Plasma Free Fatty Acid Storage in Subcutaneous and Visceral Adipose Tissue in Postabsorptive Women

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OBJECTIVE—We assessed the direct (VLDL-triglycerides [VLDL-TG] independent) storage of circulating free fatty acids (FFAs) in visceral and subcutaneous fat in postabsorptive women.

RESEARCH DESIGN AND METHODS—Twelve women (BMI 29.6 ± 6.6 kg/m^2) received an identical, intravenous bolus dose of [1-^{14}C]oleate followed by timed subcutaneous fat biopsies (abdominal and femoral) and then omental fat biopsy during tubal ligation surgery. Regional fat masses were assessed by combining dual-energy X-ray absorptiometry and computed tomography scanning. Separately, we assessed the fraction of FFA tracer entering VLDL-TG over the time representing the delay in collecting omental fat.

RESULTS—Site-specific fat specific activity (SA) (dpm/g lipid) decreased as a function of fat mass in both upper-body subcutaneous (UBSQ) and visceral fat depots. These patterns are consistent with dilution of a relatively fixed amount of FFA tracer within progressively greater amounts of fat. Interestingly, femoral SA did not vary as a function of lower-body subcutaneous (LBSQ) fat mass. [1-^{14}C]oleate storage per million LBSQ adipocytes was positively associated with LBSQ fat mass, but no significant relationships were observed in UBSQ or visceral fat depot. The fraction of [1-^{14}C]oleate stored in UBSQ, LBSQ, and visceral fat was 6.7 ± 3.2, 4.9 ± 3.4, and 1.0 ± 0.3%, respectively. Only ~4% of the tracer traversed VLDL-TG over 9.5 h.

CONCLUSIONS—The increase in FFA tracer storage per adipocyte as a function of LBSQ fat mass implies that LBSQ adipocytes, in contrast to UBSQ and omental adipocytes, store more FFA in women with greater adiposity. The direct FFA storage pathway might play a role in favoring lower-body fat accumulation in women. ***Diabetes*** **57:1186–1194, 2008**

Excess central adipose tissue is a risk factor for metabolic complications, especially type 2 diabetes and cardiovascular disease. Although progress has been made understanding the metabolic changes in obesity, there is still no satisfactory explanation(s) as to why people can differ so remarkably in body fat distribution. Regional in vivo differences in the storage of dietary fat (1,2) or adipose tissue lipolytic rates (3,4) do not seem to account for variations in human body fat distribution.

Although subcutaneous adipocytes are known to actively release substantial amounts of free fatty acids (FFAs) in the postabsorptive state, there is also simultaneous, direct uptake back to the adipose tissue. In vivo animal studies have shown that circulating FFA can be taken up and restored in subcutaneous adipose tissue, thus forming a cycle of release and reuptake (5–11). By using a combination of FFA tracers and tissue biopsies, it has been possible to assess total uptake (storage and oxidation) or, exclusively, storage of circulating FFA in adipose tissue. Fatty acid analogues, such as 14(*R*,*S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid and [^{3}H]-R-bromopalmitate have been used to assess total uptake (7,8,10,11). Fatty acids labeled with ^{14}C or ^{3}H have been used to assess tissue storage (accumulation) of fatty acids on the basis that these isotopic tracers are metabolized in the same way as natural FFA (5–7). Using [^{3}H]-R-bromopalmitate, Furler et al. predicted that FFA influx into white adipose tissue represented only a small fraction (~3%) of plasma FFA turnover in postabsorptive rats (8).

Despite the findings of direct uptake of circulating FFA by adipose tissue in rodents, until recently there was little evidence that this pathway functions in postabsorptive humans in vivo. Investigators failed to detect disappearance of circulating FFA across an adipose tissue bed in postabsorptive men using the combination of isotope dilution techniques and arteriovenous balance (12–14), although FFA disappearance across adipose tissue has been reported under postprandial or hyperinsulinemic conditions (12,14). Using an intravenous bolus of ^{14}C-labeled FFA and carefully timed subcutaneous adipose tissue biopsies to detect direct FFA storage, we estimated that ~3 and 8% of circulating FFA is incorporated into subcutaneous adipose lipid in normal-weight, postabsorptive men and women, respectively (15). Although a small fraction of FFA disposal, the pattern of storage was most interesting. The relative storage of FFA via this direct pathway in upper versus lower body fat was consistent with sex-specific differences in body fat distribution, i.e., preferential upper body fat accumulation in men but not in women and greater total storage in subcutaneous fat of women. We estimated that this physiological process of FFA recycling independent of the VLDL-triglycerides/lipoprotein lipase (VLDL-TG/LPL) pathway might serve to redistribute fat from upper body stores to lower body stores in women but not men (15). Unfortunately, we had no information regarding the presence or absence of this process in visceral fat in humans.

In the present study, we measured the storage of

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FCR, fractional catabolic rate; FFA, free fatty acid; FFM, fat-free mass; GCRC, General Clinic Research Center; HPLC, high-performance liquid chromatography; LBSQ, lower-body subcutaneous; SA, specific activity; UBSQ, upper-body subcutaneous; VLDL-TG, VLDL-triglycerides; VLDL-TGFA, VLDL-TG fatty acid; VLDL-TG/LPL, VLDL/TG/lipoprotein lipase.

