

# Splanchnic Spillover of Extracellular Lipase–Generated Fatty Acids in Overweight and Obese Humans

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**OBJECTIVE**—Triglyceride-rich lipoproteins, primarily chylomicrons, can contribute to plasma free fatty acid (FFA) concentrations via spillover of fatty acids during intravascular hydrolysis into the venous effluent of some tissues. The present study was undertaken to determine whether spillover occurs in the splanchnic bed of humans.

**RESEARCH DESIGN AND METHODS**—Arterial and hepatic venous blood was sampled in postabsorptive ( $n = 6$ ; study A) and postprandial ( $n = 5$ ; study B) obese humans during infusion of carbon-labeled ( $^{14}\text{C}$  or  $^{13}\text{C}$ ) oleate and  $^3\text{H}$  triolein, the latter incorporated into a lipid emulsion as a surrogate for chylomicrons. Spillover was determined by measuring production of  $^3\text{H}$  oleate.

**RESULTS**—Splanchnic spillover was higher than nonsplanchnic systemic spillover in both study A ( $60 \pm 7$  vs.  $24 \pm 6\%$ ;  $P < 0.01$ ) and study B ( $54 \pm 3$  vs.  $16 \pm 5\%$ ;  $P < 0.005$ ). Because portal vein sampling is not feasible in humans, assumptions regarding actual spillover in nonhepatic splanchnic tissues were required for the spillover calculation. A mathematical model was developed and demonstrated that nonhepatic splanchnic spillover rates in study A and study B of 69 and 80%, respectively, provided the best fit with the data. There was preferential splanchnic uptake of triglyceride fatty acids compared with FFAs in study B (fractional extraction  $61 \pm 3$  vs.  $33 \pm 2\%$ ;  $P < 0.005$ ).

**CONCLUSIONS**—These data confirm previous studies indicating that the transport of FFAs and triglyceride fatty acids are partitioned in tissues and indicate that splanchnic spillover from triglyceride-rich lipoproteins may be a significant source of both portal venous and systemic FFAs. *Diabetes* 56:2878–2884, 2007

**E**pidemiologic data linking visceral adipose tissue to an increased risk of cardiovascular disease and type 2 diabetes have stimulated considerable study and conjecture as to the mechanism of this relationship. Bjorntorp (1) proposed that increased delivery of free fatty acids (FFAs) to the liver via the portal vein may be the main culprit. Increased portal venous FFAs are thought to lead to increased VLDL triglyceride

production (2), to hepatic insulin resistance (3), and to impaired insulin extraction by the liver with subsequent hyperinsulinemia (4).

Visceral fat is a known source of portal venous FFAs but only a minor source of peripheral FFAs (5). Upper body fat is known to be metabolically different from lower body fat, characterized by increased lipolysis. Whether visceral fat is hyperlipolytic has been questioned (6). Although it is generally thought that the majority of FFAs are derived from the action of intracellular lipases on adipose tissue triglycerides, intravascular lipases such as lipoprotein lipase (LPL) can hydrolyze triglycerides in circulating triglyceride-rich lipoproteins and, thus, can be an additional source. It has previously been demonstrated that some fatty acids produced by intravascular lipolysis are released, or “spilled over,” into the plasma FFA pool; this phenomenon has been documented in human adipose tissue, forearm tissue, and myocardium (7–9). A recent study (10) has shown high rates of spillover in the liver and in nonhepatic splanchnic tissues of fasting dogs. In that study, there was a strong correlation between FFA release from visceral fat and spillover in nonhepatic splanchnic tissues, suggesting that spillover may be regulated by intracellular lipolysis in adipose tissue (10). To date, however, splanchnic spillover has not been studied in humans. The present study was therefore undertaken to determine the amount of fatty acid spillover from intravascular triglyceride lipolysis in the splanchnic bed of fasting and fed obese humans.

## RESEARCH DESIGN AND METHODS

Twelve overweight or obese nondiabetic subjects were studied after an overnight fast according to a protocol approved by the Mayo Institutional Review Board. Informed written consent was obtained before study participation. Data regarding cortisol metabolism from this study have been previously published (11). Subjects were healthy, weight stable, and did not engage in regular vigorous exercise. None had a first-degree relative with a history of diabetes. No subjects were taking medications that would affect lipid or glucose metabolism. The first group of subjects ( $n = 6$ ) was studied in the fasting state as part of a protocol (hereafter referred to as study A) conducted to investigate splanchnic cortisol metabolism (11), while the second group ( $n = 6$ ) was studied subsequently in a separate protocol (study B). Each subject enrolled in the study had body composition and visceral fat measured by dual-energy X-ray absorptiometry and single-slice computed tomography at the fourth vertebral level. All subjects were instructed to follow a weight maintenance diet with 55% carbohydrate, 30% fat, and 15% protein for  $\geq 3$  days before the study.

