Original Article

Direct Free Fatty Acid Uptake Into Human Adipocytes In Vivo

Relation to Body Fat Distribution

Samyah Shadid, Christina Koutsari, and Michael D. Jensen

We sought to assess whether direct uptake of circulating free fatty acids (FFAs) by adipocytes occurs in vivo in overnight postabsorptive humans and, if so, whether there are regional differences in uptake between lean and obese women and men. We used bolus injections of radiolabeled FFA tracers followed by carefully timed adipose tissue biopsies. First, we validated a method to measure direct adipocyte FFA uptake and then quantitated this process using the modified methods in normal-weight postabsorptive men and women. We then used a continuous infusion of radiolabeled FFA to measure direct and indirect (VLDL) regional adipose tissue uptake in obese men and women. Direct FFA uptake was greater in women than men: 8.2 ± 0.6 vs. 4.0 ± 0.5% ($P < 0.0001$) of FFAs were taken up by subcutaneous adipose tissue, respectively. Abdominal subcutaneous fat took up FFAs more avidly than femoral fat in men, but this did not occur in women. Similar sex and regional difference were found to occur in obese women and men. Gene expression of fatty acid transporters was greater in abdominal than femoral fat in men ($P < 0.05$) but not in women ($P = 0.80$). We observed sex- and site-specific recycling of circulating FFAs into subcutaneous fat. This is a novel FFA disposal pathway that may also play a role in the development or maintenance of body fat distribution. Regional variations in facilitated fatty acid transport may contribute to this process. *Diabetes* 56:1369–1375, 2007

Body fat distribution is an important predictor of obesity-related disease risk. A visceral/upper-body fat distribution often results in elevated plasma free fatty acid (FFA) concentrations (1,2), which can cause insulin resistance and associated metabolic complications (3). How people can differ so remarkably in body fat distribution is not yet known, but regional differences in fatty acid uptake or FFA release between various fat depots almost certainly play a role. Studies of isolated adipocytes or adipose tissue fragments have suggested that regional differences in lipolysis (4) or uptake of triglycerides via lipoprotein lipase (LPL) (5) account for the differences in body fat distribution. Unfortunately, in vivo studies have not been able to detect the regional differences in lipolysis or circulating triglyceride uptake that would be predicted on the basis of in vitro findings.

For example, lean and obese men and women with differing fat distributions do not display the variations in regional FFA release that would be expected to cause retention of fat in certain depots (6–8). Greater regional fat accumulation is associated with increased, not reduced, lipolysis rates from that depot (7–9). If regional differences in FFA release cannot account for site-specific fat retention, then it is reasonable to ask whether variations in fatty acid uptake are causative. Although women have greater femoral adipose tissue LPL activity (10) than men, in vivo studies from Mårin and colleagues (11–13) and our laboratory (14–16) have shown that they do not preferentially store dietary fat in this region, at least in the short term. Upper-body subcutaneous fat takes up meal fatty acids more efficiently than lower-body fat in both women and men (11,14–16). Finally, the efficiency of dietary triglyceride uptake into subcutaneous fat is not greater in women than men (14,16), although women have much more subcutaneous adipose tissue. Thus, human in vivo studies of regional fatty acid metabolism have not yielded findings that explain how humans develop or maintain regional variations in fat accumulation.

These studies were undertaken to determine whether an unappreciated pathway of adipocyte fatty acid uptake exists: direct uptake of circulating FFAs into adipose tissue independent of the LPL mechanism that acts on chylomicron and VLDL triglycerides. This pathway of direct FFA uptake has been recently reported to exist in the postprandial state (17) and during glucose infusion (18), but it has not been detected in the postabsorptive state (18). The results overturned our perception that adipose tissue does not take up FFAs directly under postabsorptive conditions and suggest that this process may be important in regulating or maintaining body fat distribution.

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

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

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S.S. and C.K. contributed equally to this work.

S.S. is currently affiliated with the VieCuri Medical Center, Venlo, the Netherlands.

DEXA, dual-energy X-ray absorptiometry; FFA, free fatty acid; GCRC, General Clinical Research Center; HOMA-IR, homeostasis model assessment of insulin resistance; HPLC, high-performance liquid chromatography; LPL, lipoprotein lipase; TLC, thin-layer chromatography.