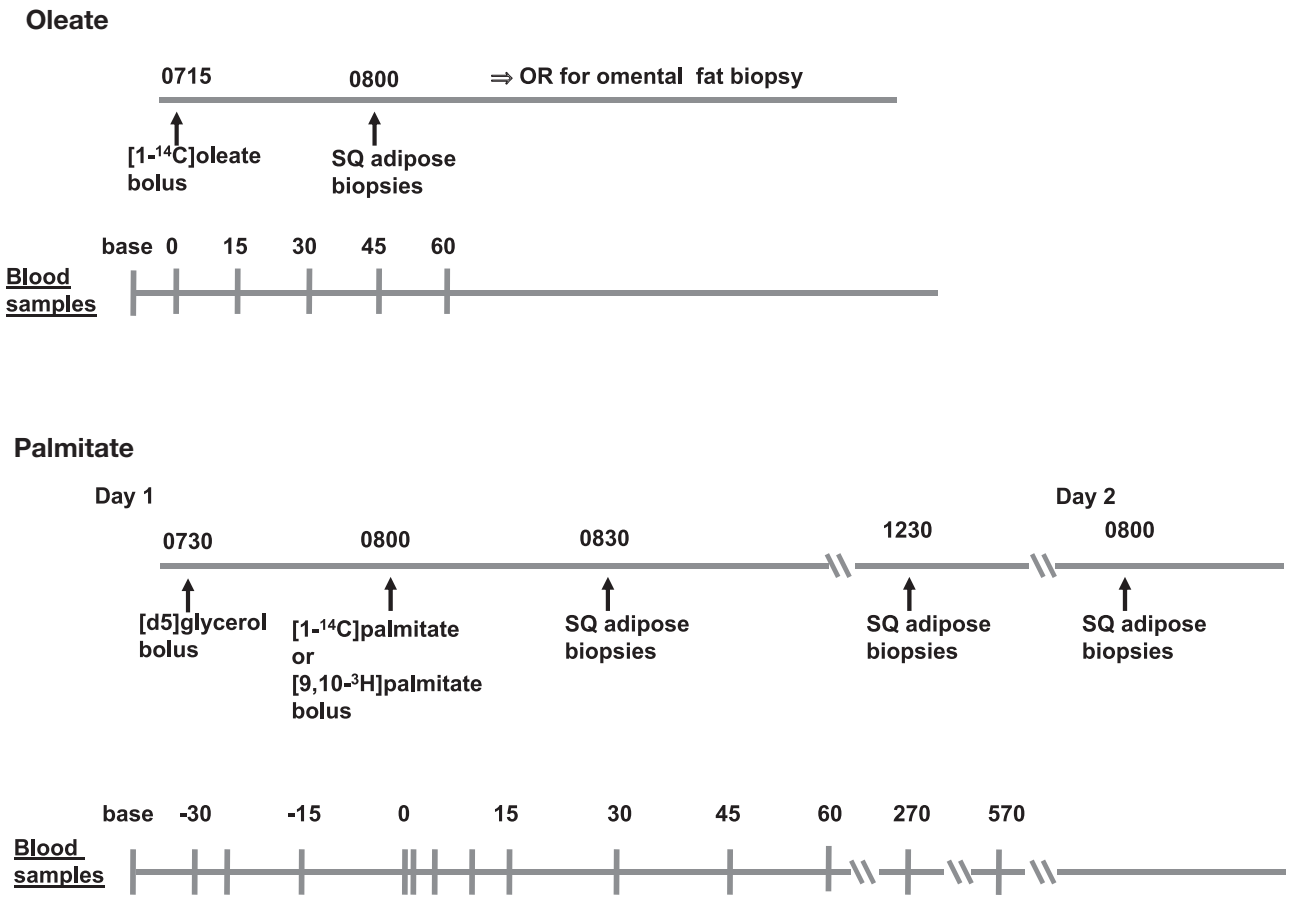

FIG. 1. The study design of the oleate and palmitate protocols. For details, please see RESEARCH DESIGN AND METHODS.

intravenously administered [^{14}C]oleate in subcutaneous and visceral adipose tissue lipid in a group of postabsorptive premenopausal women scheduled for tubal ligation surgery. In a separate protocol, we quantified the immediate (direct) and delayed (presumably via VLDL-TG/LPL) storage of an FFA tracer into subcutaneous fat depots together with its appearance rate in plasma VLDL-TG. The results of the present study support the potential role of the direct FFA uptake pathway in regulating body fat distribution in women.

RESEARCH DESIGN AND METHODS

Two studies, both approved by the Mayo Clinic institutional review board, were conducted. Informed written consent was obtained from all volunteers. For practical purposes, we used different FFA tracers in two protocols: the oleate protocol and the palmitate protocol. The two tracers should give comparable results because they give similar estimates of plasma FFA kinetics (16) and have similar fractional extraction across adipose tissue (17) and similar incorporation into plasma triglycerides (18). Because these two fatty acids are the major fatty acids found in plasma, their metabolism can be taken to be representative of the bulk of plasma FFA. Both palmitate and oleate tracers were complexed to albumin before administration as previously described (19,20).

Procedures common to both protocols

Subjects. Participants of both protocols were weight stable for at least 3 months before the study. They were all healthy and receiving no medications, with the exception of oral contraceptives (six women participating in the oleate protocol). Before study participation, a complete blood count, chemistry group, and routine urinalysis were documented as normal.

Participants received all their meals from the Mayo Clinic General Clinical Research Center (GCRC) metabolic kitchen for 3 (the oleate protocol) or 5 (the palmitate protocol) days before the study days to ensure stable energy and consistent macronutrient composition (50% carbohydrate, 35% fat, and 15% protein). The volunteers were then admitted to the GCRC at 1700 h, given a meal at 1800 h, and remained in the GCRC for the next 2 days. At 0545 h the next day (study day 1), a forearm vein catheter was inserted and kept patent with a controlled infusion of 0.45% NaCl. A second catheter was placed in a retrograde fashion in a hand vein for collecting arterialized blood using the heated hand vein technique.

Body composition measurements. Body composition (body fat, fat-free mass [FFM], and regional fat mass) was measured with dual-energy X-ray absorptiometry (DXA) (DPX-IQ; Lunar Radiation, Madison, WI) and a computed tomography of the abdomen at the L_{2-3} interspace. Leg fat by DXA was considered as lower-body subcutaneous (LBSQ) fat. The combination of single-slice computed tomography– and DXA-derived abdominal fat provides a good measure of visceral fat mass (21). Upper body fat (DXA) minus visceral fat mass was used to derive upper-body subcutaneous (UBSQ) fat mass.

Oleate protocol. In the oleate protocol, we measured [^{14}C]oleate storage in subcutaneous and visceral adipose tissue in premenopausal women scheduled for tubal ligation surgery. These 12 women are a subset of participants in a study of meal fatty acid metabolism (22). We hypothesized that *1*) there would be no regional differences in the adipose lipid specific activity (SA) (dpm/g lipid) between fat depots and that *2*) regional adipose lipid SA would decrease as a function of regional fat mass.

