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Laurence V. Basso, Richard J. Havel

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Research Article

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Hepatic uptake was proportional to net outflow transport of plasma free fatty acids in the three groups and, in each, hepatic extraction fraction was about 25%. Since specific activity of free fatty acids entering and leaving the liver was equal and their composition was closely similar in the three sites sampled, it was concluded that palmitate is a representative tracer for free fatty acids entering the liver and that the liver does not release free fatty acids into the blood.

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Hepatic Metabolism of Free Fatty Acids in Normal and Diabetic Dogs

LAURENCE V. BASSO and RICHARD J. HAVEL

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco Medical Center, San Francisco, California 94122

ABSTRACT Fasted dogs prepared with catheters in the femoral artery, portal vein, and hepatic vein and infused intravenously with palmitate-1-^{14}C were used to estimate uptake of free fatty acids in liver and their conversion to major metabolic products secreted into hepatic venous blood. Animals were studied under ordinary conditions and when fat mobilization was increased abruptly by infusing norepinephrine or for a prolonged period by withdrawing insulin from depancreatized dogs. 80% of hepatic blood flow was assumed to be derived from the portal vein.

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In norepinephrine-infused dogs, the fraction of free fatty acids secreted in triglycerides (13%) was similar to that of control animals, so that transport of triglycerides was increased. In diabetic dogs no increased transport could be demonstrated since an average of only 2% of free fatty acids was converted to plasma triglyceride fatty acids; the hyperlipemia uniformly observed therefore appeared to result from defective removal of triglycerides from the blood.

A similar fraction of free fatty acids was converted to ketones in normal and norepinephrine-infused dogs. This fraction was somewhat higher in diabetic animals and, in addition, a substantial quantity of ketones was derived from unlabeled precursors. Fractional conversion of free fatty acids to CO_2 was similar in normal and norepinephrine-infused dogs, but reduced in the diabetics.

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INTRODUCTION

The liver is an important site of removal of free fatty acids (FFA) from blood plasma. In various mammals, one-fourth to one-third of isotopically labeled FFA can be recovered in hepatic lipids shortly after intravenous pulse injection (2–4) and the liver extracts about the same fraction of labeled FFA from the blood passing through it (5–7). The major pathways taken by FFA entering the liver are reasonably well defined (8) and studies with perfused livers have shown that the extent to which FFA enter these pathways is influenced by nutritional state and other variables (9, 10). Very little is known about the regulation of hepatic metabolism of FFA in vivo. Here we report studies of intact dogs on the extent to which FFA enter various metabolic pathways in liver.

Animals were studied in the postabsorptive state under ordinary conditions and when mobilization of fat from adipose tissue was increased abruptly (infusion of norepinephrine) or for a prolonged period (uncontrolled diabetes mellitus).

METHODS

Experimental animals and procedures. Three groups of male mongrel dogs weighing 11–26 kg were studied: (*a*) healthy dogs fasted for 18 hr; (*b*) dogs fasted for 18 hr and given a constant intravenous infusion of *l*-norepinephrine, 0.5 μg/ min × kg throughout the study; (*c*) depancreatized dogs fasted 18 hr and deprived of insulin for 48 hr. The diabetic animals were depancreatized at least 2 months earlier and maintained on a diet of horse meat partially digested by incubation with crude pancreatin and given NPH insulin subcutaneously once daily to maintain urinary excretion of glucose below 15 g daily. These animals lost

10–15% of their initial body weight during the 1st month after operation, but their weight was stable for at least 1 month before study. The other dogs were fed a diet containing approximately 75% protein and 25% fat and had stable weights for at least 2 wk before study.

4–7 days before each experiment, the animals were anesthetized with sodium pentobarbital and a laparotomy was performed. The spleen was removed and a small catheter of Teflon was placed through a branch of the splenic vein so that the tip lay just below the bifurcation of the portal vein as it entered the liver. All animals were eating for 3-4 days before study. The experiments were performed under pentobarbital anesthesia. 50–60 mg/kg were injected intravenously initially and repeated doses of 3–6 mg/kg were given as needed to maintain heart rate below 100 beats/min, respiratory rate below 12/min, and to prevent shivering. Under fluoroscopic control, a catheter of Teflon, 3 mm in diameter and filled with Renografin was placed into a large left hepatic vein through an incision in the right external jugular vein. The tip of the catheter was inserted about 3 cm into the hepatic vein so that free flow of blood was obtained. Patency of this catheter was maintained by a slow infusion of 0.15 M sodium chloride. No heparin was used. A superficial leg vein and a femoral artery were cannulated and a constant infusion of 0.15 M saline containing palmitate-1-^{14}C bound to human serum albumin (11), approximately 1 μCi/ml, and Indocyanine Green (Cardio green) was given through the peripheral venous catheter at a rate of 0.25 ml/min from a pump-driven calibrated syringe. Hepatic venous, portal venous, and arterial blood were sampled simultaneously several times during a period of 3–4 hr. Less than one-fifth of estimated blood volume was removed; this was replaced with 0.15 M sodium chloride. At the end of most experiments, the liver was weighed and samples were taken from four separate lobes for extraction of lipids.

