Meal Fatty Acid Uptake in Visceral Fat in Women

Susanne B. Votruba, Rebecca S. Mattison, Daniel A. Dumesic, Christina Koutsari, and Michael D. Jensen

OBJECTIVE—Differential meal fat uptake into adipose tissue depots may be a determinant of body fat distribution.

RESEARCH DESIGN AND METHODS—We used the meal fat tracer/adipose tissue biopsy approach to compare the effects of meal fat content on the fat uptake into visceral and upper and lower body subcutaneous fat depots in 21 premenopausal women. [^{3}H]triolein was used to trace the fate of fatty acids from a normal-fat or high-fat meal.

RESULTS—The proportion of dietary fat uptake into the three depots did not differ between meals; visceral fat accounted for only ~5% of meal fat disposal irrespective of visceral fat mass. For the women consuming the normal-fat meal, the uptake of meal fatty acid into femoral fat (milligrams meal fat per gram lipid) increased as a function of leg fat mass ($r = 0.68$, $P < 0.05$), which we interpret as increased efficiency of uptake. The opposite pattern was seen in omental fat with the normal-fat meal and in all depots after the high-fat meal. For both meals, ~40% of meal fat was oxidized (^3H_2O production) after 24 h.

CONCLUSIONS—We conclude that greater thigh adipose tissue in women is associated with greater efficiency of meal fat storage under conditions of energy balance, whereas the opposite is seen with visceral fat. These findings imply that different mechanisms may regulate fatty acid uptake in different depots, which may in turn impact on body fat distribution. ***Diabetes*** **56:2589–2597, 2007**

Because of the health implications of upper body/visceral obesity we (1–4) and others (5–7) are interested in the contribution of competing regional fat storage processes in determining body fat distribution. This is of special interest given the possible role of visceral adipose tissue lipolysis in the metabolic complications of obesity (8). Under weight stable conditions, fatty acid uptake into an adipose tissue depot should equal lipolysis from this depot. Therefore, the direct measurement of regional meal fat uptake provides an indirect measure of regional lipolysis and clues as to how variations in regional fat gain develop.

We found greater efficiency of uptake (milligrams meal fat per gram lipid) in the upper body subcutaneous (UBSQ) fat than lower body subcutaneous (LBSQ) fat in both lean men and women under isoenergetic conditions (2,3). In addition, we reported that a high-fat/calorie meal increased the "efficiency" of LBSQ meal fatty acid uptake in women compared with men (4). Although the previous study provides clues to sex-specific regional fat distribution, it does not address how obese individuals may respond to a similar high-fat challenge nor does it address the role of visceral fat.

Mårin et al. (6,9) first used the meal fatty acid tracer/adipose biopsy approach to study visceral adipose tissue metabolism in men after a high-fat meal. They found greater meal fat uptake into visceral than into subcutaneous abdominal fat but were not able to assess LBSQ fat, to compare their findings to a normal-fat meal, or to measure meal fat oxidation. We suggest that by combining all of these measures, it is possible to better understand the contribution of dietary fat uptake in determining regional fat mass.

The purpose of this study was to assess the effect of meal fat content on the disposal of dietary fat in women with a wide range of body fat distribution. We measured meal fatty acid oxidation and uptake into subcutaneous and visceral adipose tissue. The null hypothesis was that the efficiency of meal fat uptake (in milligrams meal fat per gram lipid) would be similar between regional fat depots and between individuals with different amounts of body fat.

RESEARCH DESIGN AND METHODS

This study was approved by the Mayo Clinic institutional review board, and informed, written consent was obtained from 21 women. Participants were recruited if they were scheduled for voluntary bilateral tubal ligation ($n = 14$) or had existing polycystic ovarian syndrome (PCOS) ($n = 7$) undergoing research laparoscopy (1). The PCOS participants tended to have more body fat but did not have metabolic abnormalities known to confound our measures. One PCOS patient, who had no previous diagnosis of diabetes, was found to have an asymptomatic fasting glucose of 151 mg/dl the first morning of the study. We classified her as a diet-controlled type 2 diabetic patient, and her values have been distinguished in figures, where appropriate. No medications, with the exception of oral contraceptives, were allowed, and a complete blood count and a chemistry panel were documented to be within normal limits.

Protocol. The Mayo Clinic General Clinical Research Center (GCRC) metabolic kitchen provided weight maintenance research meals for 3 days before the test meal to ensure constant macronutrient composition (50% carbohydrate, 35% fat, and 15% protein), as previously described (2). The volunteers were randomly assigned to the high-fat/calorie or normal-fat meal group.

The volunteers were admitted at 1700 h, given a meal at 1800 h, and remained in the GCRC for the next 2 days. After an overnight fast (day 1; Fig. 1), subjects were given a liquid test meal at 0800 h (Ensure Plus; Ross Laboratories) that provided calories equal to 40% of their resting energy expenditure (REE) or the same meal plus an additional 80 g triolein (provided by Karlshams, Karlsham, Sweden); ~84 μCi [^{3}H]triolein was sonicated into the liquid meal (10) to trace meal fatty acid metabolism (1,11). Weighed research meals of solid food were provided at 1300 and 1800 h. Blood samples were taken hourly until 1600 h and then less frequently until 0800 h the following morning (day 2). One hour after the lunch meal and again 24 h after the consumption of the test meal, needle biopsies of the abdominal and femoral subcutaneous adipose tissue were performed to measure [^{3}H]triolein

From the Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota.

Address correspondence and reprint requests to Michael Jensen, Mayo Clinic, Endocrine Research Unit, 200 1st St. SW, Rm. 5-194 Joseph, Rochester, MN 55905. E-mail: jensen@mayo.edu.

Received for publication 30 March 2007 and accepted in revised form 19 July 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 30 July 2007. DOI: 10.2337/db07-0439.

CT, computed tomography; DEXA, dual-energy X-ray absorptiometry; FFA, free fatty acid; GCRC, General Clinical Research Center; LBSQ, lower body subcutaneous; LPL, lipoprotein lipase; PCOS, polycystic ovarian syndrome; REE, resting energy expenditure; RER, respiratory exchange ratio; TG, triglyceride; UBSQ, upper body subcutaneous.