Tubal ligation surgery was performed during the follicular phase. During the first inpatient study day (study day 1), volunteers received a research meal containing ^{3}H-triolein to study dietary fat metabolism (22). Figure 1 depicts the design of the oleate protocol, which was conducted on study day 2. An ~41 μCi intravenous bolus of [1-^{14}C]oleate was given at 0715 h after a 13-h fast. A blood sample was collected just before the tracer injection and then at 15-min intervals for the next hour. Abdominal, femoral, and gluteal subcutaneous adipose biopsies were collected at 45 min after the intravenous [1-^{14}C]oleate bolus. The subcutaneous biopsies were timed such that virtually no FFA tracer remained in the circulation and there would be insufficient tracer in VLDL-TG to accumulate in adipose tissue via the LPL pathway. The participants were then transferred to the preoperative suites and the operating room as scheduled, where omental fat was obtained as previously described (23). The volunteers were cared for by the surgical and anesthesia staff per standard Mayo protocol. In eight participants, the omental biopsy was obtained between 3 and 5 h after the [1-^{14}C]oleate bolus. In the remaining four

women, the omental biopsies were obtained at 6, 7, 8, or 11 h after the [1-^{14}C]oleate bolus as a result of unanticipated interruptions in the surgical schedule. All volunteers remained fasted until they had recovered from anesthesia.

Palmitate protocol. The palmitate protocol was adapted from a protocol already approved by the Mayo institutional review board and designed to determine *1*) the storage of an intravenously administered FFA tracer ([1-^{14}C]palmitate or [9,10-^{3}H]palmitate) in subcutaneous fat depots through direct and indirect (via VLDL-TG) uptake and *2*) the fraction of circulating FFA that traverses the plasma VLDL-TG pool in premenopausal postabsorptive women.

The palmitate protocol is schematically presented in Fig. 1. Seven healthy premenopausal women participated in this protocol. At 0730 h on study day 1, after a 14-h fast, an intravenous bolus of [1,1,2,3,3-$^{2}H_5$]glycerol (50 μmol/kg) was administered. At 0800 h (time 0), subjects received an intravenous bolus of either ~20 μCi [1-^{14}C]palmitate or ~75 μCi [9,10-^{3}H]palmitate. Blood samples were collected before tracer administrations and at intervals as described by Patterson et al. (24) for 9.5 h for measurement of plasma free glycerol and VLDL-TG glycerol tracer-to-tracee ratio (TTR) and VLDL-TG fatty acid (VLDL-TGFA) SA. Subcutaneous abdominal and femoral adipose biopsies were collected 30–45 min after the FFA tracer bolus to assess direct FFA tracer storage in adipose tissue. Additional biopsies were collected at 4.5 h (1230 h) and 24 h (0800 h on study day 2) to assess possible changes in adipose lipid SA as an index of storage of VLDL-TG–derived fatty acid label. Subjects remained in bed for most of study day 1 and received a fat-free meal at 1330 h and a normal-fat meal at 1800 h. Each meal provided ~50% of each volunteer's resting energy expenditure.

Adipose tissue biopsies. Subcutaneous adipose tissue from three (the oleate protocol) or two (the palmitate protocol) sites were obtained by needle liposuction under sterile conditions and local anesthesia. Biopsies were collected from the abdominal (just lateral to the umbilicus), the gluteal (lower lateral quadrant of the buttock), and the femoral (on the anterior-lateral aspect of the midthigh) regions. Omental biopsies were obtained laparoscopically during the tubal ligation procedure. The samples were meticulously rinsed with saline through Nitex Nylon Fiber 250/50 and processed for measurement of adipocyte size and lipid SA.

Assays

Measurement of adipocyte size and adipose tissue lipid SA. Adipocyte size was assessed using a modification (25) of the approach of Di Girolamo et al. (26). Adipose tissue lipid SA (dpm/g lipid) was assessed after extracting tissue lipids with chloroform:methanol (2:1) (15) and measuring radioactivity to <2% counting error.

Plasma oleate, palmitate and TG concentrations and SA. Plasma oleate or palmitate concentrations and SA were measured using high-performance liquid chromatography (HPLC) (20). The amount of FFA tracer (^{14}C oleate) in plasma triglyceride (the oleate protocol) was determined by extracting lipids from 1 ml fresh plasma using the Dole procedure and separating TG using HPLC (27). The plasma triglyceride concentrations (28) and the triglyceride fraction radioactivity were used to calculate plasma triglyceride SA.

Plasma VLDL-TG concentrations and SA and TTRs of plasma and VLDL-TG glycerol. To measure the entry of the FFA tracer into VLDL-TG (the palmitate protocol), 2 ml fresh plasma from each time point was layered underneath a density solution of 1.006 g/ml. The tubes were spun at 180,000*g* for 19.5 h. VLDL were isolated as described by Patterson et al. (24), and TG concentrations corrected for free glycerol (28) were measured in a small fraction. The remainder was extracted with choroform:methanol (2:1) and the TG isolated by HPLC (27) to measure lipid radioactivity and the TTR of VLDL-TG glycerol. We found >90% of the VLDL-lipid radioactivity present in the triglyceride fraction.

Plasma glycerol TTR was determined as previously described (24), except that we used chemical impact ionization, and heptafluorobutyric-glycerol TTR was determined by selectively monitoring ions at mass-to-charge ratios (*m/z*) of 680 (*m* +0) and 685 (*m* +5) by GC-MS (Agilent Technologies 5973N) operating in negative ion mode and using ammonia as reactant gas. The VLDL-TG glycerol TTR was determined according to Patterson et al. (24). Triglyceride were reacted with sodium methoxide (29), and the liberated glycerol was derivatized with heptafluorobutyric anhydride and analyzed as described above.

Materials. [l-^{14}C]oleate, [1-^{14}C]palmitate and [9,10-^{3}H]palmitate were purchased from NEN Life Science Products, PerkinElmer, Boston, MA. [1,1,2,3,3-$^{2}H_5$]glycerol was purchased from Cambridge Isotope Laboratories, Andover, MA.

Calculations. To extrapolate from the ^{14}C or ^{3}H FFA tracer in adipose tissue biopsies to the fraction of FFA tracer stored in each fat depot, we divided total body adipose tissue into UBSQ, LBSQ, and visceral fat compartments as described above. The adipose lipid SA (dpm/g lipid) was multiplied by the depot-specific adipose tissue fat mass (grams) to derive total dpm per depot.

