Hepatic and Splanchnic Uptake and Oxidation of Free Fatty Acids[1] (34198)

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Fatty acids play an important role in supplying the caloric requirements of the body. The major transport forms of fatty acids in blood are free fatty acids (FFA) and triglycerides (TG). The liver occupies a central position in regulating the metabolism of both FFA and TG (1, 2) by removing the excess circulating FFA (3–6) and by synthesizing lipoproteins (7–9).

Hepatic metabolism of lipids has been studied most frequently in liver perfusion experiments or in liver homogenates (10–13). In such studies, the lack of intact blood supply, hormonal and nervous influences, etc., may influence the interpretation of the results. Therefore, the present studies were undertaken in animals under *in vivo* conditions to gain further information concerning the fate of fatty acids removed by the liver. A further aim of these studies was to demonstrate the participation of FFA in the oxidative metabolism of the nonhepatic splanchnic area (NHSA) in the same animals, because of the paucity of available information concerning the energy metabolism of the region. A comparison of control dogs and diabetic ones was also carried out because of the known association of metabolic alterations of fatty acid metabolism with this pathological state.

Materials and Methods. Nine control and four diabetic dogs were used for the major part of these studies. The animals were rendered diabetic by the intravenous administration of 80 mg/kg of alloxan monohydrate, 2–3 days before the experiment, and exhibited a fasting blood sugar of 200 mg/100 ml or more when used. The mean weight of the control dogs was 21.9 kg, that of the diabetic group, 22.7 kg.

All animals were fasted for about 16 hr prior to the experiment. They were anesthetized with intravenously administered pentobarbital sodium (0.03 g/kg). The hepatic vein was catheterized via the external jugular vein under manual guidance following laparotomy. Catheters were also inserted into the portal vein (via mesenteric vein) and into the femoral artery (through a side branch, without obstructing blood flow in the femoral artery). The position of the cannulas was checked at the beginning, sometimes during, and always following the experiment. No anticoagulant was used in the cannulas, or was injected into the dogs. Arterial, portal venous and hepatic venous blood samples were placed into ice-cold heparinized tubes. Aliquots were immediately removed for β-hydroxybutyrate (βOHB) determinations (14). The rest of the blood was centrifuged in the cold and further handled on ice. Plasma FFA were determined in duplicates by the method of Dole and Meinertz (15), blood CO_2 and plasma glucose by the AutoAnalyzer (16, 17). The concentration of $C^{14}O_2$ was estimated in duplicates according to Passman *et al.* (18). The BSP method of Bradley *et al.* (19), was used for estimation of hepatic blood flow throughout the experiments. From this, estimated hepatic plasma flow (EHPF) was derived using the hematocrit value. Albumin-bound palmitic acid 1-^{14}C was given as a continuous intravenous infusion, as described previously (20). The various plasma

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lipids were separated by thin-layer chromatography, using a modification of Mangold's technique (21) and a solvent system of hexane, ethyl ether, and glacial acetic acid (156:40:4v/v/v). After identifying the various lipids using appropriate standards, the spots were scraped into counting vials, 10 ml of Bray's solution were added, and the radioactivity was assayed in a Packard liquid scintillation spectrometer. Radioactivity was expressed in terms of dpm, after allowing for quenching and counting efficiency.

The timing of the blood samples was as follows: From each of the 9 control dogs, 2 blood samples were taken, one about 95 min following the beginning of continuous infusion of labeled fatty acid, the other about 55 min later. Data from all the samples were treated as a group, mean values and standard errors were calculated and presented in the tables.

Four sets of blood samples were taken from each of the four diabetic dogs, about 85, 140, 200, and 260 min following the beginning of the continuous labeled FFA infusion. The means and standard errors of the data from all the samples are given in the tables.

Most of the calculations performed in obtaining the data presented in the tables were described previously (20). The dual blood supply of the liver presents a serious problem because of the fact that most of the metabolites occur in different concentrations in the arterial blood and in the portal venous blood. The ratio of portal venous to arterial blood supply to the liver has been the subject of numerous studies (22). On the basis of these and on textbook information (23), a ratio of 4:1 was used in all of the calculations of the current studies to represent the contribution of portal to arterial blood supply to the liver. The following is a brief summary of the calculations used:

$$\text{FFA extraction (\%)} = \frac{FFA^{14}C_i - FFA^{14}C_o}{FFA^{14}C_i} \times 100, \quad (1)$$

where the subscript i indicates incoming blood (assumed to be of portal venous origin in four parts and of arterial origin in one part), o indicates outgoing blood (hepatic venous). In the case of the NHSA, i equals arterial and o corresponds to portal venous blood.