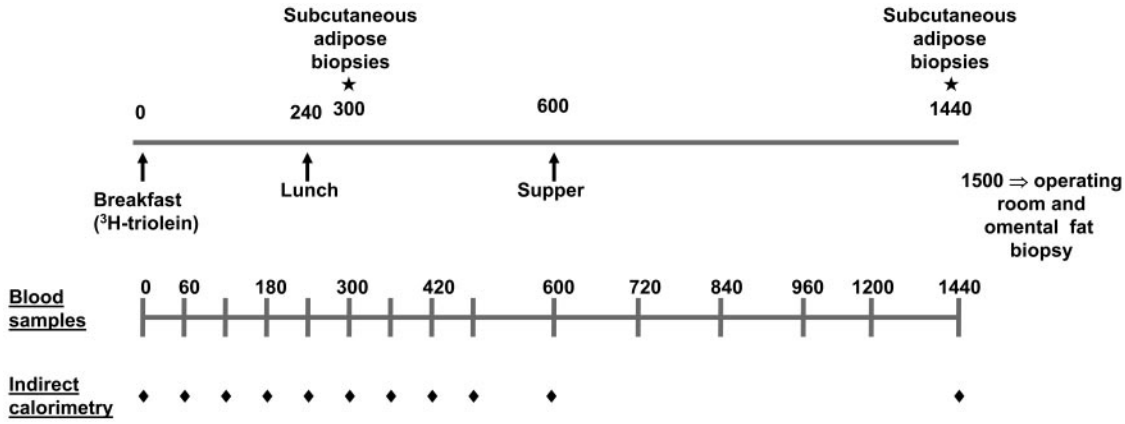

FIG. 1. Schematic of the protocol design. Time is presented as minutes from the time of test meal ingestion at 0800 h. Breakfast was either a normal-fat (~27%) or a high-fat (~69%) meal. Subcutaneous adipose tissue biopsies of the abdomen and thigh were performed at 300 and 1,440 min. Biopsies of omental fat were performed after transfer to the operating room.

content of lipid in these depots. After the subcutaneous fat biopsies, the volunteers were transferred to the preoperative suites and then to the operating room as scheduled. Immediately upon entering the abdomen, a sample of omental fat was obtained using procedures previously outlined (1). The volunteers were then cared for by the surgical and anesthesia staff per standard Mayo Clinic protocols.

Vo_2 and Vco_2 were measured hourly with indirect calorimetry using the V_{max} metabolic cart (Sensor Medics, Yorba Linda, CA) starting immediately before the test meal as depicted in Fig. 1. Urine was quantitatively collected for 24 h to assess ^3H_2O losses and urinary nitrogen excretion (4).

Materials. [9,10-^{3}H]triolein was purchased from NEN Life Science Products (Perkin Elmer, Boston, MA). ^2H_2O was obtained from Isotech (Miamisburg, OH).

Assays and methods. Total body fat, leg fat, and total fat-free mass were measured with dual-energy X-ray absorptiometry (DEXA) (DPX-IQ; Lunar Radiation, Madison, WI), and visceral fat was determined using the combination of a single-slice computed tomography (CT) image at the level of L_{2-3} in combination with DEXA abdominal fat analysis (12). DEXA and CT were performed during 1 of the 3 days before the study in concert with a visit to the GCRC for meals. Total body water was measured using ^2H_2O (13).

The quantity of [^{3}H]triolein in the liquid test meal and the methods used to measure adipose tissue lipid-specific activity have been previously reported in detail (11). Plasma chylomicron triglyceride (TG) concentrations and specific activity were determined as previously described (14), as were plasma free fatty acid (FFA) concentrations (15,16). Heparin-releasable lipoprotein lipase (LPL) activity (17) and fat cell size (18) were measured using established methods.

Calculations. The uptake of meal fatty acids into adipose tissue TG (milligrams meal fat per gram adipose tissue lipid) was calculated as the regional adipose tissue–specific activity multiplied by 1,000 and divided by meal fat–specific activity (11). The concentration of ^3H_2O in urine water at 24 h was used to determine cumulative meal fatty acid oxidation (2). The sum of the tracer in UBSQ, LBSQ, and visceral fat plus the amount in ^3H_2O was used to assess whether we could account for all of the administered [^{3}H]triolein after 24 h. "Unaccounted for" dietary fat is the difference between the tracer content of the meal and that found in body water and the three adipose compartments.

Measuring mRNA for fatty acid transporters. Adipose tissue samples were washed and flash frozen until RNA was extracted using an RNeasy lipid tissue mini kit (Qiagen, Valencia, CA). A cDNA was made using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA). Quantitative RT-PCR was performed on an ABI 7900 using primer and probe sets from Applied Biosystems. Calculations of relative transcript amounts were normalized to a "housekeeping"/endogenous control gene (cyclophilin A) and then reported relative to a calibrator sample (surgical fat) using the DDCt method described in Sequence Detection System User Bulletin #2 (2001) "Relative quantitation of gene expression" from Applied Biosystems (http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf).

Data analysis and statistics. Because the goal of this study was to understand factors that can result in the preferential retention of fat in certain depots, the primary approach to data analysis was based on uptake values relative to tissue lipid content. Thus, we compared meal fatty acid uptake (milligrams meal fat per gram adipose lipid) between adipose depots (UBSQ, LBSQ, and visceral) and between individuals with varying amounts of fat. Differences between depots or differences between individuals within the same group (high- vs. normal-fat meal) are referred to as differences in "efficiency" for the purposes of this report. Because another common means of data interpretation is to examine metabolic processes per cell, we also examined meal fatty acid uptake per cell for some analyses.

All data are presented as means ± SE and were analyzed using the JMP 6.0 statistical package (SAS Institute, Cary, NC). A P value of <0.05 was taken to be significant. The effect of meal fat content on regional meal fat disposal was assessed by a nonpaired t test. Comparisons between adipose depots were performed using a paired t test. We tested whether PCOS volunteers differed from non-PCOS volunteers in any of the adipose tissue fatty acid metabolism variables, and because they did not, subsequent analysis was performed on the entire group. The whole-body and regional uptake of a standard amount of meal fatty acids into widely varying amounts of adipose tissue lipid should follow a predictable pattern if each person and each fat depot stores dietary fatty acids in the same manner: the uptake (milligram meal fat per gram adipose lipid) should decrease as a function of body fat, and the decrease should be linear if both body fat and uptake are log transformed. To provide this standard challenge, we used a liquid meal with calories equal to 40% of each participant's REE measured the day of the study, and the high-fat meal is created by adding 80 g triolein (4). Thus, when examining meal fatty acid uptake into various regions, the data were log transformed if this relationship seemed likely from review of the scatter plots.