Subjects were admitted to the Mayo Clinic General Clinical Research Center (GCRC) the afternoon before the study. Following admission, they were given an evening meal providing 10 kcal/kg, with the same macronutrient distribution described above. No other food, except the morning study meal in study B, was consumed after the evening meal until the conclusion of the study. At 0500 h on the morning after admission, an intravenous catheter was placed in a left forearm vein for infusions of saline and isotopes. A urinary bladder catheter was inserted. Subjects were taken to the interventional radiology suite where femoral artery and hepatic venous sampling catheters were placed (11). The procedure did not involve the use of heparin. The arterial and hepatic vein catheters were used for blood sampling for the

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FE, fractional extraction; FFA, free fatty acid; GCRC, General Clinical Research Center; LPL, lipoprotein lipase.

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TABLE 1  
Subject characteristics

	Study A	Study B	P (study A vs. B)
Age (years)	52.8 ± 6.5 (36–74)	45.2 ± 3.2 (38–54)	NS
Sex (female/male) (n)	3/3	4/1	
Weight (kg)	90.5 ± 6.0 (74.9–110.0)	91.1 ± 6.0 (71.4–105.0)	NS
BMI (kg/m <sup>2</sup> )	32.2 ± 1.0 (28.5–35.1)	31.2 ± 1.1 (27.6–33.9)	NS
Glucose (mg/dl)	93 ± 2 (84–99)	91 ± 4 (81–102)	NS
Total cholesterol (mg/dl)	209 ± 14 (165–258)	208 ± 13 (166–240)	NS
HDL cholesterol (mg/dl)	51 ± 5 (31–65)	55 ± 10 (32–83)	NS
LDL cholesterol (mg/dl)	118 ± 12 (81–156)	120 ± 13 (75–156)	NS
Triglycerides (mg/dl)	200 ± 34 (96–316)	134 ± 22 (74–208)	NS

Data are means ± SE (range) unless otherwise indicated.

present study. Subjects then returned to the GCRC for the remainder of the study.

Upon return to the GCRC, study group A remained fasting, whereas B received a mixed meal (~450 kcal: 45% carbohydrate, 15% protein, and 40% fat) prepared as previously described (11). Infusions of tracer amounts of [<sup>14</sup>C] oleate (~0.3 μCi/min) and a lipid emulsion (10% intralipid) labeled with [9,10-<sup>3</sup>H] triolein (~1.2 μCi/min) were started immediately upon return to the GCRC in study A. In study B, similar tracer infusions began 3 h after meal ingestion, except that [U-<sup>13</sup>C] oleate (~0.5 nmol · kg<sup>-1</sup> · min<sup>-1</sup>) was infused instead of [<sup>14</sup>C] oleate. In both groups, tracer infusions were continued until the end of the study. Preparation of the lipid emulsion, which has been shown to be a good surrogate for chylomicron metabolism, has been described previously (10,12). Sixty minutes after catheter placement, an infusion of indocyanine green dye (Akorn, Buffalo Grove, IL) was started through the arterial sheath for measurement of splanchnic blood flow. Four sets of blood samples (arterial and hepatic vein) were collected at 10-min intervals starting 30 min after the start of the infusion for determination of indocyanine green dilution. In both groups of subjects, arterial and hepatic vein blood samples were taken at 10-min intervals beginning 2 h after the start of tracer infusion (designated as time -120 min); six paired samples were taken in study A and five in study B. The orientation between infusion and sampling sites (i.e., tracer infusion in a peripheral vein, with “downstream” sampling) in this study has been previously validated for FFA kinetics (13). Plasma samples were analyzed for triglyceride radioactivity, oleate and FFA concentration, <sup>3</sup>H oleate specific activity, and either <sup>14</sup>C oleate specific activity (study A) or <sup>13</sup>C oleate enrichment (study B). In both groups, the study was terminated after the final paired blood samples were taken.

**Analyses.** Blood samples were collected in chilled 10-ml EDTA tubes containing 0.5 mg of paraoxon to inhibit LPL (14) and kept on ice until centrifugation at 4°C. For both studies, plasma triglyceride radioactivity and FFA concentration and specific activity were determined on the same aliquot of plasma (10). Plasma FFA concentration and specific activity were determined using a modification (10) of a high-performance liquid chromatography (15) method. For study B, <sup>13</sup>C atoms percent enrichment was determined on a separate aliquot of plasma by isotope ratio mass spectrometry using a Finnegan Delta Plus mass spectrometer. A published isotope ratio mass spectrometry procedure for palmitate enrichment (16) was modified for measurement of oleate enrichment by utilizing two columns in series in order to separate oleate and elaidate.

