Storage of Circulating Free Fatty Acid in Adipose Tissue of Postabsorptive Humans

Quantitative Measures and Implications for Body Fat Distribution

Christina Koutsari, Asem H. Ali, Manpreet S. Mundi, and Michael D. Jensen

OBJECTIVE—Preferential upper-body fat gain, a typical male pattern, is associated with a greater cardiometabolic risk. Regional differences in lipolysis and meal fat storage cannot explain sex differences in body fat distribution. We examined the potential role of the novel free fatty acid (FFA) storage pathway in determining body fat distribution in postabsorptive humans and whether adipocyte lipogenic proteins (CD36, acyl-CoA synthetases, and diacylglycerol acyltransferase) predict differences in FFA storage.

RESEARCH DESIGN AND METHODS—Rates of postabsorptive FFA (palmitate) storage into upper-body subcutaneous (UBSQ) and lower-body subcutaneous (LBSQ) fat were measured in 28 men and 53 premenopausal women. Stable and radiolabeled palmitate tracers were intravenously infused followed by subcutaneous fat biopsies. Body composition was assessed with a combination of dual-energy X-ray absorptiometry and computed tomography.

RESULTS—Women had greater FFA (palmitate) storage than men in both UBSQ (0.37 ± 0.15 vs. 0.27 ± 0.18 μmol·kg⁻¹·min⁻¹, P = 0.0001) and LBSQ (0.42 ± 0.19 vs. 0.22 ± 0.11 μmol·kg⁻¹·min⁻¹, P < 0.0001) fat. Palmitate storage rates were significantly greater in LBSQ than UBSQ fat in women, whereas the opposite was true in men. Plasma palmitate concentration positively predicted palmitate storage in both depots and sexes. Adipocyte CD36 content predicted UBSQ palmitate storage and sex-predicted storage in LBSQ fat. Palmitate storage rates per kilogram fat did not decrease as a function of fat mass, whereas lipolysis did.

CONCLUSIONS—The FFA storage pathway, which had remained undetected in postabsorptive humans until recently, can have considerable, long-term, and sex-specific effects on body fat distribution. It can also offer a way of protecting the body from excessive circulating FFA in obesity.

Obesity is a major contributor to chronic disease (1). Furthermore, a recent large cohort study demonstrated a strong positive association between abdominal fat distribution and all-cause mortality independently of general adiposity (2). Participants in the lowest third of BMI (women <23 kg/m²; men <24.9 kg/m²) and the highest quintile of waist or waist-to-hip ratio had the highest relative risk of death. These findings emphasize the importance of body fat distribution not only in overweight/obese individuals but also in normal-weight individuals.

Men tend to preferentially store fat in their upper-body region and women in their lower-body region (3). Regional differences in fat accumulation must develop from imbalances in fatty acid storage and/or free fatty acid (FFA) release between fat depots. In both sexes, the upper-body subcutaneous (UBSQ) fat depot is lipolytically more active than lower-body subcutaneous (LBSQ) fat (4,5), suggesting that differences in regional lipolysis cannot explain the sex differences in body fat distribution. In addition to the well-known dietary fat storage pathway, an underappreciated storage pathway in adipose tissue also exists (6–8). It involves the uptake and storage of circulating FFAs in the postabsorptive state. The existence of this process under postabsorptive conditions is counterintuitive considering the rapid efflux of fatty acids due to active lipolysis. Interestingly, the pattern of FFA storage between fat depots corresponds to sex differences in body fat distribution (6). However, FFA storage rates in subcutaneous fat regions have not been quantitatively measured, which would allow direct assessment of its quantitative and physiological importance.

Adipose tissue buffers the daily flux of fatty acids in the circulation (9). A remarkable adaptation in obesity is downregulation of lipolysis per unit of fat mass (10–13). This is advantageous because it prevents excessive increases in plasma FFA concentrations. Unfortunately, the enlarged adipose tissue also downregulates dietary fat storage (13). Because adipocytes resist further fat storage, dietary fat may be diverted to nonadipose organs leading to ectopic fat accumulation. It is currently unknown whether postabsorptive FFA storage is also downregulated in obesity or whether the enlarged adipose tissue maintains its ability to store FFA from plasma at rates similar to those in normal-weight individuals.