RESULTS

Table 1 provides the characteristics of the study participants. There was a wide range of body fat and body fat distribution that did not differ between the meal groups. Average adipocyte size differed between all three depots (at least $P < 0.05$). Omental adipocyte size was correlated with visceral fat mass ($r = 0.84$, $P < 0.0001$), abdominal adipocyte size was correlated with UBSQ fat mass ($r = 0.70$, $P < 0.0004$), and thigh adipocyte size was weakly correlated with LBSQ fat mass ($r = 0.38$, $P = 0.08$).

Plasma chylomicron and FFA. The 24-h plasma chylomicron TG concentrations were not significantly different by meal group, although the peak concentrations, which occurred at 300 min in both groups, were approximately double in the high-fat meal compared with the normal-fat meal group (data not shown). The chylomicron-specific activity (dpm/nmol) was significantly less in the high-fat meal group, as expected (4).

Between 180 and 420 min, the plasma FFA oleate concentrations in the high-fat and normal-fat meal groups averaged 208 ± 36 and 67 ± 11 μmol/l ($P < 0.0001$), whereas plasma palmitate concentrations averaged 57 ± 11 and 38 ± 5 μmol/l ($P = 0.001$).

Whole-body energy and substrate metabolism. The hourly respiratory exchange ratios (RERs) are shown in

TABLE 1
Subject characteristics

	Normal-fat meal	High-fat meal
n	12	9
PCOS	4	3
Age	34 ± 2 (20–43)	36 ± 2 (24–44)
Weight (kg)	91.6 ± 6.1 (65–125)	90.2 ± 7.8 (52–118)
BMI (kg/m^2)	32.1 ± 1.9 (23.6–41)	33.4 ± 2.4 (20–42)
Body fat (%)	47.2 ± 1.9 (32.7–55)	44.4 ± 3.5 (22.9–56)
UBSQ (kg)	22 ± 1.7 (11.9–29.6)	21.3 ± 3 (6.1–30.4)
LBSQ (kg)	14.2 ± 1.4 (6.5–23.5)	14.9 ± 2.1 (4.9–25.1)
Visceral fat (kg)	3.8 ± 0.7 (1.2–7.6)	2.6 ± 0.5 (0.8–5.5)
Fat cell lipid (μg/cell)		
UBSQ	0.65 ± 0.08 (0.35–1.28)	0.66 ± 0.08 (0.24–0.92)
LBSQ	0.76 ± 0.07 (0.39–1.22)	0.74 ± 0.06 (0.44–0.93)
Visceral	0.36 ± 0.07 (0.11–0.86)	0.33 ± 0.06 (0.12–0.6)
Glucose (mg/dl)	92 ± 1 (84–99)	100 ± 7 (79–151)
Total cholesterol (mg/dl)	190 ± 8 (146–237)	176 ± 9 (131–214)
Triglycerides (mg/dl)	161 ± 29 (38–395)	128 ± 21 (57–260)

Data are means ± SE (range).

Fig. 2. The RER was significantly lower 240–360 min after meal test in the group receiving the high-fat breakfast ($P < 0.05$), corresponding to the first 3 h after lunch.

Table 2 provides the whole-body substrate oxidation and energy expenditure values for the 10 h beginning just before breakfast. Fat made up a significantly greater portion of substrate oxidation in the group that consumed the high-fat breakfast, whereas carbohydrate oxidation was greater in those consuming the normal-fat meal. Interestingly, the 10-h energy expenditure after a high-fat meal (698 ± 35 kcal) was less than that after a normal-fat meal (826 ± 42 kcal) ($P < 0.05$); however, the REE averaged somewhat less (~110 kcal/day) in the high-fat meal group. Protein oxidation was not different between groups.

Meal fatty acid disposal. Table 3 provides meal fatty acid storage (milligrams meal per gram adipose tissue lipid) as determined by isotope techniques. Because the amount of dietary fat consumed was normalized to each volunteer's energy needs in the normal-fat meal group and the same amount of excess fat was added to the high-fat meals, this should reflect the efficiency of meal fatty acid uptake into each adipose tissue depot. The addition of extra fat to the meal increased the absolute amount of meal fatty acid uptake into UBSQ ($P < 0.05$) and LBSQ ($P < 0.005$) adipose tissue. Although the meal fatty acid uptake into visceral adipose averaged 10-fold greater with the high-fat meal, this difference did not reach statistical significance ($P = 0.07$), likely due to the large variability.

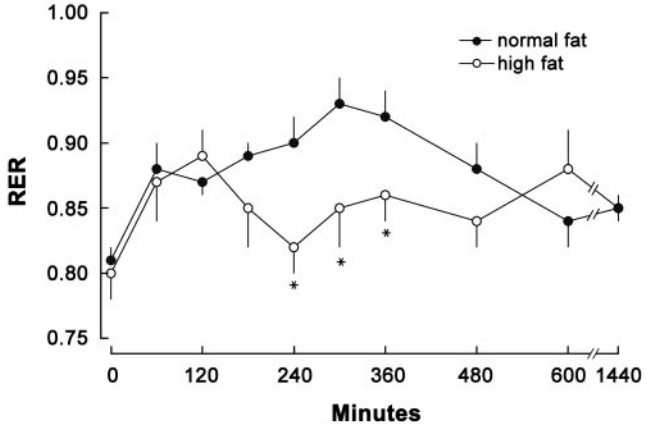

FIG. 2. RER as measured by indirect calorimetry before and after a normal- or high-fat breakfast. *$P < 0.05$; values are means ± SE.

Neither fed nor fasted LPL activity (as micromoles FFA release per hour per gram lipid or as LPL per cell) in subcutaneous adipose tissue differed based on meal type (Table 3). Furthermore, in contrast to what we observed in normal weight women (4), fed LPL activity did not correlate with the proportion of meal fatty acids stored in UBSQ or LBSQ adipose tissue (Fig. 3).