**Calculations.** Systemic oleate rate of appearance ( $R_a$ ) was calculated from the infusion rate of carbon-labeled oleate and <sup>14</sup>C oleate specific activity or <sup>13</sup>C oleate atoms percent enrichment using the equations of Steele et al. (17). The contribution of the splanchnic bed to systemic disposal of labeled triglycerides was calculated from splanchnic uptake of triglyceride tracer, splanchnic plasma flow, and <sup>3</sup>H triglyceride infusion rate, as previously described (10). Systemic fractional spillover was calculated as previously described (8). Net splanchnic spillover was calculated from arterial and hepatic venous data using splanchnic plasma flow, as previously reported in dogs (10), and analogous to measurements in the forearm (8) and heart (9) of humans. We previously demonstrated in fasting dogs that when net splanchnic spillover is calculated from arterial and hepatic venous data without use of portal venous data, an error in the estimate of “true” splanchnic spillover (as determined when portal venous data are included in the calculation) may be introduced; therefore, true spillover was calculated from net spillover using the following regression formula: true spillover = 44.04 × log<sub>e</sub>(net spillover) - 108.21, which was generated from the dog studies (10). Nonsplanchnic spillover was determined from systemic spillover and true splanchnic spillover, as previously described (10). A more detailed description of the calculations is

provided in the online appendix (available at <http://dx.doi.org/10.2337/db07-0812>).

Because of the unavailability of portal venous data, spillover in nonhepatic splanchnic tissues in humans under various conditions cannot be known with

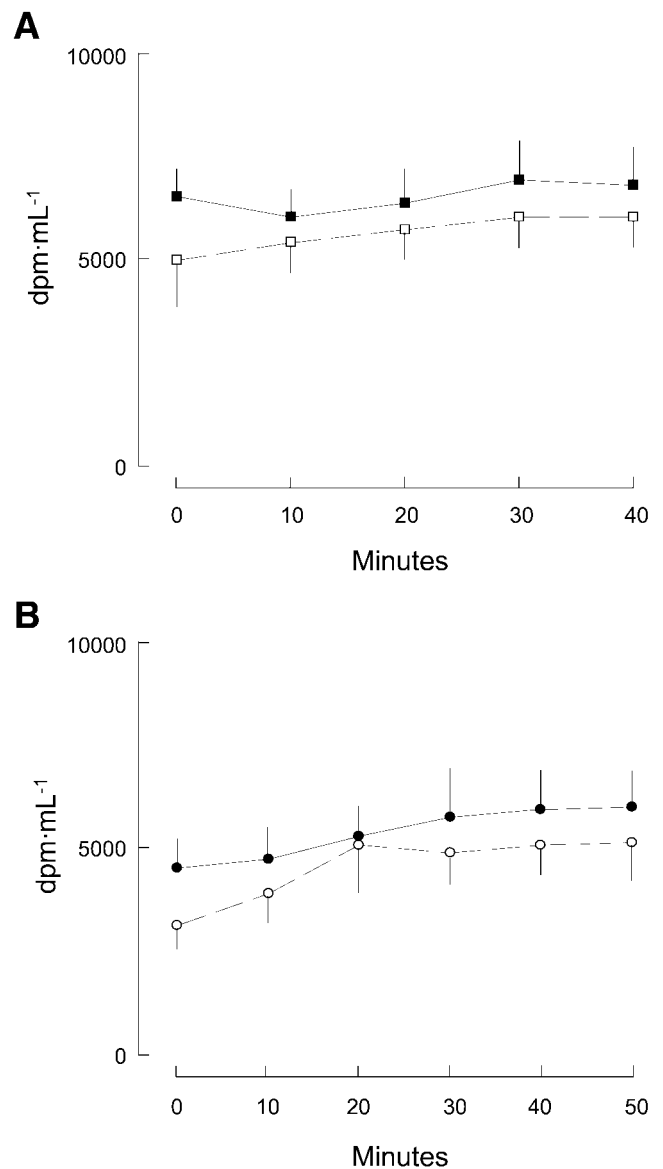


FIG. 1. Triglyceride radioactivity (dpm/ml) in arterial and hepatic venous plasma in studies A (A) and B (B). ■, arterial; □, hepatic venous.

TABLE 2  
Oleate data: study A

	Time (min)				
	0	10	20	30	40
Oleate concentration (μmol/l)					
Artery	278 ± 32	282 ± 36	282 ± 31	255 ± 29	283 ± 36
Hepatic venous	208 ± 23	200 ± 25	195 ± 25	204 ± 25	193 ± 21
<sup>14</sup> C oleate specific activity (dpm/nmol)					
Artery	1.95 ± 0.30	2.04 ± 0.31	2.00 ± 0.29	2.10 ± 0.39	2.03 ± 0.30
Hepatic venous	1.74 ± 0.31	1.78 ± 0.31	1.70 ± 0.28	1.72 ± 0.29	1.82 ± 0.27
<sup>14</sup> C oleate radioactivity (dpm/ml)					
Artery	515 ± 71	553 ± 96	556 ± 97	507 ± 71	549 ± 88
Hepatic venous	352 ± 66	346 ± 63	330 ± 66	342 ± 59	340 ± 49
<sup>3</sup> H oleate specific activity (dpm/nmol)					
Artery	3.59 ± 0.69	3.59 ± 0.65	3.80 ± 0.65	3.35 ± 0.38	3.78 ± 0.67
Hepatic venous	4.84 ± 1.04	5.01 ± 0.85	4.74 ± 0.80	4.80 ± 0.78	5.02 ± 0.81
<sup>3</sup> H oleate concentration (dpm/ml)					
Artery	984 ± 229	1,007 ± 234	1,068 ± 238	1,058 ± 245	1,094 ± 309
Hepatic venous	1,025 ± 306	1,021 ± 270	953 ± 264	1,009 ± 269	990 ± 243