$$\text{FFA uptake } (\mu\text{moles/min}) = \frac{(FFA^{14}C_i - FFA^{14}C_o) \times EHPF}{FFA^{14}C_i \; SA}. \quad (2)$$

In the case of the NHSA, estimated splanchnic plasma flow (ESPF) was employed in place of the hepatic flow.

$$\text{FFA oxidation } (\mu\text{moles/min}) = \frac{(^{14}CO_{2_o} - ^{14}CO_{2_i}) \times EHPF}{FFA^{14}C_i \; SA \times \left(1 - \frac{Hct}{100}\right)}, \quad (3)$$

where Hct = hematocrit, expressed as percent.

$$\text{FFA flux } (\mu\text{moles/min}) \; (24): \; g = \frac{F}{SA} \quad (4)$$

where g = FFA released into and taken up from plasma (μmoles/min;); F = ^{14}C palmitate infused continuously (dpm/min); and SA = FFA specific activity (dpm/μmole)

$$\text{Fatty acid carbon released as beta-hydroxybutyrate (\%)} = \frac{\text{Hepatic } \beta\text{OHB output} \times 4}{\text{Hepatic FFA uptake} \times 17}, \quad (5)$$

where the average chain length of FFA is taken as 17.

In four additional control dogs (also receiving continuous 1 ^{14}C-palmitate infusion), a splenic vein and the inferior pancreaticoduodenal vein were cannulated in addition to the portal vein and femoral artery. Five sets of blood samples (each taken simultaneously from the four sites) were removed 20 min apart from these animals.

Results. The mean arterial FFA concentration of the control dogs was 486 μmole/liter (Table I). The liver removed approximately one-third, and the NHSA about one-fifth of the FFA perfusing the region (Table II). Hepatic uptake of FFA equaled about 37%, NHSA uptake approximated 22% of the total

TABLE I. Uptake and Oxidation of FFA by the Liver.

	Control dogs[a]	Diabetic dogs[b]
Arterial FFA (μmoles/liter)	486 ± 45	1464 ± 161
FFA flux (μmoles/min)	175 ± 17	640 ± 71
Hepatic extraction of 1-^{14}C-palmitate (%)	34.6 ± 2.3	24.3 ± 2.1
Hepatic FFA uptake (μmoles/min)	67 ± 13	215 ± 42
$\frac{\text{Hepatic FFA uptake}}{\text{FFA flux}}$ (%)	37.3 ± 5.3	33.0 ± 4.3
Hepatic FFA oxidation (μmoles/min)	7.7 ± 3.0	40.4 ± 9.9
$\frac{\text{FFA oxidation}}{\text{FFA uptake}}$ (%)	13.0 ± 5.0	23.9 ± 8.0
Hepatic $\frac{^{14}\text{C TG output}}{^{14}\text{C FFA uptake}}$ (%)	13.0 ± 3.6	18.7 ± 6.4
Hepatic βOHB output (μmoles/min)	25.2 ± 8.7	73.5 ± 41.0
$\frac{\text{Hepatic } \beta\text{OHB output} \times 4}{\text{Hepatic FFA uptake} \times 17}$ (%)	6.8 ± 1.5	9.3 ± 4.0
Estimated hepatic plasma flow (ml/min)	383 ± 40	621 ± 56

[a] Mean ± SE of 18 samples from 9 control dogs.
[b] Mean ± SE of 16 samples from 4 diabetic dogs.

body FFA flux. The rate of FFA oxidation by the liver was 7.7 μmoles/min, that of the NHSA, 2.9 μmoles/min. Thus, 13% of the removed FFA was oxidized to CO_2 by the liver and 14% by the NHSA. It is also shown in Table I that 13% of the removed FFA counts are released in the blood in the form of TG by the liver. No consistent release was found by the NHSA. A consistent uptake of glucose by the NHSA is shown in Table II.

The arterial FFA level of diabetic dogs was markedly elevated (Table I). Uptake and oxidation of FFA, both by the liver and by the NHSA (Table II), were much higher than in controls. Both the hepatic and splanchnic extraction of FFA were decreased. The ratio of FFA uptake to total flux was not significantly different in the diabetic animals.

TABLE II. Uptake and Oxidation of FFA by the Nonhepatic Splanchnic Area.