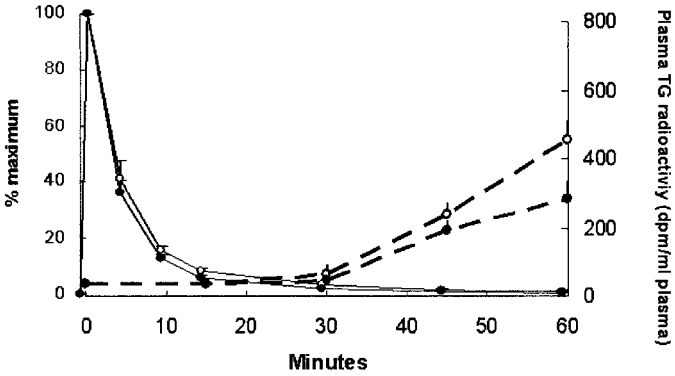

FIG. 1. Kinetics of radiolabeled FFAs in plasma and plasma triglycerides (TG). The decay of plasma FFA radioactivity (solid lines) and appearance in plasma triglycerides (dashed lines) before and after intravenous bolus administration of [^{14}C]FFA are shown. ○, values for women; ●, values for men. In protocol 2, adipose tissue biopsies were collected 40–45 min after the bolus, a time when most of the tracer has disappeared from plasma but has not substantially entered circulating triglyceride.

RESEARCH DESIGN AND METHODS

Three studies were conducted. The first was to define the optimal approach of measuring direct FFA uptake into adipose tissue. The second was to quantitate direct FFA uptake by adipose tissue in nonobese men and women. The third study was to determine total (direct and VLDL triglyceride–derived) adipose tissue FFA uptake in obese men and women. Each protocol was approved by the Mayo Clinic institutional review board, and written informed consent was obtained from the volunteers.

All participants were weight stable for at least 3 months before the study. Inclusion criteria are described below; exclusion criteria included smoking, pregnancy, breast-feeding, and use of medication that could affect lipid metabolism that could not be stopped for at least 2 weeks before the study.

The following procedures were common to all protocols. Each volunteer consumed an isoenergetic diet (50% carbohydrates, 35% fat, and 15% protein) from the Mayo General Clinical Research Center (GCRC) for at least 5 days before the study. Body composition was measured with a single-slice abdominal computed tomography (L_{2-3} interspace) and dual-energy X-ray absorptiometry (DEXA) (DPX-IQ; Lunar Radiation, Madison, WI). After admission to the Mayo GCRC, a forearm vein catheter was inserted and used for tracer infusion and a second catheter placed in a retrograde fashion in a hand vein for collecting arterialized blood using the heated hand vein technique.

Protocol 1: distribution of the FFA tracer in adipose tissue lipid fractions. Five lean and obese women and seven lean and obese men participated in studies to assess the distribution of the FFA tracer in plasma and in adipose tissue lipids. The time course of the decay of plasma FFA radioactivity and appearance of FFAs in plasma triglycerides was determined by collecting blood samples before and at 1, 5, 10, 15, 30, 45, and 60 min after a bolus of radiolabeled palmitate. Abdominal and femoral subcutaneous fat biopsies were collected 15 or 30 min after the FFA tracer bolus. The tracer distribution in lipids (phospholipids, monoglycerides, diglycerides, triglycerides, cholesterol esters, and FFAs) extracted from whole adipose tissue and from isolated adipocytes was assessed to distinguish whether the tracer in whole adipose tissue represented intracellular triglycerides or extracellular radiolabeled FFAs.

Protocol 2: LPL-independent adipose tissue FFA uptake. This study was to quantitate the direct adipose tissue uptake of circulating FFAs in normal-weight men and women. We gave an intravenous bolus of an FFA tracer and performed fat biopsies timed such that virtually no FFA tracer remained in the circulation and there would be insufficient tracer in VLDL triglycerides to allow adipose tissue accumulation via the LPL pathway. Twenty-seven (12 men and 15 women) participated in this study. The morning after admission to the Mayo GCRC, adipose tissue blood flow (14) was measured in the overnight postabsorptive state to test for site or sex differences that could affect tissue FFA delivery. The volunteers continued the same diet while staying in the GCRC until the following morning, when, after an overnight fast, an intravenous bolus of [1-^{14}C]oleate was given. Blood samples were collected as in protocol 1 to measure the decay of ^{14}C oleate in plasma and the appearance of ^{14}C oleate in plasma triglycerides. The adipose biopsies were timed such that the abdominal and femoral fat was collected between 40 and 45 min after the tracer bolus.

Protocol 3: regional and sex differences in adipose tissue FFA uptake in obesity. This experiment allowed us to examine the cumulative reentry of plasma FFAs into adipose tissue of obese men and women through the direct uptake and indirect (VLDL) routes. Ten obese men and 12 obese premenopausal women participated in this study. The volunteers were admitted to the GCRC the evening before the experiment and consumed a standardized evening meal at 1800 h. At 0300 h, a constant infusion of [1-^{14}C]palmitate (~0.3 μCi/min) was started and, at 0900 h, femoral and abdominal subcutaneous adipose tissue biopsies were obtained.