The percentage of meal fatty acids oxidized and taken up into the UBSQ, LBSQ, and visceral fat depots is also provided in Table 3. In contrast to what we observed in normal weight women (4), the proportion of meal fatty acids oxidized or taken up into the various depots did not differ based on meal fat content. The proportion of meal fatty acids that could be accounted for by oxidation and uptake into adipose tissue did not differ by meal type, averaging 89 ± 4% for both groups combined; thus, ~11 ± 4% of the meal fatty acids could not be accounted for after 24 h.

Relationship between body fat and regional meal fatty acid uptake. Figure 4 depicts meal fatty acid uptake (nanograms per cell) relative to regional fat mass in women consuming the normal-fat meal (Fig. 4, *left*) and high-fat meal (Fig. 4, *right*). There was not a statistically significant relationship between regional fat mass and adipocyte meal fatty acid uptake in the high-fat meal ($P > 0.05$ for all depots). For the normal-fat meal group, there were trends for greater uptake per cell with increasing fat mass in all depots (UBSQ, $r = 0.50$, $P = 0.097$; visceral, $r = 0.57$, $P = 0.07$; and LBSQ, $r = 0.54$, $P = 0.07$).

In Fig. 5, the relationship between regional meal fat uptake (milligrams meal fat per gram adipose tissue lipid) and regional fat cell size (micrograms lipid) is shown. After the normal-fat meal (Fig. 5), increasing omental fat cell size was associated with decreased meal fatty acid uptake in visceral fat (Fig. 5*B*; $r = -0.64$, $P = 0.034$). There were no statistically significant relationships between fat cell size and meal fatty acid uptake for the UBSQ (Fig. 5*A*; $r = -0.54$, $P = 0.073$) or the LBSQ (Fig. 5*C*; $r = 0.17$, $P = 0.60$) depots. In contrast, with the high-fat meal (Fig. 5, *right*), increasing fat cell size was associated with lesser

TABLE 2
Whole-body substrate use and energy expenditure

	Normal-fat meal	High-fat meal	*P* value
Baseline			
RQ	0.82 ± 0.01	0.80 ± 0.02	0.3738
REE (kcal/24 h)	1,649 ± 95	1,541 ± 78	0.3878
Test meal			
kcal	548 ± 29	1,248 ± 33	<0.0001
Fat (g)	16.4 ± 0.9	95.8 ± 1	<0.0001
10-h substrate oxidation			
Carbohydrate (g)	131 ± 7	75 ± 9	0.0002
Fat (g)	24 ± 4	33 ± 5	0.1731
Protein (g)	27 ± 2	26 ± 3	0.7575
Substrate use			
Carbohydrate (% of 10-h EE)	62 ± 3	44 ± 5	0.0111
Fat (% of 10-h EE)	25 ± 3	42 ± 5	0.0124
Protein (% of 10-h EE)	13 ± 1	15 ± 1	0.2564

Data are means ± SE. EE, energy expenditure; RQ, respiratory quotient.

meal fatty acid uptake per gram adipose lipid in the UBSQ (Fig. 5*A*; $r = -0.81$, $P = 0.008$), the LBSQ (Fig. 5*C*; $r = 0.71$, $P = 0.03$), and the visceral depot (Fig. 5*B*; $r = -0.45$, NS).

Figure 6 depicts the relationship between body fat stores and meal fat uptake (milligrams meal fat per gram adipose tissue) in the normal-fat (Fig. 6) and high-fat (Fig. 6) breakfast groups, and participants with PCOS are identified. There was no relationship between UBSQ fat mass and meal fatty acid uptake after a normal-fat meal (Fig. 6*A*), whereas there was a strong, negative ($r = -0.74$ for log-transformed data; $P < 0.01$) relationship between visceral fat mass and the uptake of meal fatty acids into omental adipose tissue (Fig. 6*B*). For the normal-fat breakfast group, there was a positive, linear ($r = 0.68$, $P < 0.05$) relationship between LBSQ adipose tissue and the efficiency of meal fat uptake into femoral fat (Fig. 6*C*).

Somewhat different relationships were seen in response to the high-fat meal (Fig. 6*D–F*). There was a negative relationship between UBSQ fat mass and the uptake of meal fat fatty acids (milligrams meal fatty acids per gram adipose lipid) into abdominal subcutaneous fat ($r = 0.71$; $P < 0.05$). The same was observed for omental meal fat uptake and visceral fat mass ($r = -0.77$ for log-transformed data, $P = 0.02$), and we found a similar trend for LBSQ fat ($r = -0.62$; $P = 0.08$). These trends are consistent with the expected dilution of the meal fat content within larger masses of adipose tissue in response to the larger meal fat load.

Gene expression of fatty acid transport proteins in adipose tissue. The expression of CD36 and FATP4 were correlated with femoral ($r = 0.51$, $P = 0.04$ and $r = 0.52$, $P = 0.04$, respectively) and omental fat cell size ($r = 0.62$, $P = 0.005$ and $r = 0.53$, $P = 0.02$, respectively). The

TABLE 3
Meal fat storage and utilization

	Normal-fat meal	High-fat meal	*P* value
Uptake (mg meal fat/g lipid)			
UBSQ	0.2 ± 0.02	1.32 ± 0.44	0.0342
LBSQ	0.2 ± 0.02	1.37 ± 0.29	0.0037
Visceral	0.29 ± 0.04	2.7 ± 1.17	0.0717
Fasted LPL activity (μmol FFA release · h^{-1} · g^{-1} lipid)			
UBSQ	0.3 ± 0.03	0.33 ± 0.06	0.657
LBSQ	0.61 ± 0.18	0.48 ± 0.07	0.4954
Visceral	0.17 ± 0.02	0.19 ± 0.03	0.485
Fed LPL activity (μmol FFA release · h^{-1} · g^{-1} lipid)			
UBSQ	0.52 ± 0.13	0.52 ± 0.08	0.9797
LBSQ	0.66 ± 0.12	0.83 ± 0.19	0.4562
Meal fat/regional fat (g/g)			
UBSQ	4.4 ± 0.4	20.7 ± 3.7	0.0024
LBSQ	3.1 ± 0.5	17.4 ± 2.4	0.0003
Visceral	0.8 ± 0.1	4.5 ± 0.8	0.0017
Fractional meal fat utilization (%)			
UBSQ	27 ± 3	23 ± 4	0.373
LBSQ	19 ± 3	19 ± 3	0.9942
Visceral	5 ± 1	5 ± 1	0.8618
Oxidation	40 ± 3	42 ± 3	0.5463
Unaccounted for	10 ± 5	12 ± 6	0.8022

Data are means ± SE. *P* values are for differences between the normal-fat and high-fat meal groups. The uptake did not differ significantly between depots, within groups.

