Splanchnic and hepatic triglyceride secretion during hypercaloric intravenous glucose infusion in conscious swine

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Abstract We have validated a radiochemical technique for measuring the rate of secretion of plasma triglycerides from the liver and/or splanchnic region during the consumption of glucose under isotopic steady-state conditions. Values obtained with this technique correlated closely with those based on transhepatic or transsplanchnic chemical gradients ($r = 0.95$). Likewise, values for secretion of triglycerides obtained with the radiochemical technique correlated closely with those obtained for extrahepatic or extrasplanchnic triglyceride clearance. Values for mean net splanchnic and hepatic secretion of plasma triglyceride fatty acids, transported essentially in very low density lipoproteins, were 1.9 and 2.0 μmoles/min·kg body $wt^{0.75}$, respectively, about one-half of the rate of transport of free fatty acids. However, the fraction of triglyceride fatty acids of plasma very low density lipoproteins that was derived from plasma free fatty acids averaged 9%, and that derived from glucose, though increasing with time, reached only 2% after constant intravenous infusion of radioglucose for 5 hr. Porcine hepatic secretion of plasma triglycerides is large in the glucose-fed state, and the secreted triglyceride fatty acids evidently are derived from stored fat or glycogen.

Supplementary key words extrahepatic splanchnic region · free fatty acids · glycogen · liver · metabolism · miniature swine · triglyceride clearance · very low density lipoproteins

Sustained ingestion of glucose in rats results in a marked increase in the rate of secretion of plasma TGFA and a reduction in the contribution of FFA to plasma TGFA (2, 3) compared with the fasted state. Similarly, in man, oral administration of glucose markedly diminishes the fraction of plasma TGFA derived from FFA (4); however, previous measurements of plasma TGFA secretion during glucose consumption have not taken into account the major contribution of precursors other than FFA to the synthesis of plasma TGFA (5). To validate a radiochemical technique for quantifying net splanchnic secretion of plasma TGFA in man in the fed state, we have undertaken studies in another omnivorous species, swine, which has a similar lipoprotein profile (6). We have designed experiments that permit simultaneous sampling of arterial, common portal, and hepatic venous blood during constant rates of glucose consumption in conscious, unsedated animals. The experiments have enabled us to quantify uptake and/or secretion of various metabolites by liver and EHS tissues in the glucose-fed state under physiological conditions.

METHODS

Animals and surgical preparation

Five minature swine weighing 30–47 kg and approximately 6–12 months of age were studied. Differences in weight were attributable mainly to age and growth rates rather than to degree of adiposity. None of the four females was pregnant or lactating. The diet met National Research Council minimum standards and consisted of pelleted commercial pig starter guaranteed to contain a minimum of 17% crude protein and 3% crude fat and a maximum of 6% crude fiber. Swine were housed in metal cages and maintained on approximately 36 g of pig starter daily/kg body $wt^{0.75}$, which allowed a weight gain of 1–2 kg/month.

Swine were premedicated prior to surgery with 0.8 mg of fentanyl, 40 mg of droperidol, and 0.8 mg of atropine. Anesthesia was induced and maintained with halothane and nitrous oxide according to the method of Piermattei and Swan (7). A femoral artery and a femoral vein were dissected, and the beveled tip of a silicone rubber cannula (1.02 mm ID with two silicone rubber cuffs 0.5 cm apart and placed 15 cm above beveled tip) was inserted into each vessel. Cuffed polyvinyl cannulas, 1.47 mm ID, with

A preliminary report of this work has appeared in abstract form (1).

Abbreviations: EHS, extrahepatic splanchic; FFA, free fatty acid(s); H, hepatic; HDL, high density lipoprotein(s); LDL, low density lipoproteins(s); S, splanchic; TGFA, triglyceride fatty acid(s); VLDL, very low density lipoprotein(s).

2.5- and 5.0-cm beveled tips were used for the common portal and hepatic veins, respectively (8). Suitable hepatic veins were located in either the left medial or lateral lobes (9) by palpation during laparotomy. Swine 4 and 5 also underwent cannulation of a gastrosplenic vein with a cuffed silicone rubber cannula. All cannulas were introduced along subcutaneous tracts about 30 cm long via long hubless needles and exited near the midline of the back. At the end of surgery, all cannulas were filled with 0.15 M sodium chloride solution containing approximately 300 units of heparin/ml and were taped to a cotton stockinette, which was cemented to the skin with waterproof cement. Cannulas were flushed with saline every 1–3 days to test for patency and were again filled with heparin–saline. Aqueous penicillin and streptomycin were administered intramuscularly at the time of surgery and daily for 3 days to prevent infection (10).