Materials. [l-^{14}C]palmitate, [9,10-^{3}H]palmitate, and [l-^{14}C]oleate were purchased from NEN Life Science Products (PerkinElmer, Boston, MA). [^{133}Xe] (Syncor, St. Paul, MN) was used to measure adipose tissue blood flow (14–16).

Assays and methods

Plasma concentrations and specific activity. Plasma palmitate, oleate, and total FFA concentrations and specific activity were measured using high-performance liquid chromatography (HPLC) (19,20). The amount of FFA tracer in plasma triglycerides was determined by extracting lipids from 1.0 ml plasma using the Dole procedure and separating FFAs from triglycerides using an HPLC procedure (6,21). The plasma triglyceride concentrations were measured (22), and the counts in the triglyceride fraction of the HPLC-separated plasma were used to calculate triglyceride specific activity.

Adipocyte handling. Fat cell size was measured as previously described (23). To measure the whole–adipose lipid specific activity, freshly rinsed tissue was added to 15 ml CHCl3:methanol (2:1) and incubated in a cold room for at least 2 days. Subsequently, 3.75 ml of 88% KCl was added. The lipid layer was isolated by centrifugation, dried, and weighed. The ^{14}C adipose lipid specific activity (dpm/g) from each biopsy was measured to <2% counting error as previously described (24).

To specifically measure adipocyte lipid specific activity in protocol 1, we isolated fat cells using a larger scale of the digestion process used to measure fat cell size (23). The lipid from isolated adipocytes was collected as described above, except that a second wash of the adipocyte layer with HEPES buffer was performed to eliminate extracellular FFAs.

Determining tracer distribution within adipose biopsies. Lipid extract (100–400 mg) from each adipose sample was dissolved in chloroform and applied onto one to four thin-layer chromatography (TLC) plates (silica gel 60 Å, size 20 × 20 cm, layer thickness 500 μm) (catalog no. 4861-830; Whatman, Clifton, NJ). The lipid mass applied allowed us to obtain <2% counting error with ≤100 min of counting. The lipid classes on TLC plates were identified using standards, and each class was collected by scraping and then reextracted using hexanes–chloroform–diethyl ether (5:2:1; vol:vol:vol) and counted for radioactivity. The percent contribution of each lipid class to the total number of counts was calculated.

Measuring mRNA for fatty acid transporters. Subcutaneous adipose tissue from abdomen and thigh was washed and flash frozen until extraction. RNA was extracted using 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 Applied Biosystem's Sequence Detection System User Bulletin no. 2 (2001; http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf).

Calculations. Visceral fat mass was predicted using the computed tomography measures of intra-abdominal and subcutaneous adipose tissue combined with DEXA-measured abdominal fat (25). Upper-body subcutaneous fat was calculated by subtracting visceral fat from total upper-body fat measured by DEXA. Leg fat was measured using the region of DEXA interest program.

The uptake of FFA tracer into adipose tissue was calculated by multiplying the adipose tissue triglyceride specific activity (dpm/g) by the site-specific (lower- and upper-body subcutaneous) adipose triglyceride mass (g). ^{14}C FFA uptake is presented both as an absolute value (dpm/g tissue) and as a percentage of the total tracer infused. The latter was calculated by dividing regional adipose tracer uptake (dpm) by the amount of tracer administered.

Insulin sensitivity was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) with an Excel macro (2004) courtesy of the University of Oxford.

Statistics. All data are presented as means ± SE unless otherwise stated. Statistical analyses were performed by t tests using repeated-measures ANOVA for upper- and lower-body fat within the same individuals, followed up by paired t tests as needed. Comparisons between men and women were done using a nonpaired t test. P values <0.05 were considered statistically significant.