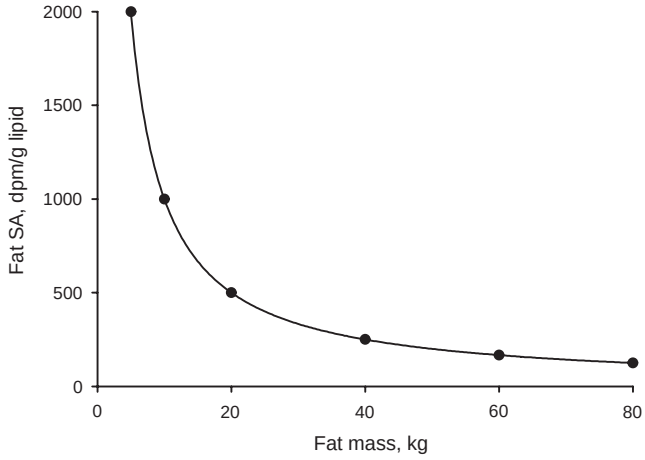

FIG. 2. This figure depicts the adipose tissue SA (dpm/g lipid) that would be observed if a fixed amount of FFA tracer, in this case 10,000,000 dpm, is taken up and stored evenly in increasing amounts of fat. SA should decrease in a hyperbolic fashion because, as fat mass increases, the FFA tracer is diluted in larger amounts of lipid. To the extent that the observed pattern deviates upward from the expected pattern, this would suggest that greater amounts of FFA tracer are taken up as body fat increases. Thus, if the pattern of adipose lipid SA as a function of fat mass does not follow a hyperbolic decrease, this should reflect a physiological process that enhances fatty acid storage.

We used the femoral adipose lipid SA for the calculation of fractional tracer storage into LBSQ fat, although using the gluteal adipose lipid SA gave similar results. The FFA tracer (dpm) in an entire depot was divided by the quantity of tracer (dpm) administered to calculate the fraction of the administered FFA tracer that was stored in that depot. FFA tracer storage in adipose tissue is presented both per unit fat mass (SA in dpm/g adipose lipid) and as a percentage of the tracer administered. Using regional adipocyte size, we also expressed FFA storage per million fat cells to present information on the basis of the adipose tissue energy storage functioning unit.

Theoretically, the distribution of an FFA tracer in widely varying amounts of adipose tissue should follow a predictable pattern if each person and each fat depot stores a fixed amount of tracer. In this case, SA—i.e., tracer retention (storage) per unit lipid weight (dpm/g lipid)—will decrease as a function of body fat mass in a hyperbolic fashion (Fig. 2). Because the dose of the FFA tracer administered was similar for all volunteers (~41 μCi in the oleate protocol), the regional FFA tracer storage per gram adipose lipid can be compared among subjects to understand whether different patterns are apparent. If the pattern of storage in our subjects with a wide range of fat mass does not follow the "expected" passive dilution (Fig. 2), we interpret this as evidence that FFA tracer storage is a dynamic process and not merely a passive retention of a fixed amount of tracer distributed equally in all fat.

The fractional catabolic rate (FCR) of VLDL-TG (in pools per hour) was determined by fitting the glycerol TTR time course in plasma and in VLDL-TG to a multicompartmental model (24). The VLDL-TG secretion was calculated as follows:

$$\text{VLDL-TG secretion rate (μmol/min)} = (\text{VLDL-TG FCR} \times C_{\text{VLDL-TG}} \times \text{PV})/60$$

where $C_{\text{VLDL-TG}}$ is the concentration of VLDL-TG in plasma and PV is the plasma volume, estimated using each subject's FFM (PV = 0.055 l × kilogram FFM).

To determine the amount (dpm) of ^{14}C or ^{3}H palmitate that traversed the VLDL-TG pool, the area under the VLDL-TGFA SA vs. time curve (dpm × min/μmol) was multiplied by the VLDL-TGFA secretion rate (μmol/min). By dividing this value by the administered dose of ^{14}C or ^{3}H palmitate, the fraction of FFA tracer disposal via VLDL-TG was calculated.

Statistics. Values are expressed as means ± SD. Statistical analyses were performed by one way ANOVA (the oleate protocol) or repeated-measures ANOVA (the palmitate protocol). For a priori hypotheses, P values of <0.05 were considered statistically significant. For the post hoc analyses, a Bonferroni correction was used based on the number of statistical comparisons performed.

RESULTS

Oleate protocol. Table 1 provides the subject characteristics of those in the oleate protocol. Nine participants

TABLE 1
Anthropometrics and fat distribution of study participants

	Oleate	Palmitate
n	12	7
Age (years)	38 ± 5	34 ± 10
Weight (kg)	81.4 ± 20.2	78.9 ± 13.4
Height (m)	1.65 ± 0.05	1.62 ± 0.07
BMI (kg/m^2)	29.6 ± 6.6	29.8 ± 4.5
Total body fat (%)	41 ± 9	40 ± 11
Total body fat (kg)	33.7 ± 14.4	31.4 ± 12
UBSQ fat (kg)	18.1 ± 7.2	15.7 ± 4.6
LBSQ fat (kg)	13.2 ± 6.4*	14.0 ± 7.2
Visceral fat (kg)	2.4 ± 1.7	1.9 ± 1.2
Abdominal fat cell size (μg lipid/cell)	0.56 ± 0.23	0.71 ± 0.15
Femoral fat cell size (μg lipid/cell)	0.66 ± 0.18	0.81 ± 0.22
Gluteal fat cell size (μg lipid/cell)	0.58 ± 0.15	N/A
Omental fat cell size (μg lipid/cell)	0.27 ± 0.15†	N/A

Data are means ± SD. *$P < 0.001$ vs. UBSQ fat. †$P < 0.05$ vs. abdominal, femoral, or gluteal fat cell size.

were overweight or obese, and three were normal weight (BMI range 19.2–42.0 kg/m^2). Plasma oleate and FFA concentrations at the time of the tracer administration were 131 ± 47 and 381 ± 136 μmol/l, respectively. Omental adipocytes were significantly smaller than subcutaneous adipocytes. Abdominal and omental adipocyte size were correlated with UBSQ and visceral fat mass ($r = 0.74$, $P = 0.006$ and $r = 0.94$, $P < 0.0001$, respectively); femoral adipocyte size and LBSQ fat mass were not as strongly related ($r = 0.54$, $P = 0.08$).