Materials

[1-^{14}C]Palmitic acid (5.4 mCi/mmole), [9,10-^{3}H]palmitic acid (320 mCi/mmole), and [U-^{14}C]glucose (5.1 mCi/mmole), certified over 98% pure, were obtained from New England Nuclear Corp., Boston, Mass. Miniature swine were obtained from the Hormel Institute, Austin, Minn. Pig starter was obtained from Master Feeds, Toronto, Ontario. Porcine serum albumin was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

Experimental procedures

None of the animals was used for experiments until at least 5 days after the laparotomy and after it had consumed three full feeds daily for at least 3 days (150% of the maintenance diet). The last feed was given at 11–12 p.m. Heparin was not used on the day of the experiment, and cannulas were kept patent by slow infusions of 0.15 M sodium chloride. All experiments were performed on unsedated animals. 8 hr postprandially, a constant infusion of glucose (1.23 ml/min) was started via the femoral vein catheter and was given at the rate of 108 μmoles/min·kg body wt$^{0.75}$ (approximately 150% of isocaloric requirement) for 9 hr; however, swine 5 received only 7 hr of glucose infusion (on the 19th day after laparotomy). After infusion of glucose for 4 hr, ^{131}I-labeled albumin was injected intravenously for determination of plasma volume, and an arterial blood sample was taken for analysis 10 min later. Subsequently, a constant infusion of 0.15 M sodium chloride containing indocyanine green (1 mg/ml) and either [1-^{14}C]palmitate or [9,10-^{3}H]palmitate (approximately 10 μCi/ml) and [U-^{14}C]glucose (approximately 3 μCi/ml) was given via the femoral vein catheter at the rate of 0.23 ml/min from a pump-driven calibrated syringe for 5 hr. Radiopalmitate was bound to porcine serum albumin (11), which had a molar ratio of endogenous FFA to albumin of 0.1 (final ratio of complexed FFA to albumin, 0.8). A pulse injection of [U-^{14}C]glucose equal to 120 times the amount infused per minute was given at the onset of the constant infusion of [U-^{14}C]glucose. Swine 1–3 received [9,10-^{3}H]palmitate and [U-^{14}C]glucose, whereas swine 4 received a constant infusion of only [1-^{14}C]palmitate. Hepatic venous, portal venous, and arterial blood samples were obtained simultaneously at approximately 20–30-min intervals during the last 3 hr of the infusions except for swine 5, from which only four samples were obtained during the last hour. In two experiments in swine 4 and one in swine 5, *p*-aminohippuric acid was constantly infused into a gastrosplenic vein for simultaneous measurements of portal and hepatic venous blood flow (12). Less than one-fifth of the estimated blood volume was removed from any animal during an experiment; this was replaced with 0.15 M sodium chloride. At the end of two experiments (swine 2 and 4), the livers were weighed and samples were taken from two separate lobes for extraction of lipids.

Analyses

Blood samples were placed either in chilled tubes containing 0.010 ml of heparin solution (1000 units/ml) per ml or in dry tubes for preparation of serum. Triplicate 0.050-ml samples were taken from the heparinized blood for analysis of glucose (13). The remainder of the heparinized blood was centrifuged for 20 min at 1000 *g* at 4°C. Triplicate 1-ml samples of fresh plasma were taken for extraction of lipids in Dole's mixture, and 4 ml was taken for separation of VLDL (14). Content and radioactivity of FFA in plasma and of TGFA in plasma, plasma VLDL, and liver were determined as described previously (14, 15). Plasma low density (d 1.006–1.063, LDL) and high density (d 1.063–1.21, HDL) lipoproteins were separated by the method of Havel, Eder, and Bragdon (16). Hepatic plasma flow was determined by the method of Ketterer, Wiegand, and Rapaport (17). Plasma concentrations of total estrogen were determined by radioimmunoassay (18). Duplicate 5-ml samples of whole blood were deproteinized with perchloric acid and neutralized with potassium hydroxide for determination of specific activity of glucose (19). Isolation of glucose as the potassium gluconate derivative (20) showed that more than 99% of the radioactivity was actually in glucose.

Coefficients of variation calculated from triplicate analyses in this study were 7.2% for FFA (pertaining to plasma concentrations averaging 0.07 mM), 3.9% for labeled FFA in whole plasma, 1.9% for plasma TGFA, 2.4% for labeled TGFA in whole plasma, 3.5% for plasma VLDL TGFA, 3.7% for labeled plasma VLDL TGFA, 1.4% for glucose, and 1.5% for ^{14}C-labeled glucose.

Calculations

The general equations used have been described (15, 21, 22).

1. Hepatic and splanchnic metabolism: (*a*) In swine 4, in which the fraction of blood entering the liver via the hepatic artery and the portal vein was determined, hepatic input (*i*) of metabolite (μmoles/min) or radioisotope (cpm/min) was calculated as

$$\left[\text{arterial concentration } (\mu\text{moles or cpm/ml}) \times \frac{\text{blood (plasma) entering liver via hepatic artery (ml/min)}}{\text{total hepatic blood (plasma) flow (ml/min)}}\right] + \left[\text{portal vein concentration } (\mu\text{moles or cpm/ml}) \times \frac{\text{blood (plasma) entering liver via portal vein (ml/min)}}{\text{total hepatic blood (plasma) flow (ml/min)}}\right]$$

In swine 1–3 it was assumed, on the basis of three experiments in swine 4 and 5, that 50% of the blood entering the liver was derived from the hepatic artery and 50% from the portal vein, and hepatic input was calculated as [arterial concentration (μmoles or cpm/ml) × 0.5] + [portal concentration (μmoles or cpm/ml) × 0.5].