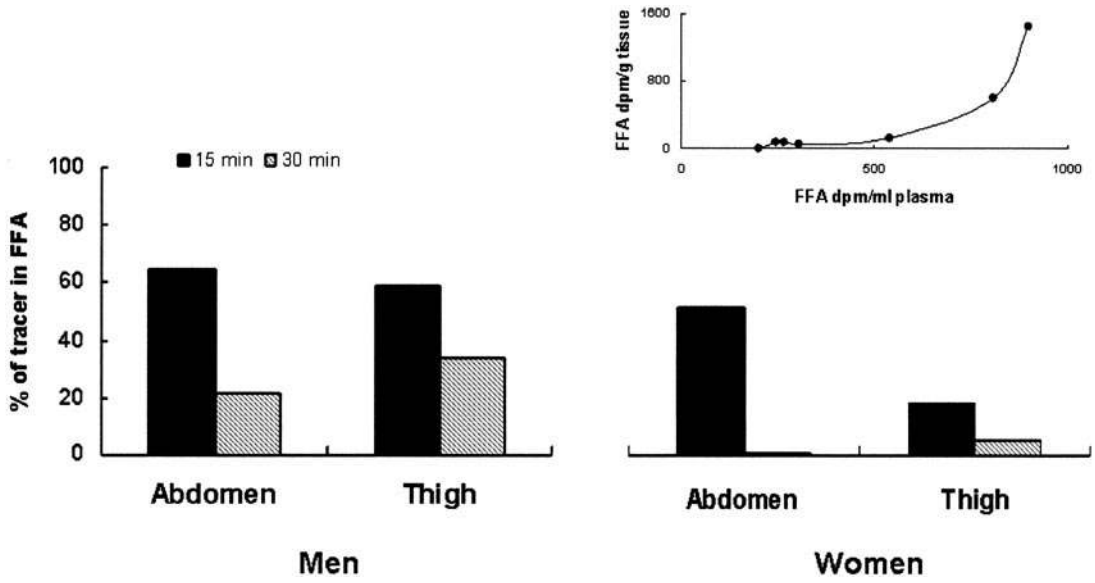

FIG. 2. ^{14}C palmitate in the FFA fraction of whole–adipose tissue lipid. Shown is the percent of ^{14}C in lipid extract of whole adipose tissue that was found in the FFA fraction in abdominal and femoral subcutaneous depots in women (*right*) and men (*left*) at 15 and 30 min after intravenous bolus administration of [1-^{14}C]palmitate. *Inset*: Relationship between plasma FFA radioactivity at the time of the biopsy (*x*-axis) and the radioactivity in the fatty acid fraction of whole adipose tissue measured using TLC (*y*-axis) in men.

RESULTS

Protocol 1: distribution of FFA tracer in adipose tissue lipid fractions. Data from volunteers in protocols 1 and 2 were used to define the disappearance of the FFA tracer from plasma and the appearance of tracer in circulating triglycerides (Fig. 1). Following the bolus administration of the FFA tracer, plasma FFA specific activity was maximum at 1 min and decreased to 8 and 3% of maximum by 15 and 30 min, respectively. No sex differences were observed in the specific activity decay curves. Significant amounts of plasma triglyceride radioactivity were first detected 45 min after the FFA tracer bolus.

In lipid extracts from isolated adipocytes, almost 100% of radioactivity was present in triglyceride, regardless of the time of the biopsy (15 or 30 min after the bolus). This is consistent with rapid esterification of FFAs into triglyceride after uptake by adipocytes. In contrast, whole–adipose tissue lipid extracts sometimes had a significant portion of the radioactivity in the FFA fraction, although >95% of radioactivity was in the FFA and triglyceride fractions combined. Biopsies collected 15 min after the tracer bolus had as much as 60% of the radioactivity in the FFA fraction (Fig. 2). Samples collected 30 min after the tracer bolus in men and women contained an average of 25 and 4% of the radioactivity in the FFA fraction, respectively (Fig. 2). There was a positive relationship between the concentration of FFA tracer in plasma (dpm/ml) at the time of biopsy and the concentration of radioactivity in the fatty acid fraction of whole adipose tissue collected from men (Fig. 2, *inset*). This suggests that the tissue included extracellular fluid/plasma FFA tracer. A similar relationship was not seen when data from women were examined; a very small proportion of tracer was present in the FFA fraction regardless of the level of residual radioactivity in plasma in women.

From these studies, we concluded that in the postabsorptive state *1*) adipocytes take up FFAs directly from the circulation; *2*) the FFAs taken up by adipocytes are, for all practical purposes, found mostly in triglycerides; *3*) whole–adipose tissue lipid extracts from biopsies taken ≤30 min after an FFA tracer bolus may contain extracellular FFAs that are present in plasma; and *4*) this problem can be overcome by isolating adipocytes or by delaying the biopsy until virtually no tracer remains in plasma.

Subject characteristics: protocol 2. Consistent with previous results, women had a greater percent body fat, more leg fat, and less visceral fat; abdominal and thigh fat cell size were not significantly different in men and women. Fasting plasma glucose, FFA, and insulin concentrations in men and women were 5.2 ± 0.1 vs. 4.9 ± 0.1 mmol/l, 463 ± 35 vs. 415 ± 24 μmol/l, and 33 ± 4 vs. 27 ± 3 pmol/l, respectively (all P = NS). The HOMA-IR values for men and women both averaged 0.5 ± 0.1 (Table 1).