Fatty acid uptake and storage in adipocytes has been well studied in vitro, but limited information is available in vivo. The initial step in fatty acid uptake involves the transmembrane transport of fatty acids. Both passive diffusion and protein-facilitated transport (e.g., fatty acid translocase/CD36, fatty acid-binding proteins, fatty acid transport proteins) are thought to contribute to the transmembrane movement of fatty acids (14–18). The transmembrane transport is intimately coupled to esterification with CoA via the action of acyl-CoA synthetases (ACSs) (19,20). This reaction decreases the intracellular concentration of fatty acids to favor import and is an essential initial step for triglyceride synthesis. Diacylglycerol acyltransferase (DGAT) catalyses the final and only committed step in triglyceride synthesis, the conversion of diacylglycerol to triglyceride, making this a critical step in vivo (21).
Our goal was to assess the effect of sex and obesity on FFA storage in UBSQ and LBSQ depots under postabsorptive conditions. We examined the potential importance of the direct FFA storage pathway in redistributing fatty acids between fat depots. We also investigated whether obesity downregulates this pathway, as it does for lipolysis and meal fat storage. Lastly, we studied whether CD36, ACS, and DGAT relate to postabsorptive FFA storage and may constitute limiting steps in this process.

RESEARCH DESIGN AND METHODS

The study was approved by the Mayo Clinic Institutional Review Board. Informed written consent was obtained from all volunteers. Participants. Healthy participants (28 men, 53 premenopausal women) receiving no medications, including oral contraceptives, participated in the study. They were weight stable for >3 months before the study with a wide range of adiposity (20–36 kg/m²). A complete blood count, chemistry group, and routine urinalysis were documented as normal.

Study protocol. Participants received all their meals from the Mayo Clinic Clinical Research Unit metabolic kitchen for 5 days before the study to ensure stable energy intake and consistent macronutrient composition (50% carbohydrate, 35% fat, 15% protein). Volunteers were dismissed from the Clinical Research Unit at 1700 h and given a meal at 1800 h. At 0545 h the next day, a forearm vein catheter was inserted and kept patent with a controlled infusion of 0.45% NaCl. Another catheter was placed in a retrograde fashion in a hand vein for collecting arterialized blood using the heated (55°C) hand vein technique. After collecting a baseline blood sample for background palmitate specific activity (SA) and enrichment, a continuous infusion of [1–13C]palmitate (2–4 nmol/kg body weight/h) (Cambridge Isotope Laboratories, Andover, MA) commenced. After 30 min for isotopic equilibration, a series of three blood samples were collected at 10-min intervals and then every 30 min until 0900 h to measure palmitate kinetics. An ~60 μCi intravenous bolus of [1–13C]palmitate or ~200 μCi [9,10-3H]palmitate (NEL Life Science Products; PerkinElmer Life and Analytical Sciences, Boston, MA) was given at 0800 h. Abdominal and femoral subcutaneous adipose biopsies were collected at 30 min after the intravenous bolus of the radiolabeled palmitate. The biopsies were timed such that virtually no radiolabeled FFA tracer remained in the circulation and there would be insufficient tracer in VLDL/TG to accumulate in adipose tissue via VLDL. (6). Participants were dismissed from the Clinical Research Unit after completing the study.

Body composition measurements. Total and regional fat masses were assessed using dual-energy X-ray absorptiometry (DXA) (Lunar Radiation, Madison, WI). Leg fat mass was considered LBSQ fat. Visceral fat mass was estimated using a combination of single-slice computed tomography (L2-L3 interspace) and dual-energy X-ray absorptiometry–measured abdominal fat (22). Total body fat minus visceral and LBSQ fat masses was UBSQ fat mass.

Adipose tissue biopsies. Subcutaneous adipose tissues biopsies were collected from (a) the abdominal (just lateral to the umbilicus) and the femoral region (on the anterior-lateral aspect of the midthigh) as previously described (6–8). 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 adipocyte lipid specific activity (SA). Adipocyte size (23) and adipocyte lipid SA (dpm/mg lipid) (6) were assessed as previously described. Plasma palmitate concentration and SA. Aliquots of the [1–13C]palmitate and [9,10-3H]palmitate infusates were counted to assess the exact amount of radiotracer administered. Plasma palmitate SA was measured using high-performance liquid chromatography (24). All other metabolic parameters were measured as previously described (25). Plasma palmitate concentration and plasma [1–13C]palmitate infusion enrichment and concentration were measured using an liquid chromatography/mass spectrometry method (26).