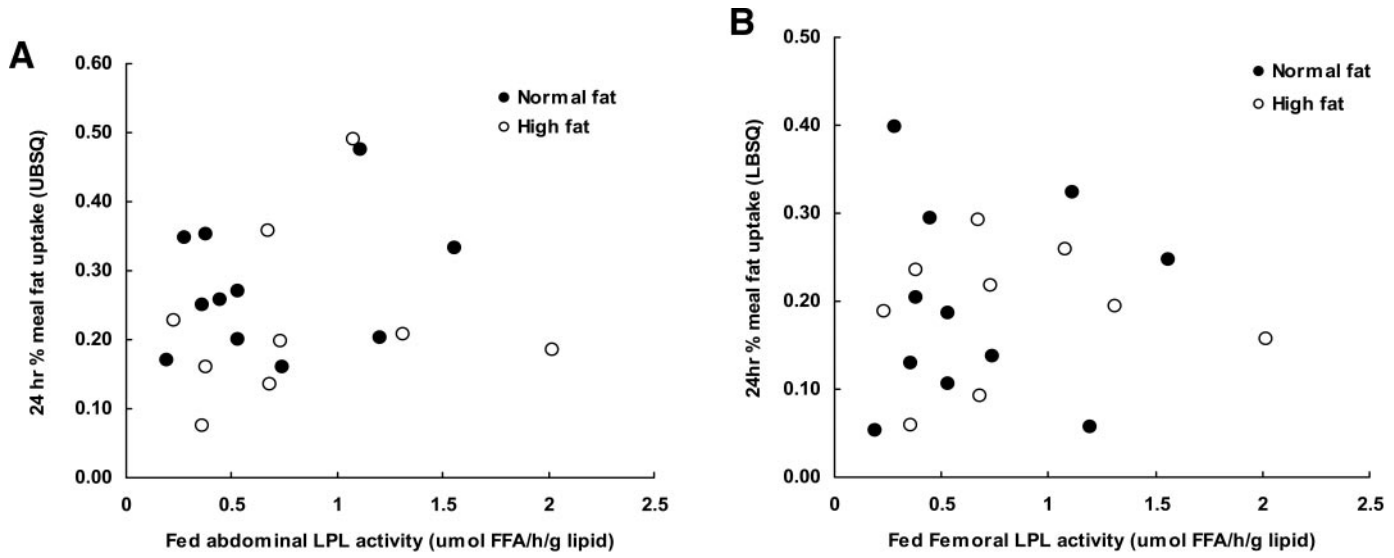

FIG. 3. Relationship between fed LPL activity and 24-h meal fatty acid uptake. *A*: The relationship in the UBSQ adipose tissue; *B*: the relationship in the LBSQ adipose tissue. Data from normal- and high-fat test meal study participants are depicted separately. No correlations were significant.

expression of FATP1 mRNA was not significantly associated with fat cell size in any depot, and abdominal fat cell size was not correlated with the expression of any of the fatty acid transport protein mRNAs.

DISCUSSION

This is the first study to compare the trafficking of dietary fat into both subcutaneous and visceral adipose tissue in women. We also tested the response to both a normal-fat and a high-fat meal (4). By sampling omental fat in this group of women, we could account for ~90% of dietary fatty acid disposal after 24 h. The most striking finding was that, under normal dietary conditions, women with greater amounts of leg fat are more efficient at taking up meal-derived fatty acids into femoral fat (milligrams meal fat per gram adipose tissue lipid). This was in stark contrast to omental fat, where those with more visceral adipose tissue were less efficient. The later result is expected if a fixed quantity of dietary fatty acids (relative to metabolic body size) is distributed in larger adipose tissue volumes. The neutral or positive relationship between dietary fatty acid storage and regional fat mass in UBSQ and LBSQ fat, respectively, implies that these tissue beds begin to compete more effectively with visceral fat for meal-derived fatty acids as body fat mass increases. This suggests that in women there are different physiological steps that drive meal fat uptake between regional depots and that these processes can be altered by a high-fat meal.

Under energy-neutral conditions, fatty acid uptake into a given depot should be offset by equal rates of release to maintain stable adipose mass. If we had found that meal fat uptake into visceral fat increases or remains stable as a function of increasing fat mass, this would provide