Abdominal adipose tissue blood flow was greater in men than women (4.5 ± 0.7 vs. 2.4 ± 0.3 ml · 100 g^{-1} · min^{-1}, P = 0.001), whereas femoral adipose tissue blood flow was not different (2.7 ± 0.5 vs. 2.7 ± 0.3 ml · 100 g^{-1} · min^{-1}, respectively, P = NS).

FFA uptake in adipose tissue. Because women and men received the same amount of tracer (41 ± 1 and 43 ± 1 μCi, respectively, P = 0.22), the concentration of tracer in adipose lipid (dpm/g) was used to compare the efficiency of adipocyte FFA uptake between groups. In all cases, the ^{14}C FFA tracer concentration was <100 dpm/ml plasma at the time of the biopsy. We readily detected ^{14}C in whole–adipose tissue lipid from the biopsy taken 40–45 min after the tracer bolus. The adipose lipid specific activity in abdominal fat was greater in women than men (455 ± 58 vs. 297 ± 35 dpm/g, P = 0.03). Likewise, the adipose lipid specific activity in femoral fat was also greater in women than in men (463 ± 42 vs. 229 ± 23 dpm/g, $P < 0.0001$). The adipose lipid specific activity in abdominal fat was greater than femoral fat (P = 0.048) in men but not in women (P = 0.72). To put it another way, the efficiency of FFA uptake (per gram of fat) was ~30% greater in abdominal fat that femoral fat in men but was not different in women. The portion of the tracer stored in upper-body subcutaneous and leg fat is depicted in Fig. 3*B*; the fraction of the tracer taken up in subcutaneous adipose lipid was greater ($P <$ 0.0001) in women than in men (8.2 ± 0.6 vs. 4.0 ± 0.5%).

TABLE 1
Characteristics of volunteers

	Protocol 2			Protocol 3		
	Women	Men	*P*	Women	Men	*P*
n	12	15		12	10	
Age (years)	29 ± 2	35 ± 3	0.04	38 ± 3	38 ± 2	0.95
BMI (kg/m^2)	22.8 ± 0.4	23.9 ± 0.4	0.04	33.0 ± 1.0	33.4 ± 0.9	0.64
Percent fat	31 ± 1	20 ± 2	<0.0001	46 ± 2	35 ± 1	<0.0001
Total body fat (kg)	18.3 ± 0.7	15.6 ± 1.6	0.11	40.8 ± 2.4	35.5 ± 2.5	0.14
Leg fat (kg)	7.7 ± 0.5	5.4 ± 0.4	0.002	15.9 ± 1.3	11.5 ± 0.9	0.009
Upper-body subcutaneous fat (kg)	10.4 ± 0.6	8.9 ± 1.3	0.24	21.0 ± 1.2	17.9 ± 1.3	0.10
Visceral fat (kg)	1.2 ± 0.3	1.0 ± 0.3	0.60	4.0 ± 0.4	6.1 ± 0.7	0.02
Abdominal fat cell size (μg lipid/cell)	0.411 ± 0.036	0.443 ± 0.049	0.51	0.858 ± 0.073	0.750 ± 0.048	0.24
Femoral fat cell size (μg lipid/cell)	0.481 ± 0.027	0.411 ± 0.029	0.11	0.813 ± 0.082	0.801 ± 0.071	0.91

Data are mean ± SE.

Subject characteristics: protocol 3. The obese men and women were well matched for BMI. The expected differences in percent body fat and regional fat mass were observed; abdominal and thigh fat cell size were not different in men and women (Table 1). Fasting plasma glucose and insulin concentrations were 5.3 ± 0.1 mmol/l and 62 ± 13 pmol/l in men and 5.2 ± 0.1 mmol/l and 69 ± 5 pmol/l in women (*P* = NS for both). The HOMA-IR values for men and women averaged 1.4 ± 0.3 and 1.1 ± 0.1, respectively (Table 1).

Fasting plasma FFA concentrations were 490 ± 24 and 384 ± 37 μmol/l (*P* = 0.03) in obese women and men, respectively.

FFA uptake in adipose tissue. The women and men received 111 ± 6 and 123 ± 8 μCi of ^{14}C palmitate, respectively (*P* = 0.25, women vs. men). The ^{14}C whole–adipose lipid specific activity from the tissue collected at the end of the tracer infusion is depicted in Fig. 4. In obese women, the adipose lipid specific activity was ~40% greater in femoral than abdominal subcutaneous fat (1,474 ± 174 vs. 1,048 ± 105 dpm/g fat, respectively, *P* = 0.02). Abdominal adipose lipid specific activity tended to be greater than the femoral specific activity in obese men (877 ± 123 vs. 721 ± 98 dpm/g, respectively, *P* = 0.15). The femoral adipose tissue lipid specific activity was greater (*P* = 0.002) in women than men, but the abdominal adipose specific activity was not statistically different (*P* = 0.30).