Analyses. Blood samples were collected with glass syringes, placed in tubes containing 0.01 ml sodium heparin (1000 U/ml) per ml blood and chilled in ice. Duplicate 0.05 ml samples were treated with zinc sulfate and barium hydroxide solutions for measurement of glucose (12). 2 ml was rapidly mixed with 2.0 ml of 30% perchloric acid. The resulting protein-free filtrate was brought to pH 7.6 with 20% KOH to minimize decarboxylation of acetoacetate. The remainder of the blood was centrifuged for 20 min at 1000 *g* at 3°C. Samples of the fresh plasma were processed for various analyses on the day of study. Separate samples of blood were taken in duplicate into 2.5-ml plastic syringes containing a small amount of dry heparin. Exactly 2 ml was delivered from the syringe through a long 22-gauge needle into a stoppered Erlenmeyer flask equipped with a center well. The well contained a 3 × 2 cm rectangle of Whatman No. 1 filter paper folded twice on its short axis and impregnated with 0.1 ml of 10 N KOH. The main part of the flask contained 0.3 ml of 5 N H_2SO_4. Carbon dioxide was allowed to diffuse onto the paper overnight at room temperature.[1] The paper was then removed and placed into counting vials together with 5 ml of methanol used to wash the center well and 10 ml of toluene containing 0.3% diphenyloxazole was added. Assay of ^{14}C was performed with a liquid scintillation spectrometer (13).

[1] CO_2 derived from decarboxylation of acetoacetate could contaminate this extract. The maximal contribution of ^{14}C from this source was calculated for each experiment and shown to have negligible effect on the reported values for production of $^{14}CO_2$.

Lipids were extracted from duplicate 1 ml samples of plasma in 5 ml of Dole's mixture (14). The procedure of Trout, Estes, and Friedberg (15) was used to purify the extract. FFA were titrated in a two-phase system by Dole's method (14); FFA and neutral lipids were then separated and assayed for ^{14}C as described previously (16). Negligible ^{14}C was present in cholesterol esters and in the very small amounts of phospholipid remaining in the neutral lipid fraction during the period of study. For separation of very low density lipoproteins (VLDL), 2–3 ml of plasma was centrifuged in the 40.3 rotor of a Beckman model L ultracentrifuge at 140,000 *g* for 15–18 hr at 12°C. VLDL were separated with a tube slicer and transfered quantitatively into chloroform-methanol, 2:1 (v/v). Glyceride glycerol was measured in duplicate samples of the extract after removing phospholipids with silicic acid (17) and on the neutral lipid fraction obtained from Dole's extract. Composition of FFA was measured by gas-liquid chromatography (18). The neutralized protein-free filtrate of whole blood was used for estimating glycerol, acetoacetate, and β-hydroxybutyrate. Glycerol was determined by the enzymatic microfluorimetric method of Chernick (19) and the ketone acids by microfluorimetric adaptation of the method of Williamson, Mellanby, and Krebs (20) in an Aminco microfluorophotometer. Because of its instability, acetoacetate was measured on the day of study as follows. To each glass cuvette is added 0.9 ml of 0.1 M phosphate buffer (pH 7.0), containing 0.06 μmoles reduced nicotine adenine dinucleotide (NADH) (Sigma) and 0.2 ml neutralized filtrate or standard solution containing 4–20 nmoles acetoacetate. An initial reading is taken and 0.02 International Unit of *d*-β-hydroxybutyrate dehydrogenase is added. The blank contains buffer, NADH, and enzyme. The reaction is essentially complete after 60 min at room temperature. The blank and initial readings are subtracted from the postenzymatic reading and the concentration of acetoacetate is estimated from a standard curve. For estimating β-hydroxybutyrate, to each cuvette is added 0.9 ml Tris buffer, pH 8.5, containing 0.15 μmole NAD, 0.1 mmole hydrazine hydrate, 0.06 μmole HCl, and 0.2 ml neutralized filtrate or 2–40 nmole *dl*-β-hydroxybutyrate. Blanks and readings are comparable to those used for acetoacetate and the reaction is complete 90 min after adding 0.02 International Unit *d*-β-hydroxybutyrate dehydrogenase. Calculations assume that only the *d*-isomer reacts with the enzyme. ^{14}C in β-hydroxybutyrate was measured by the method of Mayes and Felts (21).

Lipids were extracted from samples of liver in acetone-ethanol, 1:1, and portions of the extract were separated on silicic acid columns into two fractions, one containing phospholipids, and the other all other lipids. FFA were separated from neutral lipids in the latter fraction. Appropriate fractions were analyzed for ^{14}C and glyceride glycerol.

Hepatic plasma flow was determined by the method of Ketterer, Wiegand, and Rapaport (22). Samples of arterial and hepatic venous blood were placed into tubes containing a small amount of dry heparin and the plasma was separated at room temperature after taking a portion for measurement of volume of packed red blood cells. Lactescent plasma was centrifuged for 2 hr in the 40.3 rotor of the Spinco model L ultracentrifuge at 140,000 *g*. The plastic tube was punctured from below with a 22-gauge needle and the clear plasma was removed. Concentration of Indocyanine Green was measured in a Zeiss spectrophotometer at 807 mμ. Hemolyzed samples were discarded.

Coefficients of variation calculated from duplicate analyses in this study were 4% for FFA, 4% for FFA-^{14}C, 5% for