Data are means ± SE.

certainty; therefore, the accuracy of the calculation of true spillover using the above regression formula cannot be determined experimentally. We therefore created a model to assess the limits of the calculation. In this model, the concentrations of three tracers (<sup>3</sup>H triglyceride, carbon-labeled oleate, and <sup>3</sup>H oleate) are known for both arterial and hepatic venous plasma. Splanchnic plasma flow is also known, and hepatic blood flow is assumed to be 80% portal and 20% arterial (18). The contribution of the liver to splanchnic uptake of labeled triglyceride was assumed to be 55% in study A and 27.5% in study B, based on previous observations in animals that the liver is responsible for 55% of splanchnic triglyceride uptake during fasting (10) and that the contribution of the liver decreases by ~50% after meal ingestion (19). The contribution of the liver to splanchnic uptake of oleate was assumed to be 72% in both studies, based on results in dogs (10,18).

The following formula was used to predict portal venous <sup>3</sup>H triglyceride and carbon-labeled oleate concentrations: [T]<sub>PV</sub> = [T]<sub>A</sub> × (1 - FE<sub>NH</sub>), where [T] = tracer concentration, PV = portal vein, A = arterial, and FE<sub>NH</sub> = nonhepatic splanchnic fractional extraction.

These values for portal venous tracer concentrations were then used to estimate the contribution of portally derived tracer to hepatic venous tracer concentration using the following formula: [T]<sub>PV→HV</sub> = [T]<sub>PV</sub> × FE<sub>H</sub> × 0.8, where HV = hepatic vein, FE<sub>H</sub> = hepatic fractional extraction, and 0.8 is the relative contribution of the portal vein to total hepatic blood flow (18).

The contribution of arterial plasma tracer to hepatic venous tracer concentrations was calculated by the following formula: [T]<sub>A→HV</sub> = [T]<sub>A</sub> × FE<sub>H</sub> × 0.2, where 0.2 is the relative contribution of the artery to total hepatic blood flow (18).

Predicted hepatic venous tracer concentrations were then calculated as the following: predicted [T]<sub>HV</sub> = [T]<sub>PV→HV</sub> + [T]<sub>A→HV</sub>.

Nonhepatic splanchnic and hepatic fractional extractions for both <sup>3</sup>H triglycerides and carbon-labeled oleate were then adjusted empirically, maintaining the assumed distribution of tracer uptake between the two tissue beds as described above (e.g., 72% of splanchnic FFA uptake and 55% of splanchnic triglyceride uptake occurs in the liver in study A) until the predicted hepatic venous concentrations of <sup>3</sup>H triglycerides and carbon-labeled oleate were the same as the measured values. Portal venous tracer concentrations thus predicted by the model were then used with measured arterial and hepatic venous tracer concentrations to calculate true splanchnic spillover, as previously described (10), across a range of assumed values for nonhepatic splanchnic fractional spillover. The relationship between nonhepatic fractional spillover and true splanchnic spillover was used to define limits for true splanchnic spillover across a range of values for nonhepatic spillover.

**Statistical methods.** Data are expressed as means ± SE. Mean values were calculated by averaging results from all samples taken from each site. Comparisons of values from different sites within each group of subjects were analyzed by a *t* test for paired samples for means using the Microsoft Excel data analysis package. Between-group data were analyzed with a *t* test for two samples assuming an unequal variance using the same software. A *P* value of <0.05 was required for statistical significance.

**RESULTS**

Steady-state conditions were not achieved in one subject in study B, and the data from that subject was therefore not included in the analysis. Baseline characteristics of the subjects are shown in Table 1. There were no differences between study A and study B with respect to age, weight, BMI, fasting plasma glucose, or fasting lipid values. Fasting triglycerides were somewhat higher in study A, but the difference was not statistically significant.

There were no statistically significant differences between study A and study B for percent body fat (41 ± 4 vs. 44 ± 4%) lean body mass (50 ± 6 vs. 47 ± 5 kg), total body fat (36 ± 2 vs. 39 ± 4 kg), abdominal subcutaneous fat area (317 ± 22 vs. 331 ± 34 cm<sup>2</sup>), or visceral fat area (173 ± 27 vs. 110 ± 22 cm<sup>2</sup>) (data not shown). Splanchnic plasma flow was 882 ± 37 vs. 972 ± 118 ml/min in the two groups, respectively (*P* = NS).