	Control dogs[a]	Diabetic dogs[b]
Arterial FFA (μmoles/liter)	486 ± 45	1464 ± 161
FFA flux (μmoles/min)	175 ± 17	640 ± 71
Splanchnic extraction of 1-^{14}C-palmitate (%)	22.9 ± 1.6	16.0 ± 2.3
Splanchnic FFA uptake (μmoles/min)	32 ± 6	113 ± 28
$\frac{\text{Splanchnic FFA uptake}}{\text{FFA flux}}$ (%)	21.6 ± 3.8	16.6 ± 2.5
Splanchnic FFA oxidation (μmoles/min)	2.9 ± 1.3	36.3 ± 12.9
$\frac{\text{FFA oxidation}}{\text{FFA uptake}}$ (%)	13.6 ± 3.5	37.0 ± 6.7
Splanchnic $\frac{^{14}\text{C TG output}}{^{14}\text{C FFA uptake}}$ (%)	−0.1 ± 4.0	−8.6 ± 8.1
Arterial glucose (mm/liter)	3.88 ± 0.18	19.02 ± 2.42
Splanchnic glucose uptake (mm/min)	2.14 ± 0.58	0.30 ± 1.24
Estimated splanchnic plasma flow (ml/min)	301 ± 33	497 ± 45

[a] Mean ± SE of 18 samples from 9 control dogs.
[b] Mean ± SE of 16 samples from 4 diabetic dogs.

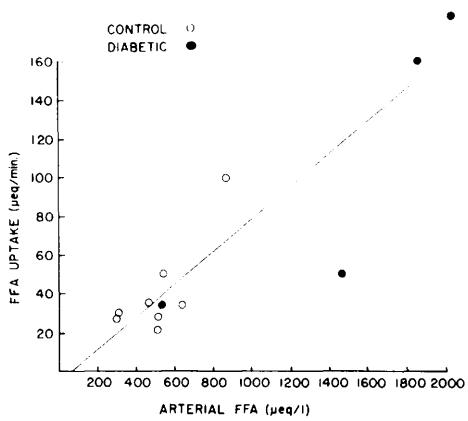

FIG. 1. Correlation between the arterial concentration and nonhepatic splanchnic uptake of FFA; each point on the graph was obtained from a different animal; $Y = -6.03 + 0.084X$, $p<0.01$.

Twenty-four percent of the hepatic FFA uptake and 37% of the splanchnic uptake were oxidized to CO_2 in the diabetic dogs. The livers of these dogs released about the same fraction of the labeled fatty acid in the form of TG as did the livers of control animals. No consistent glucose uptake was found by the NHSA.

As shown in Fig. 1, a significant direct correlation was found between the arterial concentration of FFA and the uptake of this metabolite by the NHSA. A similar relationship between arterial FFA and FFA oxidation is also demonstrated in Fig. 2.

In order to compare FFA uptake and oxidation of the spleen and pancreas with that of the gastrointestinal tract, separate venous cannulas were placed into the splenic vein, the inferior pancreaticoduodenal vein (deep in the pancreas) and in an intestinal vein (distal to the inferior pancreaticoduodenal vein) in four dogs. This enabled the simultaneous collection of venous blood from the spleen, predominantly (but not exclusively) from the pancreas, and from a small intestinal segment along with an arterial blood sample. Five sets of blood samples were taken 20 min apart from each dog. The results of the analyses are shown in Fig. 3. As shown, the spleen, pancreas, and small intestine consistently removed labeled FFA and also glucose from the blood and released $^{14}CO_2$ into the blood. The intestine and duodenum removed about the same fraction of the arterial FFA or glucose, the spleen took up a somewhat smaller fraction. Intestinal and pancreatic output of $^{14}CO_2$ per unit volume of blood was not significantly different, while $^{14}CO_2$ output by the spleen was less.

Discussion. The fate of the removed FFA by the liver has been the subject of various studies (7, 25–28). In the present investigations 67 μmoles of FFA were removed by the liver per minute. Of this amount, 13% was oxidized to CO_2 and 13% was released as TG. About 7% of the fatty acid carbon was released in the form of βOHB. Thus, even if the release of other ketones is considered and the possible appearance of some of the fatty acids in the hepatic lymph is assumed, a substantial portion of the hepatic FFA uptake still remains in the liver and presumably participates in the metabolic events in this organ. Livers of the diabetic dogs removed a much larger amount of FFA per unit time (215 μmoles/min), oxidized 24% of the removed FFA to CO_2 and released 19% of it as TG. About 9% of the fatty acid carbon was released as βOHB. Again, a substantial portion of the removed FFA remained in the liver.

In previous studies (6, 29), net changes of chemically determined FFA across the nonhepatic splanchnic region were inconsistent, suggesting that uptake, as well as release of FFA, commonly occur in this region. In the present investigations, the net removal of FFA by this area was again inconsistent (7.4

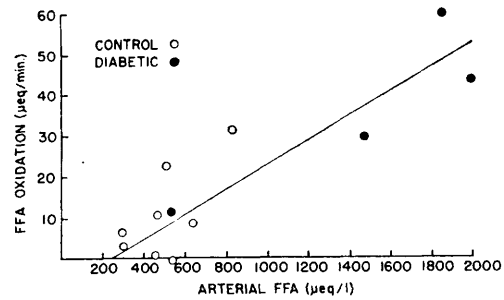

FIG. 2. Correlation between the arterial concentration and nonhepatic splanchnic oxidation of FFA; each point on the graph was obtained from a different animal; $Y = -7.0 + 0.03X$, $p<0.05$.