[^{14}C]oleate appearance in plasma oleate and triglycerides. The participants received 41 ± 3 μCi [1-^{14}C]oleate. Figure 3*B* depicts the plasma oleate concentrations and the plasma content (dpm/ml) of tracer before and after the intravenous bolus of [1-^{14}C]oleate. Plasma oleate concentrations were stable over the 60-min collection interval. The tracer in the plasma FFA pool had decreased to virtually undetectable levels by 45 min, when the subcutaneous biopsies were collected. Figure 3*A* shows the plasma triglyceride concentration and the plasma ^{14}C-triglyceride concentration before and after the FFA tracer bolus. The tracer began to appear in plasma triglyceride ~30 min after the FFA tracer bolus.

[^{14}C]oleate storage in subcutaneous and visceral adipose depots. The individual BMI, regional body fat mass, SA, and adipocyte size values are provided in Table 2. As assessed by multivariate regression analysis, oral contraceptive use did not have a significant effect on [^{14}C]oleate storage. The adipose SA tended to be different among depots (one-way ANOVA, $P = 0.058$), with the omental SA greater than subcutaneous depot SA (Table 2). Exclusion of the four participants (subject nos. 3, 8, 9, and 12) whose omental biopsies were collected 6 to 11 h after the [1-^{14}C]oleate bolus did not significantly affect the average omental SA (496 ± 229 dpm/g lipid; $n = 8$). We calculated that 6.7 ± 3.2, 4.9 ± 3.4, and 1.0 ± 0.3% of administered FFA tracer was stored in UBSQ, LBSQ, and visceral fat, respectively. [1-^{14}C]oleate storage relative to adipocyte number differed between depots (one-way ANOVA $P = 0.0009$); fatty acid tracer content in abdominal subcutaneous, femoral, and gluteal adipocytes was significantly greater than in omental adipocytes (175 ± 51, 219 ± 82, 185 ± 75, and 107 ± 31 dpm/million cells, respectively).

Site-specific adipose lipid SA decreased as a function of

TABLE 2
^{14}C SA and fat cell size (FCS) in omental and subcutaneous (SQ) abdominal and femoral adipose tissue in the women participating in the oleate protocol

Subject no.	BMI (kg/m^2)	Abdominal SQ			Femoral			Omental		Visceral fat (kg)
		SA (dpm/g lipid)	FCS (μg lipid/cell)	UBSQ fat (kg)	SA (dpm/g lipid)	FCS (μg lipid/cell)	LBSQ fat (kg)	SA (dpm/g lipid)	FCS (μg lipid/cell)	
1	19.8	750	0.25	9.3	450	0.44	6.6	900	0.16	0.8
2	22.4	504	0.23	6.1	320	0.49	4.9	630	0.12	1.0
3	23.6	*	0.37	11.9	270	0.52	6.5	390	0.17	1.4
4	26.0	240	0.66	13.6	400	0.62	9.1	370	0.26	2.9
5	26.5	357	0.35	17.1	337	0.58	11.0	546	0.11	1.3
6	27.6	480	0.35	18.6	390	0.39	15.0	670	0.16	1.2
7	30.9	230	0.74	22.5	150	0.71	11.3	250	0.60	5.8
8	31.0	240	0.77	19.9	230	0.91	15.4	510	0.32	2.4
9	32.5	270	0.70	16.5	320	0.83	13.5	690	0.15	1.2
10	35.0	238	0.92	25.6	332	0.93	16.1	306	0.36	2.5
11	38.1	420	0.70	25.9	549	0.73	23.5	293	0.35	2.7
12	42.0	171	0.68	30.4	330	0.75	25.1	256	0.45	5.5
Mean ± SD	29.6 ± 6.5	355 ± 172	0.56 ± 0.23	18.1 ± 7.2	340 ± 103	0.66 ± 0.18	13.2 ± 6.4	484 ± 207	0.27 ± 0.15†	2.4 ± 1.7

In subjects 3, 8, 9, and 12, omental biopsies were collected at 7, 6, 8, and 11 h, respectively, after the [1-^{14}C]oleate bolus. In the remaining subjects, omental biopsies were collected at 3–5 h after the [1-^{14}C]oleate bolus. *Not sufficient sample to accurately measure ^{14}C SA; †$P < 0.05$ vs. abdominal, femoral, or gluteal fat cell size; ANOVA $P = 0.058$ for regional ^{14}C SA.

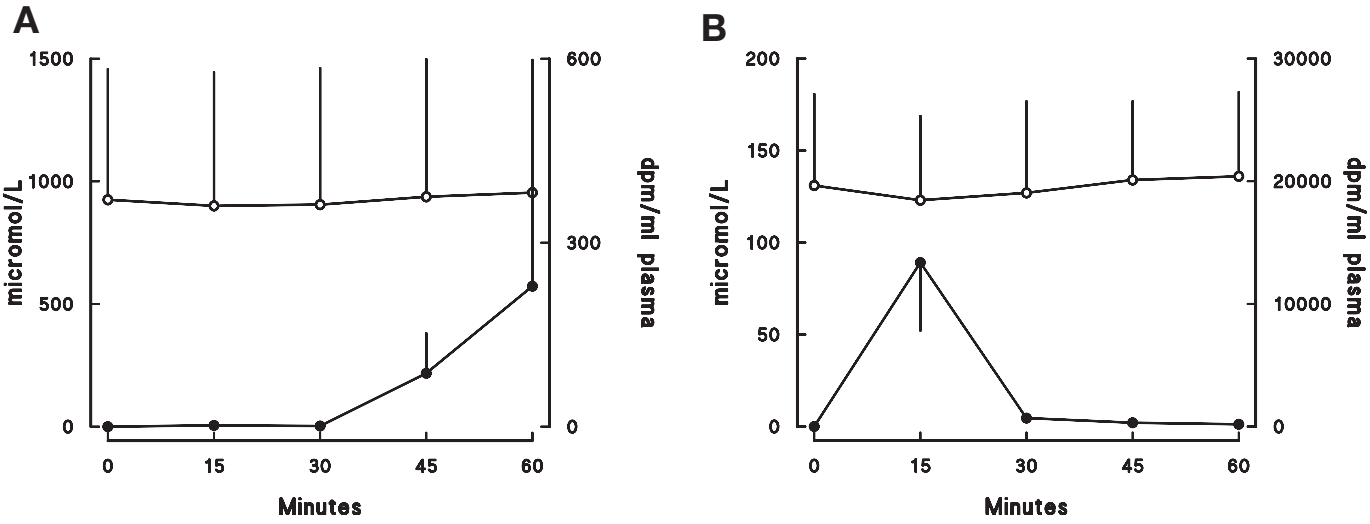

FIG. 3. *A*: ^{14}C in plasma triglycerides (TG) (●, right *y*-axis) and plasma triglyceride concentrations (○, left *y*-axis) for the same volunteers. *B*: Plasma oleate radioactivity (●, right *y*-axis) and plasma oleate concentrations (○, left *y*-axis) for the volunteers in the oleate protocol. An intravenous bolus of [1-^{14}C]oleate was given after the blood sample at 0 min was collected. All values are means ± SD.