Measurement of adipose tissue CD36 content and ACS and DGAT activities. We obtained sufficient tissue from a subset of participants to measure adipose tissue CD36 content and ACS and DGAT activities. Approximately 250 mg flash-frozen adipose tissue was homogenized in 2 mL standard homogenization buffer (20 mMol/L Tris HCl, pH 7.4, 1 mMol/L EDTA, 255 mMol/L sucrose) with proteases tablets (Roche, Indianapolis, IN). Supernatant was collected after centrifugation at 2,100 rpm at 4°C for 10 min. The fat cake of the supernatant was removed, and its lipids were extracted (chloroform:methanol) and used to normalize for protein content and enzyme activity per nulligram lipid. CD36 protein content. We used a sandwich enzyme-linked immunosorbent assay to measure adipose tissue CD36 content (27). In brief, 100 μL of the above-mentioned supernatant was denatured with SDS (final concentration 0.06%), and boiling for 10 min. The CD36 content was compared with that of a calibration standard using a five-point dilution curve for each sample and standard. ACS activity. We measured the conversion of [1–13C]palmitate to its CoA derivative using the protocol of Hall et al. (28). All reactions were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise stated. Briefly, the 150-μL reaction mix consisted of 150 mMol/L Tris HCl, 50 mMol/L MgCl2, 20 mMol/L ATP, 200 μMol/L CoA, 200 μMol/L dithiothreitol, 30 mMol/L NaCl, and 0.1 mMol/L EDTA. A total of 50 μL palmitate with [1–13C]palmitate (Perkin Elmer NET-845) mixture was added and then 50 μL sample supernatant. At 2 min, the reaction was terminated with 1.25% vol isopropanol: heptane: 1 N HSO4 (40:10:1, vol/vol/vol), and the supernatant was separated with centrifugation. The aqueous phase was counted on a scintillation counter with <2% counting error. The intra- and interassay coefficient of variation (CV) was <10%. Quality control samples were run at the beginning, middle, and end of each assay. Interassay variation was adjusted for using the mean values of the quality control samples relative to the entire set of samples analyzed. DGAT activity. We used the method of Coleman et al. (29) modified slightly to use the cytosolic fraction (30) of the adipose tissue homogenate and 20 μL of 10 mMol/L (rather than 2 mM) 1,2-dioleoyl-sn-glycerol (Sigma-Aldrich) in the reaction mixture. The intra- and interassay CVs were <10%. Interassay variation was adjusted as described above for ACS activity.

Calculations. Palmitate flux was calculated dividing the [1–13C]palmitate infusion rate by steady-state palmitate-[1–13C]palmitate). The fractional disappearance rate (Rd) of the radiolabeled adipose tissue palmitate stored per kilogram adipose tissue lipid (fat). The adipobiospalmitate was considered representative of the UBSQ depot, whereas the femoral fat biopsy was considered representative of the LBSQ compartment. Palmitate storage rates into each fat depot were measured as the product of steady-state palmitate Rd ([1–13C]palmitate) and the corresponding fraction of injected radiotracetracer stored per kilogram fat in each depot. Palmitate storage rates, CD36 content, and ACS and DGAT activities were expressed in μmol · kg−1 · min−1 and per 1,000 adipocytes.

Different means of data expression can have a significant impact on data interpretation. We focused on the per-unit fat mass expression, when aiming to understand whether one body fat depot is better at competing for the available FFA than another depot. We examined the factors that may regulate fatty acid storage within a depot, we focused on the per 1,000 adipocytes expression.

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5. Glucose and insulin were measured as previously described (25,31).

RESULTS

Subject characteristics. Table 1 provides the characteristics of the participants. Participants were well matched for age and BMI. We observed the expected differences in body fatness. Specifically, women had significantly greater body fat percentages and subcutaneous fat masses and less visceral fat than men. Women also had significantly larger femoral adipocytes than men. The average plasma palmitate concentrations did not differ between sexes, and concentrations were stable over the study interval. Adipose tissue lipolysis rates (as represented by palmitate Rd) were less in women than in men, but not when differences in resting energy expenditure were taken into account. The time course of plasma palmitate radioactivity was such that there were virtually no remaining counts in plasma palmitate after 30 min and very little in the plasma triglyceride component, similar to previous reports (6) (data not shown).

Regional palmitate storage rates in adipose tissue. Plasma palmitate storage rates in both UBSQ and LBSQ fat were significantly greater in women than in men (Table 2),
TABLE 1
Anthropometrics and fat distribution of study participants