TABLE I

*Arterial Concentration of Metabolites**

Group	Dog no.	Weight	Plasma			Blood			
			FFA	TGFA	VLDL-TGFA	Acetoacetate	β-hydroxy-butyrate	Glycerol	Glucose
		kg		*μmoles/ml*			*μmoles/ml*		*mg/dl*
Control	6	22	0.51 ±0.21	0.57 ±0.12	0.20 ±0.09	0.011 ±0.007	0.021 ±0.008	0.055 ±0.014	116 ±6
	8	24	0.42 ±0.05	1.80 ±0.51	0.40 ±0.12	0.018 ±0.004	0.025 ±0.010	0.075 ±0.021	106 ±9
	9	20	0.54 ±0.12	1.40 ±0.22	0.51 ±0.13	0.008 ±0.004	0.031 ±0.006	0.052 ±0.010	97 ±9
	11	25	0.23 ±0.04	1.25 ±0.27	0.18 ±0.09	0.002 ±0.001	0.008 ±0.002	0.026 ±0.005	107 ±7
Norepinephrine-treated	13	24	1.46 ±0.07	1.30 ±0.32	0.27 ±0.05	0.013 ±0.005	0.043 ±0.012	0.301 ±0.071	98 ±4
	14	26	1.59 ±0.08	0.78 ±0.23	0.15 ±0.03	0.11 ±0.01	0.203 ±0.039	0.114 ±0.020	97 ±3
	16	20	1.40 ±0.27	1.57 ±0.19	0.30 ±0.12	0.081 ±0.005	0.146 ±0.061	0.158 ±0.030	90 ±5
Diabetic	17	12	0.70 ±0.04	6.1 ±2.3	5.1 ±1.1	0.22 ±0.02	0.50 ±0.10	0.082 ±0.015	182 ±12
	18	11	0.71 ±0.06	2.7 ±0.8	1.6 ±0.5	0.19 ±0.04	0.48 ±0.03	0.083 ±0.021	230 ±4
	19	12	0.92 ±0.03	1.5 ±0.3	0.90 ±0.30	0.80 ±0.32	1.51 ±0.39	0.056 ±0.020	299 ±7

* In this and other tables, values are mean ±SD and represent four to eight samples.

triglyceride fatty acids (TGFA), 6% for TGFA-^{14}C in whole plasma, 5% for $^{14}CO_2$, 4% for glycerol, 3% for acetoacetate, 2% for β-hydroxybutyrate, and 5% for glucose.

Calculations: [2]

(*a*) Assuming that 80% of the blood entering the liver is derived from the portal vein and 20% from the hepatic artery (23, 24), hepatic input of metabolite or ^{14}C was calculated as: $p \times 0.8 + a \times 0.2$.

(*b*) Extraction fraction = $(a - v)/a$ where a is the concentration in blood entering and v the concentration in the vein draining the organ or region.

(*c*) Transport (uptake or production) of metabolite (except FFA) = concentration in plasma (blood) entering × extraction fraction × hepatic or portal plasma (blood) flow.

(*d*) For FFA only, production = [concentration of FFA entering × extraction fraction palmitate-^{14}C − (concentration of FFA entering − concentration of FFA in vein draining)] × hepatic or portal plasma flow.

(*e*) Net outflow transport of FFA

$$= \frac{\text{infusion rate of palmitate-}^{14}C}{\text{specific activity arterial FFA}}$$

(*f*) Net inflow transport of TGFA from liver

$$= \frac{\text{hepatic output TGFA-}^{14}C}{\text{hepatic uptake FFA-}^{14}C} \times \text{hepatic uptake of FFA.}$$

(*g*) Turnover rate of VLDL-TGFA (k) was calculated from the relation:

$$\frac{\text{specific activity VLDL-TGFA}}{\text{specific activity FFA entering}} = 1 - e^{-kt}$$

using the table provided by Zilversmit (26).

(*h*) Mean values presented were derived from 4 to 8 samples of blood obtained over a period of 3–4 hr. In calculating the fraction of FFA converted to ketones or CO_2, it was assumed that the mean chain length of the fatty acids is 17. Plasma volume was assumed to be 4.0% of body weight.

[2] Nomenclature used here is that recommended by Task Group on Tracer Kinetics of International Commission on Radiation Units (25).

RESULTS

Concentration of metabolites in blood (Table I). Values for various metabolites did not vary systematically during these studies. In control animals, plasma concentrations of FFA and glycerol were within the accepted normal range and those of VLDL-TGFA and ketones were low. In some samples, acetoacetate was almost undetectable in arterial blood (< 0.01 μmoles/ml). In the animals given norepinephrine, it caused rapid and sustained increase in levels of FFA and glycerol. This was accompanied by rapid and comparable increase in levels of acetoacetate and β-hydroxybutyrate but levels of VLDL-TGFA were not altered appreciably. Levels of FFA in diabetic animals were increased to a lesser extent than in norepinephrine-infused ones, but the concentration of ketones was much higher and these animals were consistently hyperlipemic.

Production and uptake of FFA. Net outflow transport of FFA was increased in norepinephrine-infused and diabetic animals (Table II). The composition of FFA determined in these animals was closely similar in the three sites sampled (Table III), suggesting that the extent to which palmitate was utilized in the extrahepatic splanchnic region and liver was similar to the other major fatty acid constituents. In the extrahepatic splanchnic region, the extraction fraction of palmitate-^{14}C averaged 7% in control animals and was similar in the other groups (Table IV). About 10% of plasma FFA was taken up in the region drained by the portal vein in normal and norepinephrine-infused dogs and about 5% in the diabetics. Calculated production of FFA from this region was generally comparable to uptake and both were increased in norepinephrine-infused and diabetic animals. In estimating the net out-