(*b*) Hepatic release of FFA = [(concentration of plasma FFA, in μmoles/ml, entering liver × extraction fraction of radiopalmitate) − (concentration in plasma entering liver − concentration in hepatic venous plasma)] × hepatic plasma flow (ml/min).

(*c*) Secretion of TGFA (or VLDL TGFA) from liver (or splanchnic region) = [hepatic (splanchnic) secretion of TGFA (or VLDL TGFA) (cpm/ml plasma)/hepatic (splanchnic) uptake of labeled FFA (cpm/ml plasma)] ×

$$\left[\frac{(SA_{FFA\,a+h})_{\bar{x}}}{2} / (SA_{VLDL\ TGFA})_{\bar{x}}\right]$$

× hepatic (splanchnic) uptake of FFA (μmoles/min), where $(SA_{VLDL\ TGFA})_{\bar{x}}$ is the mean specific activity of TGFA of arterial VLDL and

$$\frac{(SA_{FFA\,a+h})_{\bar{x}}}{2}$$

is the mean value for specific activity of FFA in arterial and hepatic venous blood plasma during the terminal period when values for this ratio reached a plateau.

2. Extrasplanchnic and extrahepatic clearance of plasma TGFA and VLDL TGFA: (*a*) Clearance of TGFA from plasma in the extrahepatic region over a given time interval was calculated from the same formula as for the extrasplanchnic region (22) by substituting *RI* for *RA*, where *RI* is the radioactivity in TGFA of blood plasma entering the liver.

(*b*) Clearance of plasma VLDL TGFA in the extrasplanchnic and extrahepatic regions was calculated from the above formulas by substituting values of VLDL TGFA for those of plasma TGFA.

3. Average values presented were derived from eight complete simultaneous sets of arterial, portal venous, and hepatic venous blood samples obtained serially over the last 3 hr of the studies in swine 1, 2, and 4. In swine 3, only three complete sets plus three sets of arterial and hepatic venous blood were obtained. Measurements of hepatic plasma flow were based on determination of indocyanine green in serum on three or four sets of arterial and hepatic venous blood samples. Linear regressions were calculated by the method of least squares, and their significance was tested by estimating the correlation coefficient *r*. Statistical analyses were performed according to Snedecor and Cochran (23). Variance was expressed as standard error of the mean.

4. Kg body $wt^{0.75}$ was used as a unit of body size (24) to facilitate comparisons among species differing widely in size.

RESULTS

Hemodynamic data

Values for hepatic blood flow (**Table 1**) did not change systematically and were relatively constant during the period of blood sampling from 6 to 9 hr after starting the hypercaloric intravenous infusions of glucose. **Fig. 1** shows representative results obtained in swine 2. The

TABLE 1. Hemodynamic and other data[a]

Swine No.	Sex	Weight	Metabolic Body Size	Plasma Volume	Blood Volume	Hepatic Plasma Flow	Hepatic Blood Flow
		kg	$kg^{0.75}$	*ml/kg*	*ml/kg*	$ml/min \cdot kg^{0.75}$	$ml/min \cdot kg^{0.75}$
1	M	46.7	17.9	37.5	51.4	32.8 ± 4.8	42.7 ± 6.3
2	F	40.8	16.1	47.7	63.5	69.9 ± 5.5	89.1 ± 7.2
3	F	30.1	12.9	39.4	49.7	22.4 ± 6.8	27.4 ± 8.0
4	F	37.3	15.1	44.1	65.6	40.3 ± 4.0	58.4 ± 7.1[b]
5	F	41.6	16.4	45.7	63.1	62.5 ± 5.8	79.3 ± 7.1[c]
Mean		39.3 ± 2.7	15.7 ± 0.8	42.9 ± 1.9	58.7 ± 3.3	45.6 ± 9.0	59.4 ± 11.4

[a] Means ± SEM.
[b] Portal flow was 51 ± 4% of hepatic flow.
[c] Portal flow was 46 ± 2% of hepatic flow.

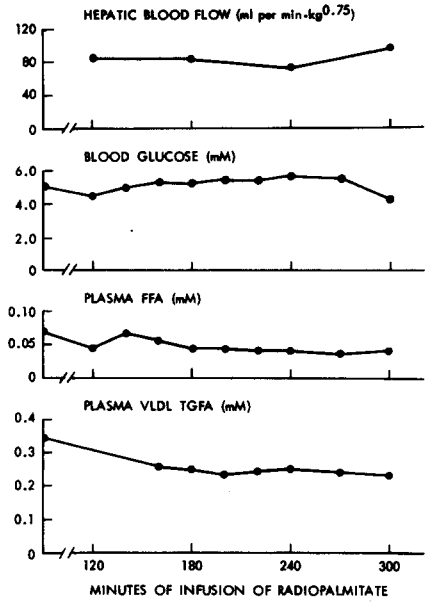

Fig. 1. Hepatic blood flow and arterial concentrations of major lipid metabolites and glucose in swine 2.

fraction of blood entering the liver via the portal vein in swine 4 was similar (51 and 56%) when determined on two separate occasions. Portal flow was 46% of hepatic flow in swine 5.