|                | Women (n=53) | Men (n=28) | P       |
|----------------|--------------|------------|---------|
| n              | 53           | 28         |         |
| Age (years)    | 36 ± 8       | 33 ± 9     | *       |
| BMI (kg/m²)    | 27.7 ± 5.0   | 27.7 ± 5.1 | *       |
| Resting energy |              |            |         |
| expenditure (kcal/day) | 1,521 ± 215 | 1,900 ± 365 | <0.0001 |
| Weight (kg)    | 75.2 ± 14.3  | 88.9 ± 16.6| 0.0002  |
| Total body fat (%) | 39 ± 8       | 26 ± 8     | <0.0001 |
| Total body fat (kg) | 29.7 ± 10.0  | 23.2 ± 10.6| 0.009   |
| UBSQ fat (kg)  | 15.8 ± 5.2   | 12.3 ± 5.9 | 0.009   |
| LBSQ fat (kg)  | 11.8 ± 6.6†  | 7.6 ± 3.1† | <0.0001 |
| Visceral fat (kg) | 2.2 ± 1.8   | 3.3 ± 2.2 | 0.015   |
| Abdominal adipocyte size (µg lipid/cell) | 0.58 ± 0.24 | 0.58 ± 0.33 | 0.90 |
| Femoral adipocyte size (µg lipid/cell) | 0.75 ± 0.20† | 0.64 ± 0.26 | 0.037 |
| HOMA-IR        | 1.36 ± 0.72  | 1.43 ± 1.20| 0.76    |
| Plasma palmitate (µmol/L) | 103 ± 28     | 109 ± 30   | 0.74    |
| Plasma palmitate Rₐ (µmol/min) | 107 ± 38     | 130 ± 50   | 0.016   |

Data are means ± SD. *Selected to be similar, not subject to statistical testing. †P < 0.001 between depots within sex.

whether expressed per kilogram fat or per 1,000 adipocytes. In addition, storage rates were significantly greater in LBSQ fat than in UBSQ fat in women using either mode of expression. Conversely, adipose tissue palmitate storage rates were greater in UBSQ fat than in LBSQ fat in men.

Plasma palmitate concentration was a positive predictor of palmitate storage rates (effect of palmitate concentration: UBSQ, P < 0.0001; LBSQ, P < 0.0001) (Fig. 1). In addition, at a given palmitate concentration, women had greater storage rates than men (sex effect: UBSQ, P = 0.0002; LBSQ, P < 0.0001). Results were similar whether the data were expressed per kilogram fat or per 1,000 adipocytes. Obesity did not significantly affect regional storage rates (effect of body fat mass on UBSQ storage, P = 0.17; LBSQ storage, P = 0.46). HOMA-IR was a significant negative predictor of storage rates, independent of sex and palmitate concentration, in UBSQ (P = 0.025) but not LBSQ fat (P = 0.63).

Regional CD36 content, ACS activity, and DGAT activity. Average values of CD36 content and ACS and DGAT activities for the participants from whom we were able to collect adequate amounts of adipose tissue are provided in Table 3. No significant differences were observed between sexes in the three lipogenic factors examined in UBSQ fat. However, women had significantly greater ACS and DGAT activity in LBSQ fat than men. In addition, women had significantly greater CD36 protein content (per milligram lipid and per 1,000 adipocytes), ACS (per milligram lipid and per 1,000 adipocytes), and DGAT activity (per 1,000 adipocytes) in LBSQ fat than in UBSQ fat. No regional differences were observed in men in any of the three lipogenic factors examined.

We examined whether adipose tissue CD36 content, ACS, and DGAT activities per 1,000 adipocytes were interrelated. We observed significant positive relationships between all of the lipogenic factors in women and between some of them in men (Table 4).

Relationship between regional lipogenic enzymes/proteins and regional adiposity. In women, ACS (P = 0.01) and DGAT (P = 0.03) activities per milligram lipid decreased as a function of UBSQ fat mass in a nonlinear fashion (Fig. 2). A similar pattern was observed for CD36 content (P = 0.02) and ACS (P = 0.005) activity in men (Fig. 2). No associations were observed between any of the lipogenic factors studied and LBSQ fat mass in either sex.

Relationship between regional lipogenic enzymes/proteins and regional palmitate storage rates. When expressed per milligram lipid, no significant correlations were observed between lipogenic factors and regional palmitate storage rates in either UBSQ or LBSQ fat, with the only exception being a weak relationship between ACS activity and palmitate storage rates in UBSQ fat in women (data not shown).

When expressed per 1,000 adipocytes, rates of palmitate UBSQ storage were significantly and positively correlated with CD36 content (women: r = 0.66, P = 0.0001), ACS activity (men: r = 0.61, P = 0.043; women: r = 0.66, P = 0.002), and DGAT activity (women: r = 0.44, P = 0.02) (Fig. 3). Therefore, in women, palmitate storage rates in UBSQ adipocytes correlated with all three lipogenic factors. In contrast, rates of palmitate storage in UBSQ adipocytes in men correlated with ACS activity only. In the LBSQ depot, rates of palmitate storage per 1,000 adipocytes were significantly and positively correlated with CD36 content (women: r = 0.43, P = 0.006), ACS activity (women: r = 0.57, P = 0.0001), and DGAT activity (men: r = 0.71, P = 0.005) (Fig. 3). Therefore, palmitate storage rates in LBSQ adipocytes correlated significantly with CD36 content and ACS activity in women but with DGAT activity in men.