TABLE II
Metabolism of FFA

Group	Dog no.	Net outflow transport	Hepatic extraction fraction of titratable FFA	Hepatic extraction fraction of palmitate-1-^{14}C	Hepatic uptake	Fraction of FFA taken up in the liver	Hepatic uptake of glycerol	Hepatic extraction fraction of glycerol
		μmoles/min ×kg			*μmoles/min ×kg*		*μmoles/min ×kg*	
Control	6	9.10 ±1.0	0.25 ±0.08	0.24 ±0.07	1.68 ±0.72	0.18	1.10 ±0.11	0.70 ±0.12
	8	4.23 ±0.8	0.31 ±0.12	0.28 ±0.10	1.56 ±0.63	0.36	1.32 ±0.20	0.65 ±0.07
	9	9.00 ±0.41	0.25 ±0.11	0.22 ±0.08	1.60 ±0.61	0.18	1.05 ±0.05	0.61 ±0.07
	11	2.92 ±0.62	0.22 ±0.07	0.20 ±0.06	0.84 ±0.13	0.29	0.40 ±0.17	0.65 ±0.20
Norepinephrine-treated	13	23.4 ±0.67	0.40 ±0.03	0.42 ±0.02	7.64 ±1.34	0.32	6.10 ±1.12	0.65 ±0.07
	14	27.7 ±1.5	0.14 ±0.04	0.14 ±0.04	5.86 ±1.71	0.21	3.20 ±0.23	0.69 ±0.10
	16	29.5 ±1.7	0.22 ±0.08	0.20 ±0.08	5.85 ±1.62	0.20	3.68 ±0.52	0.58 ±0.06
Diabetic	17	21.2 ±2.4	0.21 ±0.06	0.23 ±0.05	4.42 ±1.42	0.21	2.10 ±0.22	0.61 ±0.15
	18	17.3 ±3.5	0.19 ±0.07	0.23 ±0.02	4.87 ±0.47	0.28	1.75 ±0.32	0.58 ±0.10
	19	28.0 ±2.1	0.17 ±0.05	0.19 ±0.04	6.51 ±1.02	0.23	1.55 ±0.19	0.83 ±0.14

flow transport of FFA from the specific activity attained in arterial blood plasma, only part of the FFA released in the extrahepatic splanchnic region is included. This results from the fact that about a quarter of these FFA is removed by the liver (see below) and thus never reaches the arterial blood. Since about 8% of plasma FFA is derived from the extrahepatic splanchnic region in all groups (Table IV), the calculated transport was underestimated by about 2%. Approximately 25% of both palmitate-^{14}C and titratable FFA entering the liver was extracted. The close correspondence between these two values in all groups of animals (compare columns four and five in Table II), together with the data in Table III demonstrating similarity of composition of FFA in artery, portal vein, and hepatic vein, indicates that in these dogs the liver did not release detectable amounts of FFA into the hepatic venous blood. Fig. 1 shows individual values for FFA-^{14}C in one norepinephrine-infused animal. There were no apparent differences in efficiency of extraction of FFA in the three groups and, in all, about 25% of plasma FFA was taken up by the liver. Thus, the increase in hepatic uptake of FFA was proportional to their increased transport in norepinephrine-infused and diabetic animals.

Hepatic oxidative metabolism of FFA. In control animals, about 10% of palmitate-^{14}C extracted by the liver appeared in hepatic venous CO_2 (Table V and Fig. 1). Release of ketones from the liver could account for 12–24% of the FFA taken up. As discussed later, conversion to labeled CO_2 provides a minimal estimate

TABLE III
*Composition of FFA**

Dog	Site	Fatty acid									
		12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Control (No. 9)	Artery	—	2.5	0.4	30.2	6.2	8.3	40.5	11.9	—	—
	Portal vein	1.9	2.5	—	29.4	4.5	9.7	39.7	12.4	—	—
	Hepatic vein	3.1	2.7	—	30.9	4.7	9.9	37.8	10.9	—	—
Norepinephrine-treated (No. 14)	Artery	0.5	2.1	—	23.3	9.5	5.4	47.1	12.2	—	—
	Portal vein	0.5	1.7	—	22.5	8.6	6.6	47.7	12.5	—	—
	Hepatic vein	0.9	1.4	—	23.6	7.8	6.5	48.3	11.6	—	—
Diabetic (No. 17)	Artery	2.1	2.7	—	30.3	8.3	8.7	34.9	8.8	4.2	—
	Portal vein	1.5	2.6	—	28.5	8.1	7.9	34.5	10.8	6.1	—
	Hepatic vein	2.2	1.8	—	30.3	6.6	10.2	35.4	9.1	4.5	—

* Values expressed in moles per cent.

TABLE IV

Extrahepatic Splanchnic Metabolism of FFA

Group	Dog no.	Extraction fraction of palmitate-1-^{14}C	Uptake	Fraction of palmitate-1-^{14}C oxidized to CO_2	Production	Production/ net outflow transport
			μmoles/min ×kg		*μmoles/min ×kg*	
Control	6	0.08 ±0.02	0.59 ±0.20	0.10 ±0.12	1.82 ±0.66	0.20 ±0.07
	8	0.14 ±0.03	0.90 ±0.51	0.25 ±0.20	0.32 ±0.30	0.08 ±0.09
	9	0.06 ±0.06	0.52 ±0.45	0.09 ±0.04	0.25 ±0.53	0.03 ±0.06
	11	0.06 ±0.05	0.20 ±0.22	0.16 ±0.09	0.32 ±0.26	0.11 ±0.09
Norepi-	13	0.14 ±0.02	2.47 ±0.81	0.21 ±0.08	1.33 ±0.76	0.06 ±0.03
nephrine-	14	0.04 ±0.01	2.15 ±0.52	0.19 ±0.11	2.21 ±1.71	0.08 ±0.07
treated	16	0.10 ±0.04	2.81 ±0.41	0.12 ±0.08	2.05 ±2.22	0.07 ±0.07
Diabetic	17	0.09 ±0.04	1.40 ±0.76	−0.05 ±0.02	1.40 ±0.72	0.07 ±0.04
	18	0.08 ±0.05	1.30 ±0.67	0.11 ±0.10	1.15 ±1.34	0.07 ±0.08
	19	0.04 ±0.02	1.00 ±0.41	0.02 ±0.08	0.80 ±0.25	0.03 ±0.01