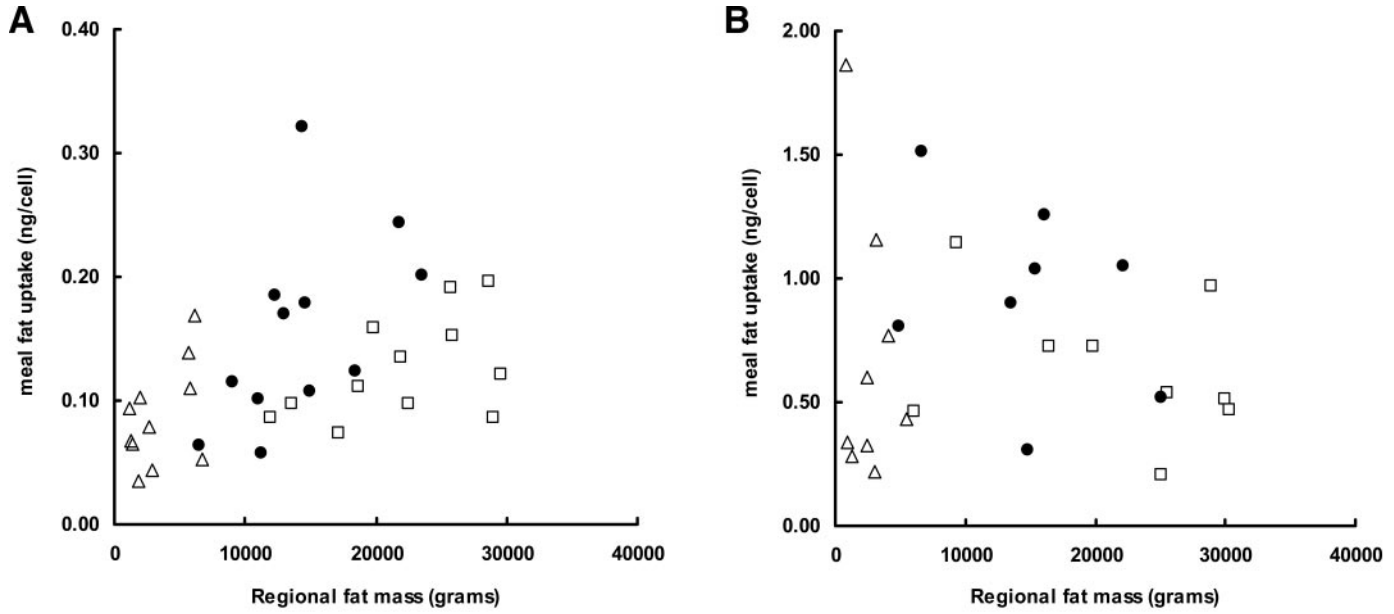

FIG. 4. The uptake of meal fatty acids (nanograms per cell) as determined by isotope tracer techniques is plotted versus regional fat mass for women consuming the normal-fat (*A*) and high-fat (*B*) meals. ●, LBSQ fat; □, UBSQ fat; △, visceral fat. None of the relationships were statistically significant.

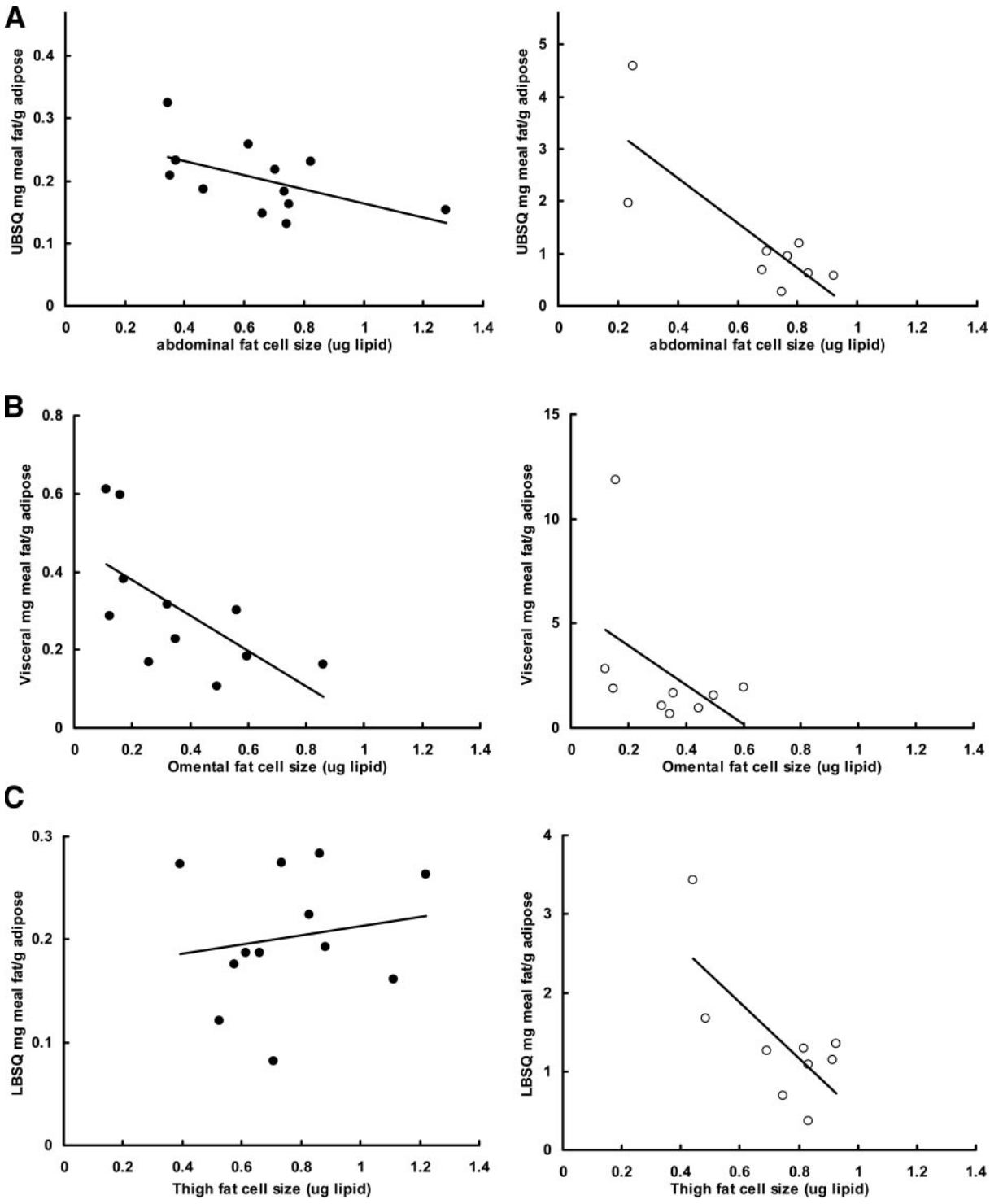

FIG. 5. Meal fatty acid uptake (milligrams meal fat per gram adipose tissue lipid) at 24 h as a function of regional fat cell size in women consuming a normal-fat (●) or high-fat (○) breakfast meal. UBSQ fat (*A*), visceral fat (*B*), and LBSQ fat (*C*) are shown.

strong evidence that FFA release into the portal vein must increase disproportionately in women with visceral obesity; the so-called "portal hypothesis" (8). Mårin et al. (6,9) assessed the uptake of dietary fat into visceral and UBSQ fat in men, and we performed similar measures in small groups of men and women (1). A consistent finding was more efficient uptake of dietary fatty acids in visceral than subcutaneous adipose tissue (1,6,9). Although this was true for less obese women in this study, there was a trend ($r = -0.54$, $P = 0.07$) for the omental adipose lipid-specific activity to fall relative to the UBSQ-specific activity with increasing BMI in the group receiving the normal-fat meal. In the most overweight women, the omental lipid-specific activity was often less than or equal to the UBSQ- and LBSQ-lipid specific activity, indicative of less efficient meal fatty acid uptake in visceral fat. This is the opposite of what would be expected if the enlarged omental and mesenteric adipocytes found with visceral obesity have