Arterial and hepatic venous <sup>3</sup>H triglyceride concentrations in the two study groups are shown in Fig. 1. Hepatic venous concentrations were lower than arterial values in both study A (5,645 ± 637 vs. 6,330 ± 688 dpm/ml; *P* < 0.001) and study B (4,368 ± 841 vs. 5,202 ± 839 dpm/ml; *P* < 0.003).

Table 2 shows plasma oleate concentrations, <sup>14</sup>C oleate specific activity, and <sup>3</sup>H oleate specific activity for study A. <sup>14</sup>C and <sup>3</sup>H oleate radioactivities (the product of oleate concentration and specific activity) are also provided. Mean hepatic venous plasma oleate concentration, <sup>14</sup>C specific activity, and <sup>14</sup>C radioactivity were lower than arterial (200 ± 23 vs. 273 ± 32 μmol/l, 1.8 ± 0.3 vs. 2.0 ± 0.3 dpm/nmol, and 342 ± 60 vs. 545 ± 83 dpm/ml, respectively, all *P* < 0.002). Hepatic venous plasma <sup>3</sup>H oleate specific activity was higher than arterial (4.9 ± 0.9 vs. 3.7 ± 0.7 dpm/nmol; *P* < 0.001). Mean hepatic venous plasma total FFA concentrations (data not shown) were also lower than arterial (484 ± 48 vs. 735 ± 84 μmol/l; *P* < 0.001).

Table 3 shows plasma oleate concentrations, <sup>13</sup>C oleate atoms percent enrichment, and <sup>3</sup>H oleate specific activity for study B. <sup>13</sup>C oleate concentration (the product of oleate concentration and enrichment) and <sup>3</sup>H oleate radioactivity are also provided. Mean hepatic venous plasma

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TABLE 3  
Oleate data: study B

	Time (min)					
	0	10	20	30	40	50
Oleate concentration ( $\mu\text{mol/l}$ )						
Artery	151 $\pm$ 26	151 $\pm$ 18	181 $\pm$ 35	183 $\pm$ 20	203 $\pm$ 25	218 $\pm$ 24
Hepatic venous	124 $\pm$ 12	133 $\pm$ 16	120 $\pm$ 16	134 $\pm$ 18	149 $\pm$ 16	164 $\pm$ 15
$^{13}\text{C}$ oleate APE ( $\% \times 1,000$ )						
Artery	21.9 $\pm$ 1.5	21.1 $\pm$ 1.9	20.6 $\pm$ 2.0	20.8 $\pm$ 1.8	19.6 $\pm$ 2.1	18.1 $\pm$ 1.8
Hepatic venous	18.9 $\pm$ 1.5	18.9 $\pm$ 1.6	18.0 $\pm$ 1.8	18.0 $\pm$ 2.1	16.3 $\pm$ 1.9	15.5 $\pm$ 2.0
$^{13}\text{C}$ oleate concentration (nmol/l)						
Artery	32 $\pm$ 3	31 $\pm$ 4	33 $\pm$ 4	34 $\pm$ 2	33 $\pm$ 4	38 $\pm$ 3
Hepatic venous	23 $\pm$ 2	24 $\pm$ 2	21 $\pm$ 2	23 $\pm$ 3	25 $\pm$ 3	25 $\pm$ 3
$^3\text{H}$ oleate specific activity (dpm/nmol)						
Artery	4.10 $\pm$ 0.35	3.82 $\pm$ 0.41	3.51 $\pm$ 0.26	3.41 $\pm$ 0.17	3.17 $\pm$ 0.15	3.94 $\pm$ 0.46
Hepatic venous	6.12 $\pm$ 0.60	5.64 $\pm$ 0.67	5.82 $\pm$ 0.87	5.65 $\pm$ 0.89	5.73 $\pm$ 0.91	5.23 $\pm$ 0.99
$^3\text{H}$ oleate concentration (dpm/ml)						
Artery	668 $\pm$ 94	550 $\pm$ 74	625 $\pm$ 78	618 $\pm$ 55	651 $\pm$ 84	685 $\pm$ 106
Hepatic venous	822 $\pm$ 137	739 $\pm$ 101	636 $\pm$ 118	749 $\pm$ 121	842 $\pm$ 144	825 $\pm$ 122

Data are means  $\pm$  SE.

oleate concentration,  $^{13}\text{C}$  enrichment, and  $^{13}\text{C}$  concentration were lower than arterial ( $137 \pm 12$  vs.  $178 \pm 19$   $\mu\text{mol/l}$ ,  $17.4 \pm 0.2$  vs.  $20.2 \pm 0.2\% \times 10^3$ , and  $23 \pm 2$  vs.  $34 \pm 3$  nmol/l, respectively, all  $P < 0.005$ ). Hepatic venous plasma  $^3\text{H}$  oleate specific activity was higher than arterial ( $5.6 \pm 0.8$  vs.  $3.9 \pm 0.4$  dpm/nmol;  $P < 0.001$ ). Mean hepatic venous total FFA concentrations (data not shown) were also lower than arterial ( $334 \pm 26$  vs.  $384 \pm 31$   $\mu\text{mol/l}$ ;  $P = 0.01$ ).