of total oxidation of FFA. In contrast, production of ketones provides an upper limit to the true value for oxidation of FFA to acetoacetate and β-hydroxybutyrate. From the following considerations, it seems likely that in control and norepinephrine-infused dogs, FFA are the dominant precursors of ketones produced by the liver. First, although there was considerable individual variation, the average increase in production of ketones in norepinephrine-infused dogs was proportional to the increased hepatic uptake of FFA (Table V). Second, changes in hepatic output of ketones closely followed those in uptake of FFA after infusion of norepinephrine was started (Fig. 2). In the diabetics, output of ketones could account for about half of FFA uptake. In these animals, the specific activity of β-hydroxybutyrate, measured at the end of the study, was only about 60% of that expected were all the circulating β-hydroxybutyrate derived from a precursor pool in equilibrium with FFA entering the liver. The mean value for oxidation of FFA to ketones, corrected for this estimate, was 33%, a value higher than that observed in all but one of the control and norepinephrine-infused animals. The fraction of FFA converted to $^{14}CO_2$ was similar in control and norepinephrine-infused animals, but substantially reduced in the diabetics. Production of glucose in diabetic dogs was about twice that of those infused with norepinephrine (Table V).

Hepatic production of triglycerides. An average of 13% of the palmitate-^{14}C taken up by the liver of control dogs appeared in hepatic venous plasma triglycerides (Table VI). This fraction was similar in norepinephrine-infused dogs, but reduced in the diabetics. The net inflow transport from liver of TGFA derived from FFA was consequently greatly increased in norepinephrine-infused animals, but not in the diabetics. In most animals, transport of VLDL-TGFA, considered to be the fraction containing newly secreted triglycerides, was also measured. Arteriovenous differences for TGFA in this fraction were similar to those for plasma TGFA,

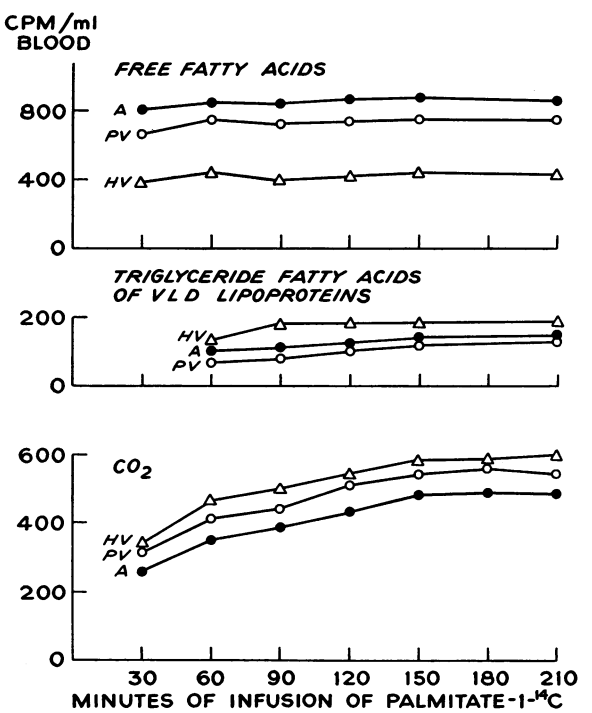

FIGURE 1 Data obtained in dog No. 13 infused at a constant rate with norepinephrine, 0.5 μg/min × kg. A = artery; PV = portal vein; and HV = hepatic vein. Note that palmitate-^{14}C was taken up in both liver and extrahepatic splanchnic region, while TGFA-^{14}C were taken up in extrahepatic splanchnic region and released from liver; oxidation to $^{14}CO_2$ occurred in both regions.

TABLE V

Hepatic Production of Glucose, Ketones, and $^{14}CO_2$

Group	Dog no.	Production of glucose	Production of ketones	Production of ketone-carbon/ uptake of FFA-carbon	Specific activity β-hydroxybutyrate/ specific activity FFA	Fraction of FFA oxidized to ketones	Fraction of FFA oxidized to CO_2
		mg/min ×kg	*μmoles/min ×kg*				
Normal	6		0.93 ±0.42	0.13 ±0.04			0.10 ±0.04
	8		1.13 ±0.12	0.17 ±0.05			0.06 ±0.03
	9		1.63 ±0.22	0.24 ±0.09			0.16 ±0.05
	11		0.43 ±0.39	0.12 ±0.04			0.08 ±0.04
Norepi-	13	2.0 ±1.8	2.27 ±0.31	0.07 ±0.04			0.12 ±0.06
nephrine-	14	1.9 ±2.0	10.7 ±0.62	0.43 ±0.05			0.11 ±0.06
treated	16	1.7 ±0.9	3.26 ±0.84	0.13 ±0.08			0.11 ±0.04
Diabetic	17	2.9 ±1.0	11.1 ±2.12	0.59 ±0.12	0.68	0.40	0.06 ±0.02
	18	3.6 ±1.8	10.6 ±1.31	0.51 ±0.08	0.53	0.27	0.05 ±0.02
	19	5.7 ±1.5	10.5 ±0.48	0.49 ±0.07	0.66	0.32	0.02 ±0.02

but variation among samples was appreciably greater. The specific activity of VLDL-TGFA always exceeded that of plasma TGFA. In two of the three control animals so studied, the terminal specific activity of VLDL-TGFA was 75–80% that of hepatic venous FFA (equivalent to that of FFA entering the liver) (Table VII). In all these animals, the specific activity of VLDL-TGFA was still rising at the end of the study. In the fourth control animal, the value was presumably higher, since the specific activity of plasma TGFA was 80% that of hepatic venous FFA. In the two norepinephrine-infused animals in which specific activity of VLDL-TGFA was measured, their terminal specific activities were 84 and 94% that of hepatic venous FFA.