Arterial concentrations

Values for serial arterial concentrations of lipids and glucose also did not vary systematically (Fig. 1 and **Table 2**). Arterial plasma concentrations of VLDL TGFA correlated significantly with those of FFA ($r = 0.90$, $P < 0.05$).

Metabolism of FFA

Rates of net inflow transport and turnover of FFA in plasma are given in **Table 3.** Mean values for extraction fraction of FFA determined radiochemically were consistently higher than those determined chemically in the liver as well as in the EHS region, although the difference was small in swine 2. Accordingly, the specific activity of FFA in hepatic venous plasma was lower than that of plasma entering liver (respective values were 89 ± 4, 95 ± 2, 85 ± 7, and 63 ± 4% in swine 1, 2, 3, and 4). Although values for specific activity of FFA in hepatic venous plasma gave a spuriously low estimate of the actual specific activity of plasma FFA entering liver (mean difference, 17 ± 7%), only in swine 4 was there a major descrepancy (37 ± 4%). A close approximation of the specific activity of plasma FFA entering liver could be obtained by averaging the specific activity of arterial and hepatic venous plasma FFA; calculated values were 99 ± 5, 99 ± 1, 97 ± 2, and 77 ± 3% of actual values for swine 1–4, respectively (mean difference, 7 ± 5%).

Secretion of plasma TGFA

On the average, 16% of the FFA removed by the S region was secreted as TGFA into hepatic venous plasma, whereas 25% of the FFA removed by liver appeared in plasma TGFA (**Table 4**). Values for S and H conversion of FFA to plasma VLDL TGFA correlated significantly with those for conversion of FFA to plasma total TGFA ($r = 0.96$, $P < 0.001$). From both radiochemical and chemical measurements, secretion of plasma TGFA was attributable to secretion of plasma VLDL TGFA (Table 4 and **Table 5**).

Mean values for S and H secretion of plasma TGFA, determined chemically, were 1.9 and 2.0 μmoles/min·kg body wt$^{0.75}$, respectively (Table 4).[1] Values for S and H secretion of plasma TGFA determined radiochemically correlated closely with those obtained by direct chemical measurement (**Table 6**). Values obtained with the radiochemical technique did not differ significantly from those obtained chemically ($P > 0.5$). Radiochemical values for S and H secretion of plasma TGFA also correlated closely with rates of clearance of TGFA from plasma in extrasplanchnic and extrahepatic tissues, respectively. Likewise, values for clearance of TGFA and of VLDL TGFA from plasma in the extrasplanchnic and extrahepatic regions correlated highly with respective rates of S and H secretion of plasma TGFA determined chemically. Values for clearance of TGFA and of VLDL TGFA from plasma were not significantly different from chemical rates of secretion of plasma TGFA ($P > 0.5$). Rates of secretion of plasma VLDL TGFA, technically more difficult to measure and based on fewer samples, were somewhat higher than those for secretion of plasma TGFA.

Values for turnover of plasma TGFA were similar in male swine 1 and female swine 2 and 3; however, TGFA turnover was appreciably higher in female swine 4. Swine 4 was in heat, which was confirmed by estrogen assays (serum estrogen at least double that of other females),[2] and this may have affected secretion of plasma TGFA (26).

Precursors of plasma TGFA and VLDL TGFA

In three of four animals (swine 1, 2, and 4), values for the ratio of specific activity of arterial VLDL TGFA to specific activity of FFA entering liver reached a plateau within 180 min of starting the infusion of radiopalmitate. In swine 3 the plateau was reached after 220 min. Results of a representative experiment from swine 2 are shown in **Fig. 2.** During the plateau period the values for the above ratio were 0.065, 0.042, 0.138, and 0.122 in swine 1–4, respectively (Table 5). Most of the VLDL TGFA (mean

[1] Attempts to estimate total transport of TGFA in plasma in swine 4 and 5 from the turnover rate of ^{3}H-labeled VLDL triglycerides after pulse injection of [2-^{3}H]glycerol (25) resulted in biphasic curves for the decay of radioactivity in plasma VLDL triglycerides.

[2] Raud, R., and B. M. Wolfe. Unpublished observations.

TABLE 2. Arterial concentrations of metabolites[a]

Swine No.	Blood Glucose	Plasma				
		FFA	TGFA	VLDL TGFA	LDL TGFA	HDL TGFA
				μmoles/ml		
1	5.7 ± 0.2	0.072 ± 0.001	0.68 ± 0.04	0.45 ± 0.03	0.18 ± 0.00	0.07 ± 0.01
2	5.1 ± 0.2	0.046 ± 0.003	0.48 ± 0.01	0.24 ± 0.00	0.17 ± 0.01	0.07 ± 0.00
3	5.8 ± 0.1	0.076 ± 0.003	0.94 ± 0.06	0.33 ± 0.03	0.42 ± 0.01	0.16 ± 0.01
4	3.5 ± 0.1	0.094 ± 0.008	1.07 ± 0.10	0.72 ± 0.08	0.16 ± 0.01	0.06 ± 0.01
5	3.9 ± 0.1	0.056 ± 0.003	0.35 ± 0.02	0.23 ± 0.02		
Mean	4.8 ± 0.5	0.069 ± 0.009	0.70 ± 0.14	0.39 ± 0.09	0.23 ± 0.06	0.09 ± 0.02

[a] Means ± SEM. Mean values were derived from six to eight samples except for swine no. 5, from which four samples were obtained.