The fractional extraction (FE) of LPL-generated  $^3\text{H}$  oleate (calculated as  $100 - \text{fractional spillover}$  in percent) was slightly but not significantly higher than the FE of  $^{14}\text{C}$  oleate in study A ( $51 \pm 10\%$  vs.  $39 \pm 4\%$ ;  $P = \text{NS}$ ), whereas the FE of LPL-generated  $^3\text{H}$  oleate was significantly higher than the FE of  $^{13}\text{C}$  oleate in study B ( $61 \pm 3$  vs.  $33 \pm 2\%$ ;  $P < 0.005$ ) (Fig. 2). Systemic oleate  $R_a$  was  $305 \pm 71$  and  $235 \pm 33$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in studies A and B, respectively ( $P = \text{NS}$ ). The fractional contribution of the

splanchnic bed to systemic disposal of labeled triglyceride was  $25 \pm 4$  and  $33 \pm 6\%$  in studies A and B, respectively ( $P = \text{NS}$ , data not shown). Systemic fractional spillover was  $38 \pm 4$  and  $34 \pm 2\%$  in studies A and B, respectively ( $P = \text{NS}$ , data not shown). Net splanchnic spillover was  $49 \pm 10$  and  $41 \pm 3\%$  in studies A and B, respectively ( $P = \text{NS}$ , data not shown).

Figure 3 shows true splanchnic spillover and nonsplanchnic spillover in studies A and B. True spillover was  $60 \pm 7$  and  $54 \pm 3\%$ , respectively ( $P = \text{NS}$ ). Nonsplanchnic spillover was  $24 \pm 6$  and  $16 \pm 5\%$ , respectively; these values were both significantly lower than true splanchnic spillover ( $P < 0.01$ ) but were not different from each other ( $P = 0.4$ ). There was no correlation between visceral fat or total body fat and systemic, splanchnic, or nonsplanchnic spillover.

The model-determined relationship between nonhepatic spillover and true splanchnic spillover is shown in Fig. 4. Assuming plausible limits of 10 and 90% for nonsplanchnic

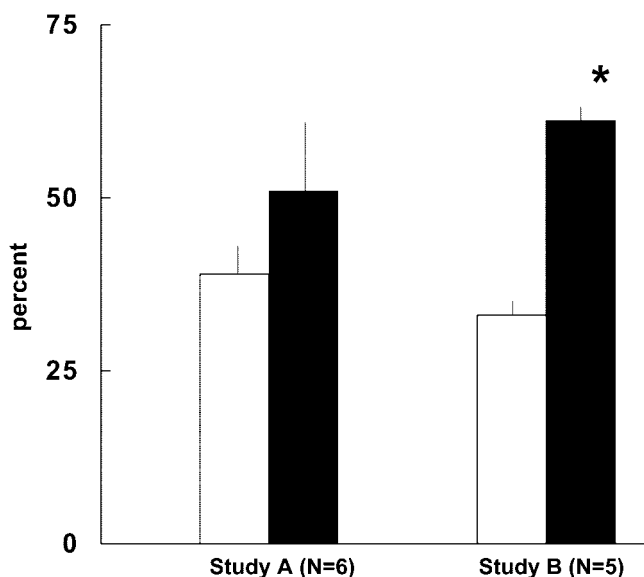


FIG. 2. Fractional extraction of FFAs ( $^{14}\text{C}$  oleate) and triglyceride-derived fatty acids (locally generated  $^3\text{H}$  oleate) in studies A and B.  $\square$ ,  $^{14}\text{C}$  oleate FE;  $\blacksquare$ , locally generated  $^3\text{H}$  oleate FE; \* $P < 0.005$  vs.  $^{14}\text{C}$  oleate.

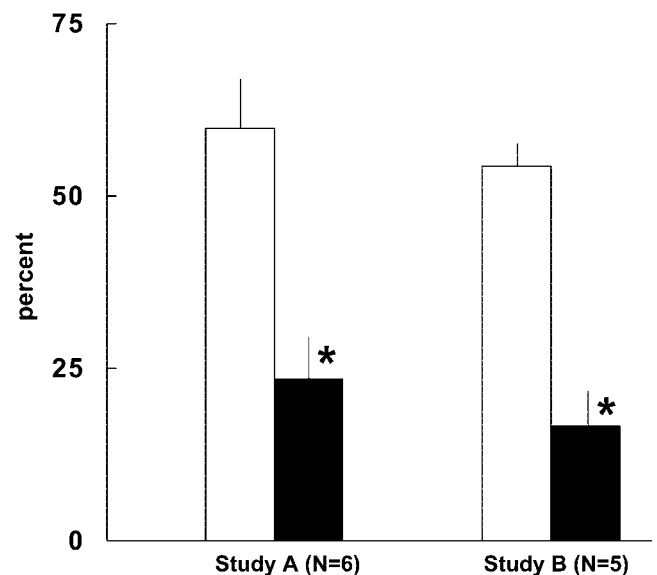


FIG. 3. True splanchnic spillover (calculated from regression formula) versus nonsplanchnic spillover in studies A and B.  $\square$ , true splanchnic;  $\blacksquare$ , nonsplanchnic.



