would appear to be the inverse of that for biosynthesis and secretion (or both) of TG. In contrast to production of CO$_2$, rates of ketogenesis did not seem to be similarly correlated with the structure of the fatty acid. Rather the total output of ketone bodies by livers from fed animals, which is of a small magnitude, was generally the same for most of the fatty acids. Although in our experiments, the output of ketone bodies was less with 16:0 than with the other FFA, the meaning of this observation remains to be evaluated since ketogenesis is a minor pathway in the fed state. When the rate of ketogenesis is increased, as it is in livers from fasting rats (31), the production of ketone bodies from a variety of fatty acids was of a similar order of magnitude (63). The reason for this lack of specificity for ketogenesis remains to be determined. The more rapid oxidation to CO$_2$ of short and medium chain saturated fatty acids, and of unsaturated long chain fatty acids, in comparison to the saturated long chain fatty acids, and the reverse order for biosynthesis and secretion of TG, may help to explain the observations that in vivo medium chain triglycerides and polyunsaturated fats tend to lower the level of TG in plasma.

If we accept as fact that diet influences the level of plasma TG, in part, by regulation of hepatic secretion of triglycerides secondary to the quantity and structure of the FFA taken up by the liver, we must still explain how the concentration of phospholipid and cholesterol in plasma is altered simultaneously. It has been reported (13) and confirmed (22) that the VLD-lipoprotein is secreted as a unit particle in which the molar ratio of TG to phospholipid to cholesterol bears some constant relationship to one another; that is, the secretion of phospholipid and cholesterol into the VLD-lipoprotein is proportional to the output of TG by the liver. This hypothesis required that secretion of cholesterol and phospholipid into the VLD-lipoprotein must also be regulated by the quantity and structure of FFA perfusing the liver. This hypothesis does not require that the ratio TG to phospholipid to cholesterol has the same numerical values under all circumstances, but, rather, that under any given set of physiological conditions specific constants can be determined. We have observed recently that different fatty acids can change the numerical value of these ratios. For example, the VLD-lipoprotein secreted by the liver when 16:0 was the substrate contained a higher percentage of cholesterol and phospholipid than did the lipoprotein secreted when 18:1 was the substrate; stated differently, these observations mean that were equal quantities of TG to be secreted by the isolated perfused rat liver into the VLD-lipoprotein, approximately twice as much cholesterol and phospholipid would be secreted when 16:0 is the substrate than when 18:1 is the substrate. If we accept the theory that hepatic secretion of TG as a VLD-lipoprotein particle requires concomitant secretion of phospholipid and cholesterol, it should be considered that the FFA substrate may have some directive influence on the biosynthesis of phospholipid and therefore of secretion of the VLD-lipoprotein; it may also be hypothesized that the fatty acid may be a regulator of the rate of biosynthesis and secretion of cholesterol. The most common class of human hyperlipemias, type IV of Fredrickson (64, 65), is associated with a marked increase in the concentration of the pre-β-lipoprotein (VLD-lipoprotein) and of...
all the associated lipids. It is conceivable that the regulatory effects of the quantity and structure of the FFA on the secretion of the VLD-lipoprotein described for the perfused rat liver in vitro may be qualitatively similar to those control mechanisms present in man.

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