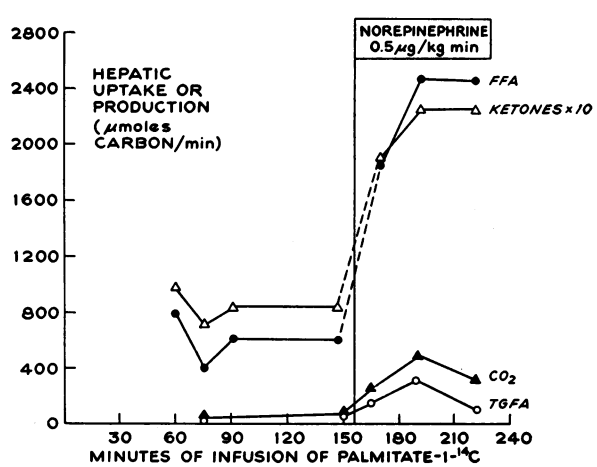

FIGURE 2 Effect of infusing norepinephrine on hepatic uptake of FFA and production of TGFA, ketones, and CO_2. Increased production of ketones closely accompanied increased hepatic uptake of FFA when norepinephrine was infused.

In these animals, such values were actually attained as early as 1 hr after starting infusion of palmitate-^{14}C. These results indicate that in the control and norepinephrine-infused animals, plasma FFA were the predominant precursors of VLDL-TGFA. Thus, the transport values shown in Table VI probably reflect closely the actual rates of production of triglycerides by the liver for these groups. In the diabetic animals, the fraction of palmitate-^{14}C released as TGFA was exceedingly small. For this reason, a separate estimate was made of the turnover rate of TGFA, based upon the rate at which the specific activity of product TGFA approached that of its precursor. Multiplied by the estimated pool size of VLDL-TGFA (concentration × plasma volume), this provided a separate estimate of the transport of TGFA (Table VI). Such calculations were also possible in two of the control animals and agreed well with the estimates based upon fractional conversion of FFA entering the liver to TGFA. In the diabetic animals, the estimates from fractional conversion are subject to large error since they were so low. The low values were, however, confirmed by the alternate estimates, from which fractional conversion can be calculated to be 0.08, 0.03, and 0.02. Both of these estimates assume that FFA entering the liver are the sole precursor of VLDL-TGFA, an assumption for which no evidence is available in the diabetic animals since the terminal specific activities of VLDL-TGFA were only 14–33% that of hepatic venous FFA (Table VII). Hepatic content of triglycerides was greatly elevated in the diabetic animals (Table VIII) and their specific activity was usually lower than that of VLDL-TGFA. In the norepinephrine-infused and diabetic animals, the amount of palmitate-1-^{14}C in hepatic lipids at the end of the study was measured. This quantity, added

TABLE VI
Hepatic Metabolism of TGFA

Group	Dog no.	Production of TGFA-^{14}C/ uptake of FFA-^{14}C	Net inflow transport of TGFA	Transport*
			μmoles/min ×kg	*μmoles/min ×kg*
Control	6	0.21 ±0.08	0.35	
	8	0.10 ±0.04	0.15	0.12
	9	0.09 ±0.10	0.14	0.18
	11	0.10 ±0.14	0.084	
Norepinephrine-treated	13	0.16 ±0.04	1.22	
	14	0.15 ±0.06	0.88	
	16	0.09 ±0.08	0.53	
Diabetic	17	0.01 ±0.08	0.04	0.37
	18	0.05 ±0.03	0.24	0.14
	19	0.00 ±0.05	0.00	0.14

* Calculated from turnover rate (k), estimated from equation: $\frac{\text{specific activity VLDL-TGFA}}{\text{specific activity FFA entering}} = 1 - e^{-kt}$, assuming plasma FFA are sole precursors.

to that secreted from the liver as esterified or oxidized products, reasonably accounted for the palmitate-^{14}C estimated to have been taken up by the liver (Table VIII).

Extrahepatic splanchnic metabolism. Oxidation of palmitate-^{14}C to CO_2 was observed in all groups (Table IV and Fig. 1). Uptake of ketones was too small to measure in control animals but was readily shown in the other groups (Table IX). The fraction of ketones extracted was much lower in diabetic than in norepinephrine-infused animals. Uptake of TGFA-^{14}C was demonstrable in norepinephrine-infused dogs but not in the other two groups.

Metabolism of glycerol. In all groups of animals, the extrahepatic splanchnic region (Table IX) extracted about one-fifth and the liver (Table II) about two-thirds of the glycerol entering their vascular beds. Thus, the liver accounted for about 80% of net splanchnic uptake. The efficiency and narrow range of variation of hepatic uptake of glycerol served as a useful check on correct placement of the hepatic venous catheter.

DISCUSSION

The three-catheter preparation used in this study is more complicated than that used by others (*27*) to study hepatic metabolism in intact animals. We elected not to use an Eck fistula preparation in order to preserve potentially crucial functional relationships, such as primary delivery of pancreatic endocrine secretions to the liver, and to determine whether certain rates could be determined without sampling portal venous blood.