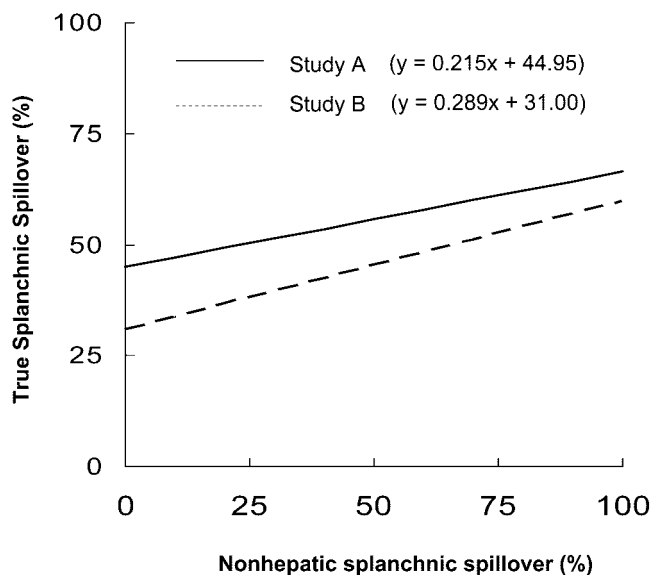


FIG. 4. Model-predicted true splanchnic spillover over a range of nonhepatic splanchnic spillover in studies A and B.

spillover, true splanchnic spillover ranged from  $47 \pm 9$  to  $64 \pm 9\%$  for study A and  $34 \pm 3$  to  $57 \pm 4\%$  for study B. The values for nonhepatic splanchnic spillover that produced model-determined estimates of true spillover closest to those determined by the regression formula were 69% for study A and 80% for study B. True splanchnic spillover remained greater than nonsplanchnic spillover ( $P < 0.05$ ) when values for nonhepatic splanchnic spillover  $\geq 40\%$  in study A and  $\geq 41\%$  in study B were used. There were no significant differences in model-predicted true spillover between study A and study B.

## DISCUSSION

The present study demonstrates for the first time in humans that fatty acids derived from splanchnic metabolism of triglyceride-rich lipoproteins spill over into the plasma FFA pool. The spillover phenomenon has previously been demonstrated systemically and in adipose tissue, forearm, and myocardium (7–9). We found that true splanchnic fractional spillover was  $\sim 50$ – $60\%$  in overweight and obese humans in both study A (the postabsorptive state) and study B (4–5 h after meal ingestion). Further, nonsplanchnic spillover, which represents the sum of spillover in subcutaneous adipose tissue, skeletal muscle, myocardium, and other nonsplanchnic tissues, was significantly lower than splanchnic spillover.

We used a formula derived from a study in dogs to calculate true spillover because it is not possible to access the portal vein in humans. In those animal studies, net spillover was  $\sim 20\%$  lower than true spillover (9). Because of the inherent uncertainty of interspecies comparisons, we developed a model to assess for possible errors in the calculation of true splanchnic spillover in humans. Specifically, we investigated the impact of different nonhepatic splanchnic spillover values on the calculation of true spillover, since the former cannot be independently determined without access to the portal vein. For the purposes of the model, we assumed that in postabsorptive subjects (study A), 45% of splanchnic triglyceride uptake was

nonhepatic and 55% occurred in the liver and that the distribution changed to 72.5:27.5 during meal absorption (study B), based on data from animal studies (10,19). It was also assumed that the liver was responsible for 72% of splanchnic FFA uptake (10,18). With these assumptions, the values for nonhepatic splanchnic spillover that produced estimates of true spillover similar to those generated by the regression formula were 69% in study A and 80% in study B. When nonhepatic splanchnic spillover was varied over a physiologically plausible range (10–90%), the model-derived estimates of true splanchnic spillover were within 3–20% of values calculated from the regression formula. Such extremes in nonhepatic splanchnic spillover are probably nonphysiological, based on previous studies in animals (10).