TABLE 3. Splanchnic (S), hepatic (H), and extrahepatic splanchnic (EHS) metabolism of free fatty acids[a]

Swine No.	Net Inflow Transport	Turnover Rate	Region	Extraction Fraction		Release[b]	Uptake	Uptake/ Net Inflow Transport
				Titratable FFA	Radio-palmitate			
	μmoles/ min·kg$^{0.75}$	*min^{-1}*				*μmoles/ min·kg$^{0.75}$*	*μmoles/ min·kg$^{0.75}$*	
1	3.2 ± 0.2	0.45 ± 0.02	S	0.13 ± 0.02	0.25 ± 0.03	0.27 ± 0.08	0.59 ± 0.05	0.18
			H	0.13 ± 0.02	0.23 ± 0.02	0.21 ± 0.09	0.51 ± 0.04	0.16
			EHS	0.02 ± 0.02	0.08 ± 0.02	0.06 ± 0.03	0.09 ± 0.02	0.03
2	2.4 ± 0.1	0.46 ± 0.02	S	0.18 ± 0.03	0.23 ± 0.02	0.10 ± 0.10	0.72 ± 0.06	0.30
			H	0.17 ± 0.02	0.19 ± 0.01	0.04 ± 0.06	0.58 ± 0.04	0.24
			EHS	0.03 ± 0.07	0.09 ± 0.01	0.07 ± 0.10	0.14 ± 0.02	0.06
3	3.8 ± 0.2	0.55 ± 0.02	S	0.00 ± 0.06	0.33 ± 0.02	0.37 ± 0.07	0.50 ± 0.11	0.13
			H	0.11 ± 0.07	0.27 ± 0.03	0.20 ± 0.13	0.40 ± 0.11	0.11
			EHS	−0.02 ± 0.10	0.16 ± 0.04	0.18 ± 0.08	0.10 ± 0.02	0.03
4	7.5 ± 0.7	0.78 ± 0.03	S	0.26 ± 0.03	0.60 ± 0.02	1.37 ± 0.21	2.50 ± 0.36	0.33
			H	0.23 ± 0.02	0.53 ± 0.03	1.06 ± 0.15	2.00 ± 0.29	0.27
			EHS	0.08 ± 0.04	0.25 ± 0.01	0.31 ± 0.09	0.51 ± 0.08	0.06
Mean	4.2 ± 1.1	0.56 ± 0.08	S	0.14 ± 0.05	0.35 ± 0.09	0.53 ± 0.29	1.08 ± 0.48	0.24 ± 0.05
			H	0.16 ± 0.03	0.31 ± 0.08	0.38 ± 0.23	0.87 ± 0.38	0.20 ± 0.04
			EHS	0.03 ± 0.02	0.15 ± 0.04	0.16 ± 0.06	0.21 ± 0.10	0.04 ± 0.01

[a] Means ± SEM. Mean values presented were derived from eight sets of samples of arterial and portal and hepatic venous blood obtained over 3 hr with the exception of swine no. 3, from which three complete sets plus three sets of arterial and hepatic venous blood samples were obtained.

[b] Portal flow assumed to be 50% of total hepatic flow in swine 1, 2, and 3.

TABLE 4. Splanchnic (S), hepatic (H), extrasplanchnic (ES), and extrahepatic (EH) metabolism of plasma TGFA

Swine No.	Region	Secretion of Labeled TGFA[a]/ Uptake of Labeled FFA	Secretion of TGFA[a]		Region	Clearance of TGFA from Plasma[a]
			Chemical	Radio-chemical[b]		
			μmoles/min·kg$^{0.75}$			*μmoles/min·kg$^{0.75}$*
1	S	0.16 ± 0.03	1.3 ± 0.2	1.3 ± 0.2	ES	1.3
	H	0.23 ± 0.05	1.3 ± 0.2	1.6 ± 0.3	EH	1.5
2	S	0.06 ± 0.02	1.7 ± 0.2	1.1 ± 0.3	ES	1.1
	H	0.08 ± 0.02	1.8 ± 0.3	1.1 ± 0.2	EH	1.0
3	S	0.15 ± 0.02	0.57 ± 0.15	0.69 ± 0.29	ES	0.66
	H	0.36 ± 0.06	1.0 ± 0.2	1.1 ± 0.3	EH	1.2
4	S	0.27 ± 0.02	4.0 ± 0.6	4.3 ± 0.7	ES	5.2
	H	0.34 ± 0.02	4.0 ± 0.5	4.3 ± 0.7	EH	5.1
Mean	S	0.16 ± 0.04	1.9 ± 0.7	1.8 ± 0.8	ES	2.1 ± 1.0
	H	0.25 ± 0.06	2.0 ± 0.7	2.0 ± 0.8	EH	2.2 ± 1.0

[a] Means ± SEM derived from eight complete sets of arterial, portal venous, and hepatic venous blood samples except from swine 3, from which three complete sets plus three sets of arterial and hepatic venous blood samples were obtained.