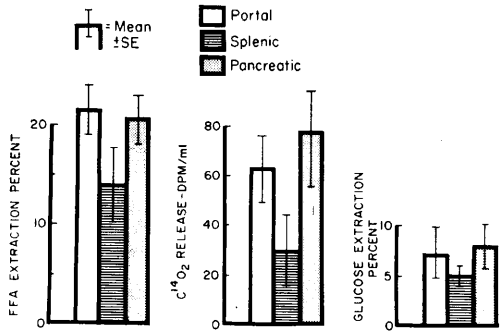

FIG. 3. FFA and glucose extraction and $^{14}CO_2$ release by the portal, splenic, and pancreatic regions.

± 6.3 μm/min in control and 35.3 ± 18.5 μmoles/min in the diabetic dogs), as indicated by the large SE. However, labeled palmitic acid was consistently removed by the area indicating that unlabeled FFA was released at the same time. The source of the released FFA has not been determined. Adipose tissue present in the splanchnic region appears to be the most likely source of the released FFA, although intestinal wall may also contribute. It was recently shown that intestine may remove, as well as release, FFA under *in vitro* conditions (30). The removal and oxidation of FFA *in vivo* by this area was found to be dependent on the arterial level (Fig. 1) as has been demonstrated previously for the myocardium (31), the liver (5), skeletal muscle (32), and the kidney (33).

In control animals, 22% of the total palmitate flux was due to splanchnic removal and 37% to hepatic uptake. Under similar experimental conditions, the myocardium was found to account for approximately 6% (20), and the kidneys for about 5% (33), of the FFA flux. This would leave about 30% of the total FFA turnover unaccounted for, and most of this portion could reasonably be attributed to skeletal muscle. It should be remembered that these values represent approximations and are based on conditions that exist under anesthesia.

The nonhepatic splanchnic area, as defined in the present study, includes the gastrointestinal tract, spleen, pancreas, and miscellaneous structures including some adipose tissue. Although it is not known to what extent these organs contribute to the overall metabolism of the region, it is important to consider that 80–85% of the portal venous blood flow originates from the gastrointestinal tract (34). Therefore, it is very likely that most of the metabolic changes discussed above are due to the gastrointestinal tract, unless another organ exhibits extremely active fatty acid oxidation. Furthermore, ileum of various species had been found to oxidize FFA to CO_2 *in vitro* (35), and it has also been shown (36) that acid secretion and O_2 consumption of the gastric mucosa are stimulated by long-chain fatty acids and ketones.

Perhaps metabolically the most active organs in the NHSA, in addition to the gastrointestinal tract, are the pancreas and the spleen. Therefore, the removal of FFA and output of $^{14}CO_2$ by these two organs were also studies. As shown in Fig. 3, neither the removal of FFA nor the production of $^{14}CO_2$ was significantly higher by these organs than by the intestine. Therefore, it seems that most of the metabolic changes noted in the NHSA were due to the contribution of the organs that supply most of the blood to the portal vein, i.e., the gastrointestinal tract.

Summary. The uptake and oxidation of free fatty acids by the liver and by the nonhepatic splanchnic area were investigated in anesthetized, control and diabetic dogs. The animals received a constant infusion of albumin-bound 1-^{14}C-palmitate. The liver of the *control dogs* removed 67 μmoles of FFA/min, the NHSA took up 32 μmoles/min. Hepatic extraction of the labeled FFA was 35%, splanchnic extraction 22% of the arterial level. About 13% of the removed FFA was oxidized both by the liver and by the NHSA. The liver released 13% of the removed labeled carbon as TG, and 7% of the fatty acid carbon as βOHB. Hepatic FFA uptake accounted for 37%, NHSA uptake for 22% of the total body FFA flux. NHSA removed glucose in the control, but not in the diabetic animals. Uptake and oxidation of FFA, both by the liver and by the NHSA, were considerably elevated in the *diabetic dogs*. However, the ratios of FFA uptake to total FFA flux were not significantly different. The fractional release of TG from the

liver was not significantly changed. A direct correlation was shown between the arterial FFA level and either the uptake or oxidation of FFA by the NHSA. Since neither the pancreas nor the spleen exhibited a more active FFA metabolism than did the intestine, it seemed that most of the metabolic changes noted in the NHSA were due to the gastrointestinal tract.

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