TABLE VII
Specific Activities of Fatty Acids at Termination of Experiments

Group	Dog no.	Min	Hepatic venous FFA*	Arterial VLDL-TGFA	Arterial plasma TGFA	Hepatic TGFA
Control	6	240	3650	—	2940	—
	8	240	7300	5400	2050	—
	9	240	5400	4280	2240	—
	11	210	8500	3670	1130	—
Norepinephrine treated	13	240	1220	1030	460	750
	14	205	1280	1210	660	1250
	16	180	1640	—	550	1020
Diabetic	17	135	2960	460	450	500
	18	140	4520	640	510	170
	19	180	2990	980	690	170

* Mean values (cpm/μmole) during last hour of study.

TABLE VIII

Recovery of Palmitate-^{14}C Extracted by the Liver

Group	Dog no.	Hepatic content of TGFA	Fraction of palmitate-1-^{14}C stored in hepatic lipids	Fraction of palmitate oxidized and secreted as VLDL-TGFA*	Total per cent recovered
		μmoles/g			
Control	6	—	—	0.44	—
	8	—	—	0.33	—
	9	—	—	0.49	—
	11	—	—	0.30	—
Norepinephrine-treated	13	56	0.60	0.35	95
	15	45	0.62	0.69	131
	16	42	0.48	0.33	81
Diabetic	17	171	0.72	0.47	119
	18	549	0.64	0.37	101
	19	647	0.60	0.34	94

* Value for diabetic animals corrected for measured fraction of ketones (as β-hydroxybutyrate) derived from FFA.

Our assumption that 80% of hepatic blood flow derived from the portal vein is based upon measurements with electromagnetic flowmeters in pentobarbital-anesthetized dogs before and during infusion of norepinephrine (23, 24). A greater fraction may derive from the hepatic artery in conscious animals (28). It might have been more desirable to determine portal and hepatic arterial flow separately. Whether distribution of hepatic arterial and portal venous flow through the hepatic lobule differs is not certain (29–31), so estimates of transport of metabolites based upon any measure of distribution of flow between portal vein and hepatic artery may be subject to an error for which there is no obvious remedy. For all the substances studied here (excepting extrahepatic production of FFA) contribution of the liver to uptake or production considerably exceeded that of the extrahepatic splanchnic region. Therefore, differing estimates of distribution of inflowing blood to the liver have only a small influence on calculated hepatic fluxes. For example, were the fraction of hepatic blood flow contributed by the hepatic artery 33% rather than 20%, our estimates of hepatic extraction of palmitate-^{14}C would increase about 10%.

Our results confirm earlier studies with respect to extraction fraction of FFA in liver (5–7) and the fraction of plasma FFA which is taken up in the

TABLE IX

Extrahepatic Splanchnic Metabolism of Various Metabolites

Group	Dog no.	Ketones		Glycerol		TGFA-^{14}C, extraction fraction
		Extraction fraction	Uptake	Extraction fraction	Uptake	
			μmoles/min × kg		*μmoles/min × kg*	
Control	6	—	—	−0.07 ±0.14	−0.10	0.03 ±0.04
	8	—	—	0.16 ±0.08	0.27	0.02 ±0.04
	9	—	—	0.28 ±0.11	0.41	0.01 ±0.05
	11	—	—	0.25 ±0.05	0.15	0.00 ±0.08
Norepinephrine-treated	13	0.25 ±0.10	0.36	0.34 ±0.12	2.55	0.11 ±0.07
	14	0.20 ±0.05	1.98	0.12 ±0.05	0.44	0.02 ±0.04
	16	0.14 ±0.05	1.03	0.16 ±0.07	0.81	0.06 ±0.04
Diabetic	17	−0.04 ±0.09	−0.97	0.17 ±0.06	0.47	0.01 ±0.02
	18	0.03 ±0.07	0.64	0.18 ±0.06	0.48	0.00 ±0.01
	19	0.01 ±0.09	0.92	0.18 ±0.09	0.27	0.00 ±0.03

liver (2–4). We found that extraction of FFA in both liver and extrahepatic splanchnic tissues was similar in the three metabolic states. It has been suggested that the liver releases FFA into the blood (9). We were unable to observe this in any animal. Because of the appreciable error of the titrimetric method for FFA, particularly when levels are low, estimates of fractional uptake based on only one or two measurements are, of course, subject to appreciable error. Our estimates are based upon several duplicate analyses of blood plasma from the three sites in each animal. This observation of unidirectional flux of FFA in liver has considerable practical importance, since it means that when labeled FFA are given as a constant infusion hepatic venous FFA are in isotopic equilibrium with FFA entering the liver. Thus, in dogs, the specific activity of FFA entering the liver can be estimated without sampling portal venous blood.

Fractional conversion of FFA to major products secreted into hepatic venous blood was not altered when hepatic uptake of FFA was increased about fourfold by norepinephrine. Smythe, Gilmore, and Handford reported that infusion of norepinephrine, 1 μg/min × kg into dogs anesthetized with sodium pentobarbital produces a 35% increase in splanchnic oxygen consumption with no change in blood flow (32). Our results suggest that increased oxidation of FFA contributes to this splanchnic calorigenesis, since fractional oxidation to $^{14}CO_2$ was unchanged.

Our data do not establish that ketones produced by the liver were derived entirely from FFA in the dogs infused with norepinephrine, but the rapid and proportionate increase in uptake of FFA and production of ketones suggests that this is the case. In postabsorptive humans given palmitate-1-^{14}C intravenously, measurements of specific activities indicate that plasma FFA are the sole precursors of the carbonyl carbon of acetoacetate.[3] As discussed below, measurements of hepatic production of $^{14}CO_2$ provide minimal estimates of complete oxidation of FFA. Thus, quantitative interpretation of measurements of fractional oxidation of FFA is not possible. The reasonably good recovery of ^{14}C taken up by the liver as stored or secreted products (Table VIII) does suggest that the overall estimates are not grossly in error. The large fraction of FFA stored in hepatic lipids of control and norepinephrine-infused animals suggests net accumulation of triglycerides during the experimental periods. This is consistent with reports that hepatic triglyceride content increases during fasting (3) and that this process is accelerated when fat mobilization is increased further by norepinephrine (33, Table VIII).