fat mass in both UBSQ ($r = 0.62$; $P = 0.04$) and visceral ($r = 0.92$; $P < 0.0001$) fat depots (Fig. 4*A* and *B*). These patterns are consistent with dilution of a relatively fixed amount of FFA tracer within progressively greater amounts of adipose tissue (see Fig. 2). The relationship with respect to visceral adipose tissue remained significant excluding the results from four participants whose omental biopsies were collected at 6, 7, 8, or 11 h after the [1-^{14}C]oleate bolus. In contrast, there was no significant relationship between femoral lipid SA and LBSQ fat mass (Fig. 4*C*). [1-^{14}C]oleate storage per million femoral adipocytes was positively associated with LBSQ fat mass ($r = 0.64$; $P = 0.02$) (Fig. 4*F*), but no significant relationships were observed in either the UBSQ or visceral fat depots (Fig. 4*D* and *E*).

Palmitate protocol. Table 1 provides the characteristics of the participants in the palmitate protocol; five were overweight or obese, and two were normal weight (BMI range 23.8–35.6 kg/m^2). Adipocyte size did not differ between the two subcutaneous fat depots.

FFA tracer appearance in plasma palmitate and VLDL-TG. Figure 5 depicts the decay of plasma palmitate SA after the [1-^{14}C]- or [9,10-^{3}H]palmitate intravenous bolus. SA was maximum at 1 min following the bolus and decreased to 6 and 3% of maximum by 15 and 30 min, respectively. Figure 5 also depicts the plasma VLDL-TG concentrations and VLDL-TGFA SA (dpm/μmol) after the tracer bolus. Plasma VLDL-TG concentrations were stable over the 570-min collection interval. The peak VLDL-TGFA SA occurred at ~60 min after the FFA tracer bolus and decreased to 20% of peak values by 270 min and 12% of peak values by 330 min.

VLDL-TG FCR and secretion rates were 0.9 ± 0.3 pools/h and 9.8 ± 4.2 μmol/min, respectively. We estimated that 3.2 ± 1.3 and 3.6 ± 1.5% of the palmitate tracer traversed the VLDL-TG pool in 4.5 and 9.5 h after the tracer intravenous bolus, respectively.

Immediate and delayed FFA tracer storage in subcutaneous adipose tissue. Regional fat SA values measured at 30–45 min, 4.5 h, and 24 h after the palmitate tracer bolus are presented in Table 3. We posited that increases in SA from 30 min to 4.5 h and 24 h would reflect deposition of FFA tracer delivered via VLDL-TG. The changes in subcutaneous abdominal and femoral fat SA over 24 h were not statistically significant, and there were no significant differences between abdominal and femoral fat SA. The fraction of injected palmitate tracer stored in UBSQ and LBSQ depots increased slightly from 30 min to 4.5 h and 24 h, but did not reach statistical significance (repeated-measures ANOVA $P = 0.16$ for UBSQ and $P = 0.46$ for LBSQ). Likewise, the portion of FFA tracer that was stored in whole-body subcutaneous fat did not increase significantly over 24 h (ANOVA $P = 0.11$).

DISCUSSION

Adipose tissue lipolysis provides the vast majority of circulating FFA in postabsorptive humans. Despite the rapid FFA outflow, ~8% of circulating FFA are directly stored back into subcutaneous adipose tissue in normal-weight women (15). Although this pathway contributes a relatively small portion to total fat storage, it seems to favor fat redistribution to lower body fat specifically in women but not men (15). In the present study, we assessed the storage of intravenously administered FFA tracer in subcutaneous and visceral adipose tissue in lean and obese women. In our previous report, we termed this pathway as uptake (15); however, we now employ the term "storage," given that we have documented that we measure the fatty acid tracer incorporation into complex lipids. We found that ~11 and ~1% of circulating FFA was stored in subcutaneous and visceral fat, respectively. We also conducted studies to understand whether additional substantial net tracer storage occurs through delayed pathways (presumably VLDL-TG). This was necessary because the delay between the tracer administration and the omental fat biopsies in the first set of studies permitted tracer recirculation via VLDL-TG, which may have confounded the visceral adipose storage data.

The adipose SA was similar in the three subcutaneous fat regions studied (Tables 2 and 3). This is the same pattern present in normal-weight women (15) and contrasts with the greater abdominal than femoral SA in normal-weight men (15).

If body fat takes up and stores circulating FFA tracer in proportion to its mass, the relationship between fat SA and