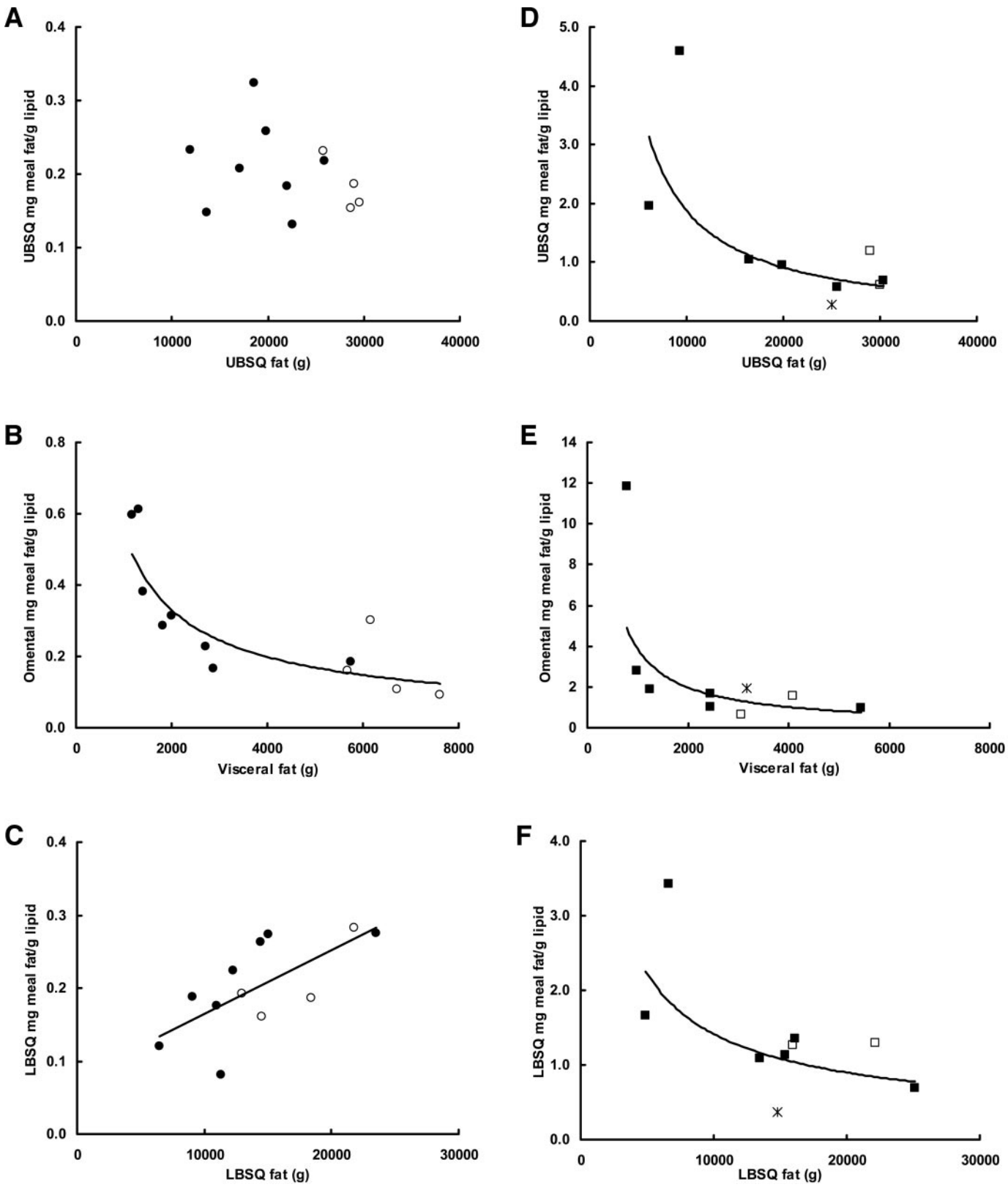

FIG. 6. The efficiency of regional meal fat uptake (milligrams meal fat per gram adipose tissue) at 24 h as a function of regional adipose tissue mass (grams) for UBSQ fat (*A* and *D*), visceral fat (*B* and *E*), and LBSQ fat (*C* and *F*). Normal-fat test meal study participants with (○) and without (●) PCOS are shown on the *left*, and high-fat test meal study participants with (□) and without (■) PCOS are shown on the *right*. One patient receiving the high-fat meal (*) was classified as a diet-controlled type 2 diabetic patient.

accelerated lipolysis in vivo and strongly suggests that visceral fat is not "hyper-lipolytic" relative to subcutaneous fat in obese women. The fact that only an average of ~20–30% of hepatic FFA delivery in viscerally obese women originates from visceral fat (19) is consistent with the meal uptake data from the present study. Furthermore,

Tchnerof et al. (20) showed in vitro that the absolute rate of lipolysis does not differ between subcutaneous and omental fat cells.

The uptake of normal amounts of dietary fat into UBSQ and LBSQ adipose tissue as a function of regional fat mass was unexpected because we anticipated a logarithmic, negative relationship between regional fat mass and meal fatty acid uptake expressed as milligrams meal fat per gram adipose tissue lipid, such as was seen in omental fat. The greater efficiency of meal fatty acid uptake in women with more leg fat and the lack of a negative relationship with UBSQ fat suggest that these depots are usually regulated differently than visceral fat. Whereas in normal weight women omental fat was more efficient at taking up chylomicron TG than either subcutaneous depot sampled, an opposite trend was seen in very obese women. We interpret this as an indication that the primary driving processes for adipose tissue TG uptake in women may differ in each of these depots as a function of body fat mass.