We calculated that 11 ± 3% of the total amount of tracer infused was taken up by lower-body adipose tissue in obese women and only 3 ± 1% was stored in lower-body fat in obese men (*P* = 0.02, women vs. men), reflecting both a greater efficiency of uptake and the greater leg fat

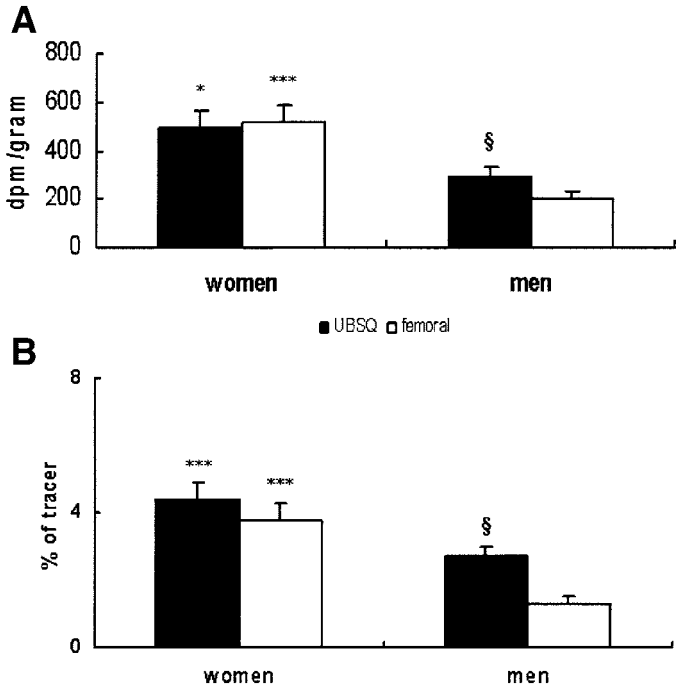

FIG. 3. Sex and depot differences in adipose tissue FFA tracer uptake. The lipid specific activity of abdominal and femoral adipose tissue in nonobese men and women (*A*) and the percent of the tracer stored in upper-body subcutaneous and lower-body fat (*B*). In both depots, tracer uptake is greater in women than in men. **P* < 0.05, ****P* < 0.001, men vs. women; §*P* < 0.05, upper-body subcutaneous (UBSQ) vs. leg fat.

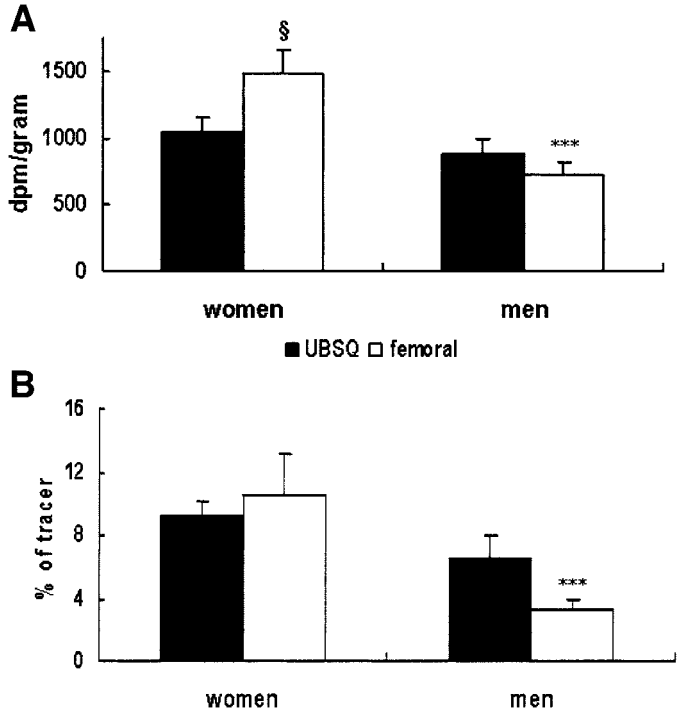

FIG. 4. Sex- and depot-specific tracer uptake in adipose tissue of obese men and women after continuous tracer infusion. Specific activity of abdominal and femoral fat depots in obese men and women (*A*) and the percent of the tracer stored in upper-body subcutaneous (UBSQ) and lower-body fat (*B*). §*P* < 0.05, upper-body subcutaneous vs. leg fat; ****P* < 0.001, men vs. women.