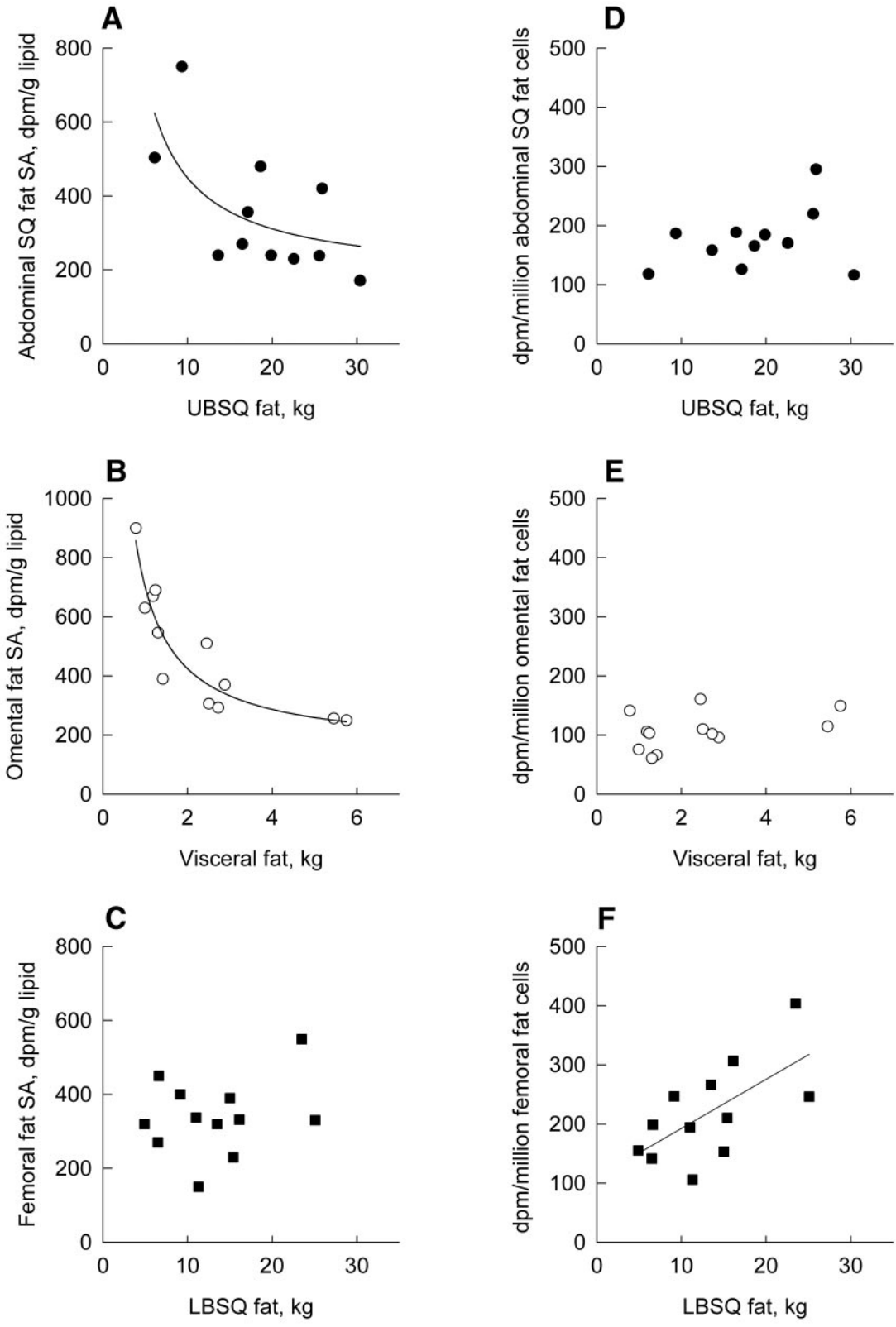

FIG. 4. Relationship between abdominal subcutaneous (SQ) fat ^{14}C SA (dpm/g lipid) and UBSQ fat mass (n = 11 women) (A), omental fat SA and visceral fat mass (n = 12 women) (B), femoral fat SA and LBSQ fat mass (n = 12) (C) and between ^{14}C dpm/million abdominal SQ fat cells and UBSQ fat mass (n = 11 women) (D), ^{14}C dpm/million omental fat cells and visceral fat mass (n = 12 women) (E), and ^{14}C dpm/million femoral fat cells and LBSQ fat mass (n = 12) (F) in the oleate protocol. Adipose tissue biopsies were collected at ~45 min (subcutaneous biopsies) or at 3–11 h (omental biopsies) after an intravenous bolus injection of [1-^{14}C]oleate.

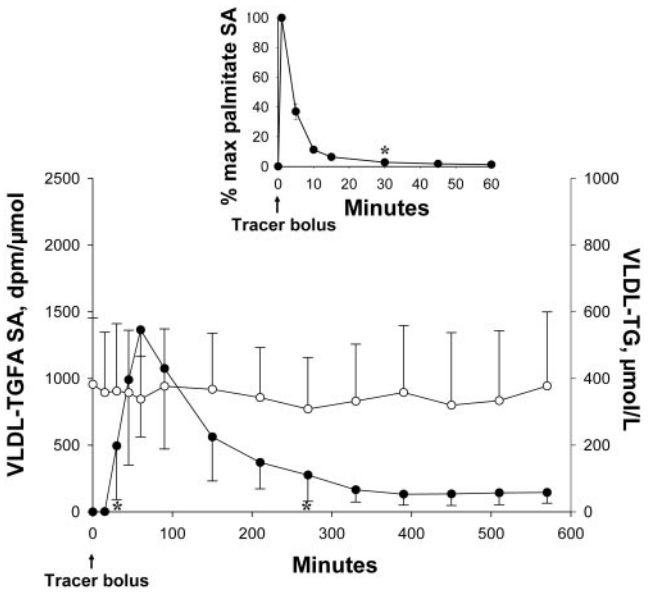

FIG. 5. The radioactivity in VLDL-TGFAs (●, left *y*-axis) and VLDL-TG concentration (○, right *y*-axis) for the volunteers in the palmitate protocol are shown. The timing of the intravenous FFA tracer bolus (arrow) and the first two subcutaneous adipose biopsies (*) are depicted. Insert: the decay of plasma palmitate radioactivity (percent of maximum SA) after the intravenous bolus administration of [^{14}C] or [^{3}H]palmitate in the palmitate protocol. All values are means ± SD.

fat mass should have deviated upward from the theoretical pattern as adiposity increased (Fig. 2). Instead, the omental and abdominal ^{14}C SA following the [^{14}C]oleate injection decreased as a function of visceral and UBSQ fat mass (Fig. 4), as might be predicted (Fig. 2). We interpret this as an indication that as these depots expand, the amount of stored tracer per unit fat mass decreases such that relatively constant amounts of the administered tracer were stored/distributed in the visceral and UBSQ depots. The remaining tracer was taken up by lean tissue and LBSQ fat. Of interest, a different relationship was seen for [^{14}C]oleate storage in leg fat (Fig. 4*C*). The femoral adipose tissue lipid SA did not decrease as LBSQ fat mass increased across a range of ~20 kg. The fraction of FFA tracer stored in LBSQ increased from 2 to 13% between lean and obese women. This pattern of LBSQ tracer storage suggests that leg fat in women can in effect steal FFA from other, possibly lean, tissues. The pattern of FFA tracer storage per adipocytes (Fig. 4*D–F*) further highlights the distinct properties of leg fat. The increase in FFA tracer per adipocyte as a function of LBSQ fat mass implies that leg adipocytes, in contrast to UBSQ and omental adipocytes, stored more FFA tracer in women with greater adiposity. We suggest that direct FFA storage in leg fat might play a role in the maintenance of a relatively gynoid body habitus in women as obesity develops.