[b] Value for fraction of arterial VLDL TGFA derived from FFA used in this calculation was based on $SA_{VLDL\ TGFA}/SA_{FFA\ a+h/2}$.

TABLE 5. Splanchnic (S), hepatic (H), extrasplanchnic (ES), and extrahepatic (EH) metabolism of plasma VLDL TGFA

Swine No.	Region	Secretion of Labeled VLDL TGFA[a]/ Uptake of Labeled FFA	$SA_{VLDL\ TGFA}$[b]/ SA_{FFA_i}	$SA_{VLDL\ TGFA}$/ SA_{FFA}[c] $_{a+h/2}$	Secretion of VLDL TGFA[a] Chemical	Secretion of VLDL TGFA[a] Radiochemical[d]	Region	Clearance of Plasma VLDL TGFA[a]
					$\mu moles/min \cdot kg^{0.75}$			$\mu moles/min \cdot kg^{0.75}$
1	S	0.19 ± 0.04	0.065 ± 0.002	0.068 ± 0.003	0.82 ± 0.15	1.5 ± 0.3	ES	1.5
	H	0.33 ± 0.04			1.4 ± 0.1	2.2 ± 0.3	EH	2.2
2	S	0.08 ± 0.03	0.042 ± 0.001	0.043 ± 0.001	0.74 ± 0.09	1.4 ± 0.4	ES	1.3
	H	0.13 ± 0.04			1.5 ± 0.2	1.9 ± 0.5	EH	2.2
3			0.138 ± 0.003	0.128 ± 0.004				
4	S	0.28 ± 0.07	0.122 ± 0.004	0.161 ± 0.010	3.6 ± 1.0	4.4 ± 1.5	ES	5.1
	H	0.38 ± 0.10			3.7 ± 1.4	4.7 ± 1.7	EH	5.4
Mean	S	0.18 ± 0.06	0.092 ± 0.023	0.100 ± 0.027	1.7 ± 0.9	2.4 ± 1.0	ES	2.6 ± 1.2
	H	0.29 ± 0.08			2.2 ± 0.8	2.9 ± 0.9	EH	3.3 ± 1.1

[a] Means ± SEM derived from three to five complete sets of arterial, portal venous, and hepatic venous blood samples.
[b] Specific activity of arterial VLDL TGFA relative to that of FFA entering the liver during the time interval when the value for this ratio was constant.
[c] Value based on average specific activity of FFA of arterial and hepatic venous blood plasma.
[d] Value for fraction of VLDL TGFA derived from FFA used in this calculation was based on $SA_{VLDL\ TGFA}/SA_{FFA\ a+h/2}$.

91 ± 2%) evidently was derived from precursors other than circulating FFA. Allowance for the fraction of VLDL TGFA derived from precursors other than FFA permits calculation of the total rate of VLDL TGFA secretion.

The specific activity of plasma LDL TGFA rose steadily throughout the period of sampling in each experiment and by the end of the experiment either approached or exceeded that of plasma VLDL TGFA (Fig. 2). Values for the ratio of terminal specific activity of arterial plasma LDL TGFA to terminal specific activity of arterial plasma VLDL TGFA were 1.6, 2.0, 0.75, and 0.89 in swine 1–4, respectively. Comparable values for the ratio of terminal specific activity of total plasma TGFA to terminal specific activity of plasma VLDL TGFA were 1.6, 2.1, 0.85, and 1.0 in swine 1–4, respectively.

TABLE 6. Correlations between different techniques for measuring turnover of plasma TGFA and VLDL TGFA

Comparison	Coefficient of Correlation r
Radiochemical vs. chemical rates of secretion of plasma TGFA from splanchnic region	0.97[a]
Radiochemical vs. chemical rates of secretion of plasma TGFA from liver	0.95[a]
Radiochemical rates of secretion of plasma TGFA from splanchnic region vs. extrasplanchnic clearance of plasma TGFA	1.00[a]
Radiochemical rates of secretion of plasma TGFA from liver vs. extrahepatic clearance of plasma TGFA	1.00[a]
Extrasplanchnic clearance of plasma TGFA vs. chemical rates of secretion of TGFA from splanchnic region	0.97[a]
Extrahepatic clearance of plasma TGFA vs. chemical rates of secretion of TGFA from liver	0.95[a]
Extrasplanchnic clearance of plasma VLDL TGFA vs. chemical rates of secretion of TGFA from splanchnic region	0.98[b]
Extrahepatic clearance of plasma VLDL TGFA vs. chemical rates of secretion of TGFA from liver	0.98[b]

[a] Values based on four swine. Significant correlation, $P < 0.05$.
[b] Values based on three swine.

The terminal specific activity of hepatic TGFA was 97% of that of arterial VLDL TGFA during the last hour of the experiment in swine 2; in swine 4 it was 60%. 53% of the [1-^{14}C]palmitate taken up in the liver in swine 4 was stored in hepatic lipids, so that 91% of the radiopalmitate was recovered in hepatic lipids or in VLDL TGFA secreted from liver. Hepatic content of TGFA (results not shown) was similar to that reported for fasted dogs (21).