TABLE 2
Fatty acid transporter gene expression

	Women		Men	
	Abdomen	Thigh	Abdomen	Thigh
CD36	2.51 ± 0.51	2.74 ± 0.64*	2.42 ± 0.48	2.17 ± 0.35
FATP1	1.85 ± 0.54	1.71 ± 0.46	2.12 ± 0.42	1.74 ± 0.36
FATP4	1.48 ± 0.56	1.55 ± 0.30*	1.40 ± 0.29	1.26 ± 0. 20
Average	2.51 ± 0.51	2.00 ± 0.42	1.98 ± 0.26	1.72 ± 0.24†

Data are mean ± 1 SD for gene expression relative to a calibrator sample. Abdominal and thigh samples from 12 normal-weight women and 7 normal-weight men were analyzed. *$P < 0.05$ vs. thigh for men; †$P < 0.05$ vs. abdomen.

mass in women (Table 1). The tracer uptake in upper-body subcutaneous fat was somewhat greater in women than men (9 ± 1 vs. 7 ± 2%, $P = 0.15$).

Gene expression of fatty acid transport proteins in adipose tissue. Abdomen and thigh adipose tissue from 12 normal-weight women and 7 normal-weight men (6 women and 3 men who participated in protocol 2) were analyzed for mRNA abundance for CD36, FATP1, and FATP4. The transcript levels for the three fatty acid transporters relative to a calibrator sample are provided in Table 2. We tested the hypothesis that regional differences in the messages for the fatty acid transporters would follow the same pattern as the regional differences in direct FFA uptake. The gene expression of the three fatty acid transporters was greater ($P = 0.04$) in abdominal adipose than femoral adipose tissue in men, whereas no difference ($P = 0.80$) was seen in women. Thigh adipose CD36 and FATP4 mRNA were greater in women than men (both $P = 0.02$).

DISCUSSION

Neither regional differences in lipolysis (6–9,26–28) nor regional differences in adipose tissue uptake of meal-derived fatty acids (11,12,14–16) can explain how differences in body fat distribution are maintained between men and women. Herein we report that adipocytes directly take up circulating FFAs in the postabsorptive state and that differences in FFA uptake efficiency are related to body fat distribution. A significant fraction of FFA disposal is direct adipocyte FFA uptake, and there are important differences between lean and obese men and women. Direct FFA uptake in subcutaneous fat differs from meal fatty acid uptake in two respects: *1*) direct FFA uptake is more efficient in women than men and *2*) there is no preferential direct FFA uptake in upper-body subcutaneous fat compared with lower-body fat in women. These sex-based differences are consistent with this process as a mechanism to develop or maintain variations in body fat distribution between men and women, both lean and obese.

The great majority of the FFA tracer recovered in lipid extracts from isolated adipocytes was in triglycerides, whereas significant amounts of radioactivity were found in the FFA fraction of whole–adipose tissue lipid, unless the biopsy was performed 40–45 min after the FFA tracer injection. The radioactivity in the FFA fraction of whole–adipose tissue lipids collected at earlier time points can be explained by the residual radioactivity in the extracellular fluid that inevitably accompanies adipose tissue. Although direct FFA uptake was detected in biopsies performed ≤30 min after tracer injection, this required isolation of adipocytes from several hundred milligrams of adipose tissue or the isolation of triglycerides from the whole–adipose tissue lipid. A much simpler approach was to wait until 40–45 min after the tracer bolus to collect the tissue samples.

By administering the same dose of FFA tracer to normal-weight men and women, we could use the concentration of ^{14}C in adipose lipid as a direct measure of FFA uptake efficiency. Direct FFA uptake in subcutaneous adipose tissue was ~70% more efficient in women than in men (protocol 2). Fat cell size was not different between men and women, and abdominal adipose blood flow was greater in men than in women, which would be expected to favor greater, not lesser, direct FFA uptake in men. Edens et al. (29) reported that subcutaneous adipose tissue fragments from women took up and esterified extracellular radiolabeled FFAs twice as well as subcutaneous fat tissue from men. This suggests that adipocyte properties, rather than external factors, account for the greater FFA uptake in women in vivo. Because passive transmembrane transport of FFAs (the so-called "flip-flop" mechanism) seemed unlikely to explain our findings, we looked for evidence for regional difference in facilitated transport. Thigh adipose tissue expressed the mRNA coding for fatty acid transport proteins more so in women than men, and, on average, there was greater mRNA expression for transport proteins in abdominal than femoral subcutaneous fat in men. To the extent that message levels relate to protein content/function, the greater direct FFA uptake in abdominal than femoral fat in men could be due to greater facilitated inward fatty acid transport.