Because of the need to coordinate our research studies with clinical care, the omental biopsies collected in the oleate protocol were performed 3 to 11 h after the subcutaneous biopsies. Therefore, some FFA tracer stored in visceral fat may have arrived via the VLDL-TG/LPL pathway, although our results suggest that the contribution of this pathway was probably small. First, in the palmitate protocol, we found that only ~4% of the FFA tracer traversed the VLDL-TG pool over 4.5–9.5 h, which is consistent with previous reports (18,30). This was accompanied by a small and nonsignificant increase in FFA tracer accumulation in subcutaneous fat from 30–45 min to 24 h after the tracer bolus (Table 3). Secondly, excluding/including the four subjects whose omental biopsy was collected later (6–11 h after the tracer bolus) than than that in the other eight participants (3–5 h) did not affect the group average visceral SA or the outcome of any other statistical analysis performed. Because only ~1% of the FFA tracer accumulated in visceral fat, irrespective of whether it arrived from the direct or indirect pathway, we conclude that visceral fat is not a major site of endogenous FFA storage in women.

The palmitate protocol studies cannot assess whether there is label loss between biopsies, i.e., release of tracer via lipolysis after incorporation into adipose lipids. Loss of label could occur via reentry into the circulation and/or local oxidation. In humans, the subcutaneous adipose triglyceride turnover period is ~6 months (31); therefore, unless there is preferential recycling of a subpopulation of newly deposited triglycerides, it is unlikely that substantial amounts of stored label could have been lost over 24 h. Regarding the possibility of a subpopulation of rapidly turning-over triglycerides in adipocytes, Stein et al. (32) found that perfused adipose tissue from starved rats took up, esterified, and quickly stored plasma FFA in lipid mature droplets. In line with this observation, Öst et al. (33) showed in human adipocytes that newly synthesized triglycerides are rapidly transferred from the site of synthesis (plasma membrane) to the central lipid droplet. However, both studies were performed under high insulin conditions (32,33); thus, studies of this type under normal insulin conditions would help to assess the existence of different, nonuniform intracellular lipid pools in postabsorptive humans. Loss of adipose label via local oxidation can probably be neglected because of the tissue's low-energy requirements and the finding that glucose rather than fatty acids supplies the majority of its energy (34).

Potential mechanisms for the observed differences in FFA incorporation into adipose lipids include regional

TABLE 3
SA in subcutaneous abdominal and femoral adipose tissue lipid at 30–45 min, 4.5 h, and 24 h after the FFA tracer bolus injection in the women participating in the palmitate protocol

	30–45 min	4.5 h	24 h	Repeated-measures ANOVA *P*
Abdominal SA (dpm/g lipid)	528 ± 384	614 ± 557	681 ± 479	0.24
Femoral SA (dpm/g lipid)	708 ± 532	748 ± 604	890 ± 703	0.43
% FFA tracer taken into UBSQ fat	5.1 ± 2.3	5.4 ± 3.9	6.1 ± 2.3	0.16
% FFA tracer taken into LBSQ fat	6.9 ± 6.1	7.1 ± 6.7	8.8 ± 8.2	0.46
% FFA tracer taken into SQ fat	12.0 ± 8.3	12.5 ± 10.3	14.9 ± 9.7	0.11

Data are means ± SD. SQ, whole-body subcutaneous.

differences in blood flow, fatty acid transport, or fatty acid intracellular trafficking. Blood flow per unit subcutaneous adipose tissue mass has been shown to be lower in obese (abdomen ~1.7 ml · min^{-1} · 100 g^{-1}; thigh ~1.8 ml · min^{-1} · 100 g^{-1}) than in lean (abdomen ~4.1 ml · min^{-1} · 100 g^{-1}; thigh ~2.4 ml · min^{-1} · 100 g^{-1}) women (35). Therefore, the lower [^{14}C]oleate retention per gram UBSQ fat in the obese participants (Fig. 4) may have been to some extent due to reduced delivery of the FFA tracer to the region. The fact that femoral adipose blood flow is also less in obese than lean individuals, however, suggests that delivery cannot solely explain the different patterns we observed between UBSQ and LBSQ fat (Fig. 4). Evidence for an intrinsic role of adipocytes comes from studies of radiolabeled palmitate storage in isolated pieces of epididymal adipose tissue from mice with smaller pads/smaller fat cells and from animals with larger fat pads/larger fat cells; the adipose lipid SA was higher in the former samples than the latter, although all samples were incubated in the same amount of FFA tracer (36).

There are several limitations to the present study that need to be taken into consideration. We assessed FFA tracer storage in visceral fat by sampling omental adipose tissue. Although omental fat well reflects meal fatty acid storage in mesenteric fat (23), it is unknown whether this also holds true for plasma FFA storage. To estimate FFA tracer storage, we extrapolated from biopsy samples of relatively small size to an entire regional depot. Although this seems reasonable based on the limited heterogeneity of meal-derived fatty acid uptake (37), further studies are needed to be confident that extrapolation is not in error. As mentioned above, the timing of the laparoscopic surgery did not allow the collection of omental biopsies before the appearance of the FFA tracer in VLDL-TG. We measured fractional tracer storage and not absolute fluxes of plasma FFA storage in adipose tissue. The latter would allow a direct comparison of regional FFA disposal between volunteers with a wide range of adiposity and provide solid evidence as to whether obese women store greater amounts of FFA in their lower-body fat region than their lean counterparts. Finally, it will be necessary to perform similar studies in men to understand if this is a sex-specific phenomenon.

In summary, we found that in women, postabsorptive FFA storage in leg adipose tissue displays a unique pattern that distinguishes it from UBSQ and visceral fat. Our findings suggest that FFA storage (direct or indirect) is not a significant pathway for the maintenance of visceral obesity in women. The observed heterogeneity in FFA tracer storage between fat depots suggests different regulatory mechanisms that may play a causal role in the regulation/maintenance of body fat distribution in women. Studies of both men and women with different obesity phenotypes will be needed to understand the role of direct FFA storage in regional adipose tissue metabolism and body fat distribution.

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