Nonsplanchnic spillover was significantly lower than true splanchnic spillover in both postabsorptive (study A) and postprandial (study B) subjects. Utilizing the model for splanchnic spillover, this discrepancy remained significant when nonhepatic splanchnic spillover was decreased from the best-fit values of 69 and 80% in the two studies to as low as 40%; with lower values for nonhepatic spillover, the difference between true splanchnic and nonsplanchnic spillover was not significant. Assuming actual nonhepatic spillover values  $\geq 40\%$ , the fact that true splanchnic spillover was higher than nonsplanchnic spillover in study B is consistent with relative nonsuppressibility of visceral lipolysis by meal-induced insulin secretion in overweight and obese subjects, considering the strong correlation between nonhepatic spillover and visceral lipolysis previously observed in animals (10). Because 1) we did not study lean subjects and 2) the visceral fat depot in our subjects was considerably greater than that observed in lean individuals (5), further study will be required to determine whether such high rates of postprandial splanchnic spillover (study B) are unique to overweight and obese individuals. In any case, our data suggest that high rates of splanchnic spillover do occur and may augment portal FFA delivery to the liver during meal absorption. They may also contribute to systemic FFA availability, especially if, as suggested by animal data (10,19), a significant portion of spillover is occurring in the liver.

FFA concentrations are increased in obese subjects, especially those who have predominantly upper body fat accumulation and do not suppress normally during insulin infusion compared with lean subjects (20). It is not known whether fractional spillover (either splanchnic or systemic), which is essentially a reflection of the efficiency of fat uptake/storage, is higher in obese subjects compared with lean individuals. If this were the case, it would mean that a defect in fat storage can be added to impaired suppression of intracellular lipolysis as a cause of elevated FFA concentrations in obese people. Since spillover results in the addition of fatty acids derived from triglyceride-rich lipoproteins to circulating FFAs, abnormally elevated spillover rates could contribute to ectopic fat accumulation in liver, skeletal muscle, and myocardium (21).

The spillover process occurs primarily as a result of intravascular metabolism of chylomicrons, since spillover from VLDL is minimal (22,23) and the amount of triglycerides that traverses the circulation in the form of chylomicrons is so much greater than that in VLDL in most individuals (8). The contribution of dietary fat to plasma FFAs can be expected to continue for most of the 24-h

period, considering the long duration of fat absorption after ingestion of a mixed meal (24) and the fact that intervals between meals are not >14 h in most individuals. Data on the impact of varying meal fat content on spillover are not available, but it seems likely that reduced total spillover of fatty acids from chylomicrons would be a salutary effect of reduced dietary fat intake.

The potential impact of splanchnic metabolism of dietary fat on total fatty acid delivery to the liver can be estimated. Assuming a daily energy intake of 2,000 kcal, a daily fat intake of 78 g (35% of energy intake), net splanchnic spillover of 41%, and a 30% contribution of the splanchnic bed to whole-body dietary triglyceride uptake (as was observed in study B), we would estimate that 12–13% of systemic FFA availability derives from splanchnic spillover. Although this is a relatively small amount, the contribution of splanchnic spillover to delivery of FFAs to the liver would be considerably greater. Assuming that portal venous FFA concentrations in these human subjects are ~13% higher than arterial values due to lipolysis in visceral fat as was observed in dogs (10), we estimate that spillover in nonhepatic splanchnic tissues could increase portal vein FFA concentrations by roughly one-third over 24 h compared with what would be expected on a fat-free diet. The relative contribution could actually be greater during the 4- to 6-h period after a meal, when FFA concentrations are suppressed and fat absorption is at its peak.

A previous study in rodents suggested that there is complete mixing of LPL-generated fatty acids with circulating FFAs, and therefore a distinction between triglyceride-derived fatty acids and FFAs cannot be made (25). However, the present study indicates that this is not the case in humans. Net splanchnic FE of newly generated <sup>3</sup>H oleate (the reciprocal of fractional spillover) was somewhat (~30%) greater than that of FFAs (determined by the carbon-labeled oleate tracer) in study A, but the difference was not statistically significant. However, during meal absorption (study B), <sup>3</sup>H oleate FE was nearly 100% higher than FFA FE ( $P < 0.005$ ). These data are strong evidence for partitioning of fatty acids from the two sources (FFAs and chylomicron triglycerides) for cellular uptake during meal absorption and are consistent with a recent report by Bickerton et al. (24) showing preferential uptake of triglyceride-derived fatty acids compared with circulating FFAs. We have reanalyzed data in dogs (10) and found that locally generated <sup>3</sup>H oleate FE was greater than FFA FE in both nonhepatic splanchnic tissues ( $50 \pm 10$  vs.  $7 \pm 2\%$ ;  $P < 0.005$ ) and the liver ( $55 \pm 9$  vs.  $18 \pm 3\%$ ;  $P < 0.01$ ) (R.H.N., D.S. Edgerston, R.B., J.C. Roesner, A.D. Cherrington, J.M.M., unpublished data), indicating that partitioning of fatty acids occurs throughout the splanchnic bed. Considering the evidence for lower spillover of fatty acids from VLDL (23), it is likely that the partitioning is greater when this lipoprotein is the source of fatty acids.

In summary, this study demonstrates that splanchnic spillover of fatty acids from triglyceride-rich lipoproteins is of potential importance in contributing to the delivery of FFAs to the liver and systemically. Studies in healthy lean subjects are needed to put these results into perspective.

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