[3] Havel, R. J., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. To be published.

While the concentration of ketones increased rapidly in norepinephrine-infused dogs, that of VLDL-TGFA did not. This must be explained by a substantial increase in the turnover rate of VLDL-TGFA, which is supported by the rapid equilibration of its specific activity with that of precursor FFA in these animals, and the increased extraction fraction of TGFA-^{14}C in extrahepatic splanchnic tissues, but the mechanism is not apparent. Increased production of VLDL-TGFA in dogs infused with norepinephrine agrees with morphological studies on livers of such animals by Hamilton (34). He observed increased accumulation of particles associated with elements of the Golgi apparatus which have tentatively been identified as VLDL precursors (35).

The diabetic dog presents a considerably more complex situation. In these animals, it may be assumed that prolonged deficiency of insulin had led to increased rate of the gluconeogenesis (36). These animals had grossly fatty livers and their ketosis was out of proportion to the rate of fat mobilization. The magnitude of the ketonemia is explained, in part, by the observation that hepatic production of ketones was greater than expected from the rate of hepatic uptake of FFA. The fractional extraction of ketones in the extrahepatic splanchnic region was much lower in diabetic than in norepinephrine-infused animals; this suggests that peripheral utilization of ketones was also impaired. In studies inspired by these observations it has been shown that utilization of ketones is impaired in such diabetic dogs.[4] Our data also indicate that not all ketones produced by the livers of the diabetic dogs were derived immediately from plasma FFA. Two additional sources must be considered. First, ketones could be derived from fatty acids contained in the large quantity of triglyceride stored in these livers. As shown in Table VII, hepatic triglyceride pools of very low specific activity were present. Second, they could be derived from ketogenic amino acids as part of the general increase in protein catabolism characteristic of uncontrolled diabetes. The first possibility is supported by the observations of Heimberg and his associates (37, 38). They observed decreasing content of hepatic triglycerides together with high rates of production of ketones when livers from alloxan diabetic rats were perfused with small quantities of palmitic acid.

Relatively less palmitate was oxidized to CO_2 than to ketones in the diabetic than in the control and norepinephrine-infused animals (Table V). In the latter two, the ratio, production ketone carbon: production CO_2, varied between 0.6 and 3.9 (mean 1.8) while in the former it varied from 5.4 to 16 (mean 9.4). Earlier studies in simpler systems have suggested that accelerated oxidation of fatty acids may be accompanied by in-

[4] Balasse, E. O., and R. J. Havel. To be published.

creased fractional conversion of resulting acetyl CoA to ketones (39). The results obtained in the norepinephrine-infused animals suggest that this is not necessarily the case in vivo. Whether the results in the diabetic animals represent such a shift is uncertain. Krebs, Hems, Weidemann, and Speake have shown that in tissues possessing a pathway for gluconeogenesis, a substantial fraction of fatty acid–carbon may be found in glucose even when the net fate of such carbon is oxidation to carbon dioxide (40). This apparently results from equilibration of this carbon, in oxaloacetate, with carbon atoms derived from pyruvate and other precursors of glucose. It is possible that a greater fraction of carbon in acetyl CoA derived from beta oxidation of fatty acids was deposited in hepatic glucose in the diabetic animals because a greater fraction of oxaloacetate was directed to glucose rather than to citrate. In any case, it is clear that the fraction of FFA taken up in the liver which was released as $^{14}CO_2$ represents a minimum value for hepatic oxidation of FFA in all groups.

The estimate of hepatic production of VLDL-TGFA in diabetic dogs presents some similar problems. Clearly, a very small fraction of FFA taken up in their livers was released in triglycerides. However, it is not certain that FFA were the sole precursors of plasma TGFA, particularly since this did not appear to be the case for ketones. It is not known whether fatty acids arising from hydrolysis of stored triglycerides in liver enter a pathway leading to secretion of VLDL-TGFA, but there is no evidence to discount such a possibility. If it is assumed that the specific activities of β-hydroxybutyrate in these dogs (Table V) equal that of the precursor pool for VLDL-TGFA, the production rates shown in Table VI underestimate the actual values by about 40%. The data do, however, permit the conclusion that the hyperlipemia in these animals was not primarily the consequence of increased hepatic production of triglycerides. This is consistent with the observation that incorporation of palmitate-1-^{14}C into VLDL-TGFA is decreased in perfused livers from alloxan-diabetic rats (38). Other data in experimental animals (41) and in man (42) indicate that peripheral utilization of plasma triglycerides is impaired by prolonged deficiency of insulin. In contrast, we have observed that in short-term insulin deficiency in dogs, produced by injection of anti-insulin serum, hyperlipemia is accompanied by increased hepatic production of triglycerides.[4] Our present results as well as others in man (43, 44) indicate that changes in concentration of triglycerides in blood plasma in states of increased fat mobilization may provide little indication of their hepatic production rate.

Like FFA, utilization of glycerol was proportional to arterial level in liver and extrahepatic splanchnic tissues in the three groups of animals. Fractional extraction was higher in liver, but uptake in intestinal mucosa, which contains an active glycerokinase (45), may have been comparably high, since only a fraction of portal blood drains this tissue and glycerol presumably was released with FFA from splanchnic adipose tissue.

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