Values for the ratio of specific activity of plasma VLDL [^{14}C]TGFA carbon to specific activity of blood [^{14}C]glucose carbon rose progressively and did not plateau. Values for this ratio at the termination of the experiments in swine 1–3 were 0.014, 0.022, and 0.015, respectively. Results of a representative experiment are shown in **Fig. 3.** An average of 9% of plasma VLDL TGFA, is derived from glucose after a 24-hr infusion of hypercaloric amounts of radioglucose (27).

DISCUSSION

The glucose-fed state in miniature swine is characterized by a low rate of transport of FFA, as in man (4), and a high rate of transport of TGFA, of which only a small fraction is derived from FFA, the other sources remaining uncertain (Tables 3–5). The lower rate of transport of FFA in miniature swine compared with swine fed ad lib. (28) may relate to enhanced insulin secretion that inhibited lipolysis and promoted high rates of glucose utilization and fat deposition. Contrary to earlier reports that swine

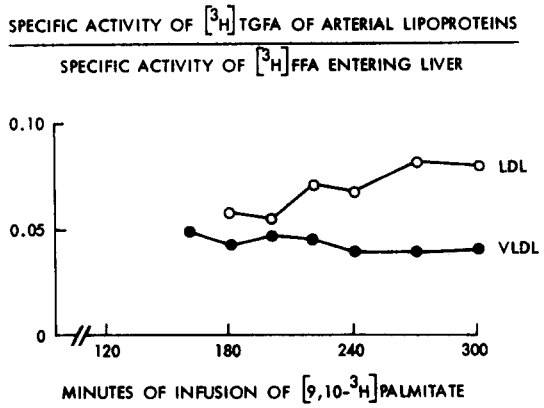

Fig. 2. Ratios of specific activity of TGFA of arterial plasma VLDL and LDL to that of FFA entering the liver in swine 2.

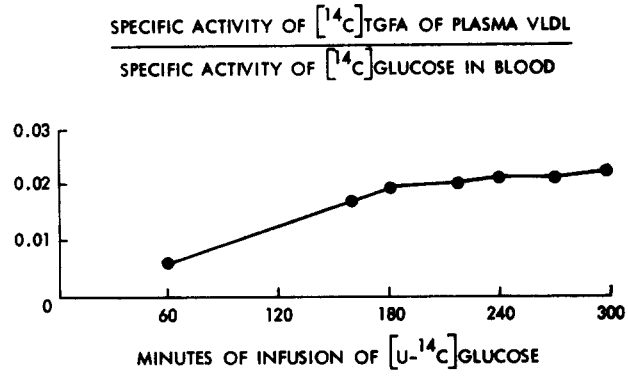

Fig. 3. Ratio of specific activity of carbons of TGFA of plasma VLDL of arterial and portal venous blood to those of blood glucose in swine 2.

have poor glucose tolerance (29), we found that the mean blood glucose concentration of miniature swine during hypercaloric infusion of glucose (antecedent diet high in carbohydrate) was only 1.6 mM higher than that of swine receiving 2400–8000 kcal/day ad lib. (30).

Metabolism of FFA

The fraction of net inflow transport of FFA taken up in the splanchnic region of glucose-fed miniature swine was similar to that reported in fasted anesthetized dogs (14), and the fraction of radiopalmitate taken up in the splanchnic bed that was extracted by liver was also similar (0.82 ± 0.01 vs. 0.75 ± 0.03, $P > 0.1$). In contrast with the situation in fasted dogs, we found that hepatic uptake of radiopalmitate exceeded that of titratable FFA. Possible explanations for such differential uptake of FFA and labeled palmitate include (*1*) different uptakes of individual FFA, (*2*) the existence of more than one pool of albumin-bound FFA, (*3*) hepatic hydrolysis of cholesteryl esters and TGFA in remnants of VLDL with release of their component fatty acids, and (*4*) immediate release from liver of FFA synthesized de novo.

Studies with perfused rat liver (31) suggest the existence of two "pools" of albumin-bound FFA, one with a rapid and one with a slow turnover rate. Because our values for fractional turnover of FFA (Table 3), like those reported in a previous study of FFA metabolism in the fed state (4), are higher than those observed in the postabsorptive state (22), a disproportionately large fraction of radiopalmitate may have been contained in a rapidly turning over "pool" in the present studies. The ratio of FFA to albumin in our radiopalmitate infusion was similar to that previously used in studies in the fasted state but higher than that present in the plasma of our miniature swine. The albumin-bound radiopalmitate infused into the animal may not have been distributed sufficiently rapidly among its albumin molecules and may have been extracted more readily by liver and other tissues than endogenous FFA. In that case, our values for net inflow transport, turnover rate, and radiochemical uptake of FFA may be high, even though they are fivefold lower than values calculated for swine fed ad lib. (28).