There are a variety of opinions as to the best means of expressing data regarding adipose tissue physiology. Although we often express fatty acid uptake or release data relative to the adipose tissue depot mass or per gram adipose lipid (to understand the metabolic functionality of the tissue as a whole), it can be helpful to also examine meal fatty acid uptake data relative to the functional units, fat cells. In that regard, we have provided the relationships between meal fat uptake (nanograms per cell) and regional fat mass (Fig. 4) and meal fat uptake (milligrams meal fat per gram adipose lipid) and regional fat cell size (Fig. 5). When meal fat uptake is expressed as nanograms per cell, there are no significant relationships between uptake and regional fat mass after a high-fat meal, which hints at a flooding of the TG-uptake mechanisms of the fat cells. After a normal-fat meal, we observed trends toward a positive relationship between meal fat uptake per cell and regional fat mass, with the LBSQ depot appearing to increase to a greater extent than the UBSQ depot. This finding is consistent with the greater uptake with increasing leg fat in women (Fig. 6). Unfortunately, the range of visceral fat mass of our volunteers is too narrow to draw comparisons with other depots for this analysis. In contrast, meal fat uptake (milligrams per gram adipose lipid) decreases as a function of fat cell size in all but the LBSQ depot under normal meal fat conditions (Fig. 5). Interpreting these relationships is difficult because of the confounding relationship between fat cell size and fat mass; the meal fatty acids from normal meal fat are diluted in greater amounts of adipose lipid. To understand whether larger cells can inherently take up more dietary fat, it would be necessary to study individuals with differing fat cell sizes but the same regional fat mass.

Uptake of circulating TG into adipose tissue can potentially be rate limited by delivery (blood flow $\times$ concentration), LPL activity, adipocyte cell membrane transport, or intracellular processing to TG. The fact that the high-fat meal resulted in severalfold greater meal TG storage in each depot (milligrams meal fat per gram adipose lipid) suggests that intracellular processing of fatty acids was not rate limiting after the normal-fat meal. In addition, the lack of a correlation between regional, postprandial LPL activity and meal fat uptake suggests that LPL is not a rate-limiting step, unlike what we observed in nonobese women (4). If adipose blood flow in women increases as a function of leg fat, that could explain the more efficient femoral meal fat uptake in obese women. Unfortunately, we did not measure blood flow in these studies, and we found no reports of postprandial femoral adipose tissue blood flow in obese women.

The relationships between regional fat mass and the efficiency of meal fat uptake displayed the expected negative relationship for all depots in the high-fat meal situation. The response to the high-fat meal is of particular interest for LBSQ fat because the change in pattern of uptake indicates that leaner women have a great capacity for incremental TG trafficking to leg adipose tissue when challenged. This implies that the mechanism limiting leg adipose tissue fatty acid uptake after normal meals in lean women is not intracellular processing steps and that the regulatory step can be overwhelmed by consuming excessive dietary fat. A more proximal step regulating fatty acid uptake could be adipose tissue delivery/membrane transport. After lunch, chylomicron TG concentrations were approximately twofold greater after the high-fat meal than the normal-fat meal, and the postprandial plasma FFA oleate concentrations were approximately fourfold greater (five- to ninefold greater in nonobese women). Perhaps fatty acid transport into leg adipocytes is primarily via fatty acid transport proteins (CD36, FATP1, and FATP4) under normal-fat meal conditions, which would be consistent with the greater mRNA expression for these proteins as thigh fat cell size increases. The high-fat meal may increase unbound FFA in the extracellular milieu of adipocytes to the extent that meal-derived fatty acids enter in larger quantities via the passive (flip-flop) mechanism (21). Perhaps meal fatty acid uptake into UBSQ fat and visceral fat is controlled through different mechanisms than leg fat under normal meal fat conditions.

The greater LBSQ uptake of dietary fat with more leg fat suggests that fat retention in this depot in women is driven primarily by uptake rather than defective lipolysis. LBSQ fat is less lipolytically active than UBSQ fat (22); however, as leg fat increases, leg FFA release increases (19). If defective lipolysis were primarily responsible for leg fat gain in women, one would expect that to maintain stable fat mass, LBSQ adipose uptake of fatty acids would be reduced to offset the defective lipolysis. Instead, the greater efficiency of meal fatty acid uptake in those with more leg fat suggests that uptake drives greater leg adipose tissue FFA release, perhaps as fat cell size increases. One interpretation of the reduced efficiency of meal fatty acid uptake in those with greater amounts of visceral fat is that meal fatty acid uptake is not a major factor driving fat accumulation in women in this region. Understanding whether visceral fat gain is related to uptake of fatty acids from VLDL-TG, direct uptake of FFA (23), or relative defects in FFA release will have major implications for the treatment of visceral obesity.

An advantage of this study was the ability to obtain visceral (omental) fat biopsies to determine meal fat uptake into this usually elusive store. To our surprise, only $\sim$5% of meal fatty acids was found in the visceral fat, regardless of meal fat content. This indicates that the size of the visceral fat depot is not an indicator of its ability to store dietary fat in women. Note that we could not account for $\sim$11% of the fatty acids after 24 h. Other possible sites for the fatty acids include liver, muscle, and plasma. Although we did detect some tracer in plasma TGs 24 h after the experimental meal, it was somewhat $<$1%, similar to our previous findings (1). Muscle is a large potential site for fat storage given its large mass, and some dietary fatty

acid retention in liver seems likely given the presence of [^{3}H]oleate in VLDL 1 day after the experimental meal.

Some limitations to this study exist. First, given the differences between these results in mostly overweight and obese women versus those we reported in nonobese women (2), as well as the difference in meal fat uptake between the normal- and high-fat meals, measures of regional adipose tissue blood flow would have been helpful. Although this may be possible in the UBSQ and LBSQ fat regions in future studies, the measurement of blood flow in visceral fat in humans does not seem possible. Second, future studies should include more comprehensive measures of adipose tissue enzymes and proteins involved in meal fatty acid uptake to provide a better understanding of potential rate-limiting steps. Last, the test meal that we used in this study was high in fat and calories and therefore the high-fat meal group had a greater caloric intake than the normal-fat group. Thus, we cannot be certain that the differences we observed were due to the greater energy intake or the greater fat intake.

In conclusion, this is the first study to directly compare the subcutaneous and visceral adipose tissue uptake of dietary fat in women. We found that after either a normal-fat or high-fat meal, visceral fat accounts for a small fraction of meal fat disposal. Furthermore, we found that there is a direct negative relationship between meal fat uptake efficiency and visceral fat mass, suggesting that visceral fat mass size is not a determinant of meal fat disposal. Of equal interest are the dramatic differences in patterns of efficiency of meal fatty acid uptake between different depots and different meal challenges.

ACKNOWLEDGMENTS

We thank the volunteers who participated in this study and Carol Siverling, RN; Rebekah Herrmann, RN; Deborah Harteneck; Darlene Lucas; Jessica Eastman; and the staff of the Mayo GCRC.

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