We tested whether total recycling of tracer from FFAs back into adipocytes (both direct FFA uptake and any contribution from VLDL triglyceride uptake) maintained this sex-specific pattern in obese men and women by performing fat biopsies following a continuous ^{14}C palmitate infusion. The efficiency of FFA storage in adipose triglycerides was greater in obese women than obese men, and women showed a preference for femoral adipose uptake. A portion of the FFA tracer could have entered adipose via VLDL triglycerides, although the relatively slow turnover of VLDL triglycerides compared with FFAs (30) makes it unlikely that this pathway contributed substantially. Irrespective of the relative contribution of direct uptake versus VLDL triglyceride uptake into subcutaneous adipose tissue in obese women, the pattern supports a preferential lower-body fat distribution.

The implications of our findings for body fat distribution relate to the discrepancy between regional lipolysis and regional direct FFA uptake into adipocytes. Upper-body subcutaneous fat contributes ~70% of systemic FFAs in lean men and women, whereas the leg contributes only ~20% (9); meal fatty acid uptake follows a similar pattern (14). In nonobese men, the direct uptake/storage of FFAs in abdominal and leg fat mirrors this regional difference in FFA release, whereas in lean women, direct FFA uptake

was similar in abdominal and femoral adipose tissue. This imbalance between release and direct reuptake in women could redistribute fatty acids toward leg fat. In the postabsorptive state, the systemic FFA flux in men and women averages ~500 μmol/min (~8.9 g/h or 107 g during a 12-h postabsorptive state) (10,21), of which 8.2 and 4.0% would be recycled to subcutaneous adipose tissue via direct uptake in lean women and men, respectively. If the ~8% of direct FFA uptake in subcutaneous fat in women was in proportion to rates of regional FFA release, 6.8 and 1.9 g of FFA should be recycled into upper- and lower-body fat, respectively. Instead, we calculate that 4.7 and 4.1 g of FFA would be directly taken up into upper- and lower-body subcutaneous fat in nonobese women. Thus, in postabsorptive women, the direct adipose FFA uptake pathway could result in the redistribution of ~2.1 g/day (779 g/year) of fatty acids from upper-body to lower-body fat compared with what would occur if direct reuptake occurred in proportion to release. Using the same approach, we estimate that ~0.5 g/day (P = NS vs. 0 g/day) of lipid would be redistributed from upper- to lower-body fat in lean men. In obese women, the total FFA reuptake in leg fat was also significantly greater than in abdominal fat.

One might question how adipocytes, which are actively exporting FFAs in the postabsorptive state, can simultaneously take up FFAs. Because in these studies the tracer is administered intravenously, it must mix in the central venous circulation with FFAs released from (mostly) adipose tissue, enter the arterial circulation, and then be taken up as blood perfuses adipose tissue. Given that in men ~4% and in women ~8% of FFAs are directly taken up in subcutaneous fat and that subcutaneous fat accounts for ~85% of systemic FFA flux (splanchnic release accounting for the other 15% [9]), we calculate that 5 versus 10% of FFAs released by subcutaneous fat cells are subsequently taken up directly and independent of the LPL pathway. This suggests a form of partitioning between adipocyte FFA release and FFA uptake. The partitioning could be at the level of each adipocyte, with the arterial-capillary delivery being somewhat anatomically distinct from the capillary-venous effluent. Alternatively, it may be that some populations of fat cells, such as the smaller adipocytes, take up but do not as actively release FFAs, whereas larger fat cells briskly release FFAs and do not take up FFAs under postabsorptive circumstances. Finally, it is possible that there are microcollections of adipocytes that undergo cyclic, temporary inhibition of lipolysis that allows them to shift to fatty acid uptake. This shift to direct FFA uptake has been reported to occur on a larger scale in the postprandial state (17), when suppression of lipolysis is accompanied by direct FFA uptake. In addition, in the postprandial state, the substantial inflow of chylomicron-derived fatty acids into adipose tissue, which still remains the major source of adipocyte triglyceride fatty acids, could "carry" FFAs along the same pathway.

A limitation of these studies is that to estimate total, direct FFA uptake, we have extrapolated the regional tracer uptake from anterior abdominal subcutaneous adipose tissue to all upper-body subcutaneous adipose tissue. Although this seems reasonable based on the limited heterogeneity of meal-derived fatty acid uptake (24), further studies are needed to be confident that extrapolation is reasonable. In addition, we have no data regarding omental or mesenteric adipose tissue direct FFA uptake, which would be an important next step to understand the relevance of this mechanism.

In summary, we report an unappreciated mechanism for adipocyte fatty acid uptake and storage that is independent of LPL and not thought to exist in the postabsorptive state. A novel aspect of this pathway is the greater efficiency in direct FFA uptake in subcutaneous adipose tissue of women compared with men. Of equal interest is that the direct uptake pathway does not favor upper-body subcutaneous fat in women the way it does in men. The direct FFA uptake pathway can redistribute fat from upper-body stores to lower-body stores in women but not in men.

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