Additional evidence suggests that FFA derived from several possible sources, mentioned above, may be released from liver in the glucose-fed state. The ratios of palmitic acid to linoleic acid and oleic acid to linoleic acid of FFA were higher in hepatic venous than in arterial plasma of two hypertriglyceridemic human subjects receiving hypercaloric glucose,[3] to the same extent as previously reported for postabsorptive dogs (14); these findings could represent differential hepatic uptake or release of individual FFA (32). Fatty acids synthesized de novo in liver would be expected to consist largely of palmitate or oleate, linoleate not being formed (33). FFA derived from hydrolysis of chylomicron TGFA in liver may be released into blood (34). Because the miniature swine were 14 hr postprandial and their plasma triglycerides were low, it is unlikely that chylomicrons were present in the circulation. However, hydrolysis in liver of TGFA and of cholesteryl esters (35) derived from "remnants" of VLDL analogous to chylomicron "remnants" (36) may contribute FFA released from liver. Although our findings suggest that liver may release FFA into blood during the glucose-fed state in amounts up to 7% of net inflow transport of FFA, this should be considered to be a maximum value because of possible preferential uptake of radiopalmitate over other FFA.

Secretion of plasma TGFA

The mean fraction of FFA removed by liver that appeared in hepatic venous TGFA in fed swine (Table 4) was more than double that of fasted dogs (21). In addition to promoting hepatic synthesis of fatty acids (37), greater availability of glucose for synthesis of L-α-glycerophosphate may promote hepatic esterification of FFA. As in the postabsorptive state (15), most plasma TGFA secreted from liver in the glucose-fed state are transported in VLDL (Tables 4 and 5). Overestimation of hepatic extraction and uptake of FFA would not affect our radio-

[3] Carroll, K. K., and B. M. Wolfe. Unpublished observations.

chemical estimates of rates of secretion of TGFA and VLDL TGFA because values for the fraction of plasma VLDL TGFA derived from FFA would be overestimated proportionately (see Calculations, 1 [*c*]). Uptake of plasma TGFA in EHS tissues accounted for an average of 14 ± 6% of hepatic secretion, so that values for splanchnic secretion of TGFA slightly underestimated hepatic secretion.

Rates of secretion of plasma TGFA from the S and H regions determined radiochemically were in close agreement with those determined by direct chemical measurement. The lack of agreement between chemical and radiochemical rates of secretion of plasma TGFA from the splanchnic region in one reported study in man (38) may reflect failure to take into account the contribution of precursors other than FFA. The radiochemical technique for measuring TGFA secretion promises to be particularly useful in hypertriglyceridemic states, in which it has been difficult to determine transsplanchnic chemical gradients of TGFA.

Comparison with results obtained in other species suggests that rates of hepatic secretion of TGFA in swine, dogs, and rats in the glucose-fed state are similar. Values for splanchnic secretion obtained in conscious glucose-fed miniature swine were of the same order as those reported by Gross, Eigenbrodt, and Farquhar (39) for anesthetized dogs that had been maintained on high carbohydrate diets and that received approximately isocaloric (average, 80 μmoles/min·kg body $wt^{0.75}$) infusions of glucose during serial sampling of liver and plasma VLDL triglycerides. Combined hepatic and intestinal secretion of plasma TGFA may be as high as 6 μmoles of TGFA/min·kg body $wt^{0.75}$ in rats given a sustained glucose diet (2); however, as much as 40% of total TGFA secretion may be derived from the intestine in the latter species (40).

Clearance of plasma TGFA

Rates of clearance of plasma TGFA reflect hepatic secretion, influx of TGFA from the intestine via the thoracic duct, and changes in plasma TGFA pool size. Calculated values for clearance of TGFA would accurately reflect total clearance if TGFA from the intestine were unlabeled; however, they would be low to the extent that labeled TGFA were secreted from the intestine. In the present studies, all measures of transport of TGFA were similar and highly correlated (Table 6). The close correlation between plasma levels of FFA and VLDL TGFA in the glucose-fed state may reflect derivation of plasma FFA from hydrolysis of VLDL TGFA.

Precursors of plasma VLDL TGFA

Assuming a liver weight equivalent to 2.5% of body weight in minature swine (2.55 and 2.51% in swine 2 and 4, respectively), it can be calculated that hepatic synthesis of TGFA from glucose in porcine liver in vitro is about 0.01 μmoles/min·kg body $wt^{0.75}$ (41), or less than 1% of total hepatic production of plasma TGFA in vivo (Table 4). Although this apparent discrepancy between rates of hepatic TGFA synthesis obtained in vivo and in vitro may be partly attributable to in vitro changes in enzymatic activity (41), the delay in equilibration of glucose carbon with carbon of VLDL TGFA (Fig. 3) suggests that glucose may mix in vivo with a large hepatic pool of unlabeled substrate, such as glycogen, before it is incorporated into VLDL TGFA. Because incorporation of labeled palmitate into secreted VLDL TGFA was maximal within 220 min, incorporation of glucose carbons into plasma VLDL TGFA appeared to be delayed disproportionately. However, there was no evidence of excessive retention of newly synthesized TGFA in liver. The time required to achieve full equilibration between carbons of blood glucose and those of plasma VLDL TGFA is likely to be substantially longer than 24 hr (27). Measurement[4] of hepatic uptake of glucose, glycerol, and amino acids in the glucose-fed state indicates that glucose is the preponderant potential source of carbon for synthesis of plasma VLDL TGFA.

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