Predictors of regional palmitate storage rates. As mentioned, plasma palmitate concentration and sex were independent predictors of palmitate storage rates (Fig. 1). In addition, we assessed whether the lipogenic factors would provide an additional predictive value on palmitate storage above and beyond sex and palmitate concentration. When the three lipogenic factors were included in the multivariate regression analysis, palmitate concentration (P = 0.0002) and CD36 content (P = 0.047) were the only significant independent predictors of palmitate storage in UBSQ adipocytes. In LBSQ adipocytes, both palmitate

TABLE 2
Palmitate storage rates in subcutaneous adipose tissue depots

|                | Women (N=49) | Men (N=25) | P (women vs. men) |
|----------------|--------------|------------|------------------|
| Palmitate storage in UBSQ fat (µmol · kg fat⁻¹ · min⁻¹) | 0.367 ± 0.153 | 0.267 ± 0.181 | 0.001 |
| Palmitate storage in LBSQ fat (µmol · kg fat⁻¹ · min⁻¹) | 0.418 ± 0.188† | 0.219 ± 0.107† | <0.0001 |
| Palmitate storage in UBSQ fat (µmol · 1,000 adipocytes⁻¹ · min⁻¹) | 0.202 ± 0.114 | 0.153 ± 0.146 | <0.0001 |
| Palmitate storage in LBSQ fat (µmol · 1,000 adipocytes⁻¹ · min⁻¹) | 0.284 ± 0.169‡ | 0.135 ± 0.086 | <0.0001 |

Data are means ± SD. Statistics were performed on log-transformed data. ††† vs. UBSQ fat within sex; *P = 0.01; †P = 0.04; ‡P < 0.0001.
concentration ($P = 0.003$) and sex ($P = 0.0003$) remained significant independent predictors of palmitate storage, whereas none of the lipogenic factors did.

**Patterns of palmitate release and storage rates relative to adiposity.** Figure 4 depicts palmitate release (lipolysis) and storage per kilogram fat versus total subcutaneous fat mass. As opposed to the downregulation of lipolysis per kilogram fat that occurred with increasing body fat mass, palmitate storage per kilogram fat exhibited an identical range between individuals of low and high adiposity. In individuals with $>40$ kg subcutaneous fat, palmitate release was on average $\sim 2.5 \text{ mol} \cdot \text{kg fat}^{-1} \cdot \text{min}^{-1}$, whereas palmitate storage was on average $\sim 0.8 \text{ mol} \cdot \text{kg fat}^{-1} \cdot \text{min}^{-1}$ (Fig. 4). Therefore, in these cases, palmitate storage was approximately one-third of palmitate release.

**Estimates of body fat redistribution attributable to the direct FFA storage pathway.** Using the palmitate storage data, we can assess whether postabsorptive FFA storage rates in UBSQ and LBSQ fat follow the same pattern as that for postabsorptive regional lipolysis. If an imbalance exists between regional FFA storage and lipolysis, it could result in redistribution of fatty acids from one fat depot to another. We calculated palmitate storage into whole UBSQ and LBSQ fat depots by multiplying the regional storage per kilogram fat (Table 2) by the corresponding regional fat masses (Table 1). In women, palmitate storage was $5.7 \pm 2.8$ and $5.2 \pm 3.6 \text{ mol/min}$ into whole UBSQ and LBSQ fat, respectively. Corresponding values in men were $3.0 \pm 2.4$ and $1.6 \pm 1.1 \text{ mol/min}$.

Although we did not measure regional lipolysis rates in these volunteers, on average, UBSQ and LBSQ fat contribute $\sim 75$ and $\sim 18 \%$, respectively, of systemic lipolysis in normal-weight individuals and $\sim 57$ and $\sim 28 \%$, respectively, in obesity (5). Using these values, we estimate that, if palmitate storage in total subcutaneous fat in women ($\sim 10.8 \text{ mol/min}$) was distributed into UBSQ and LBSQ regions in proportion to rates of release from these regions (5), $\sim 7.7$ and $\sim 3.1 \text{ mol/min}$ palmitate would be stored into UBSQ and LBSQ fat, respectively. Compared with the actual storage rates (5.7 and 5.2 $\text{mol/min}$), the FFA storage pathway could redistribute $\sim 2.1 \text{ mol/min}$ palmitate from the UBSQ to LBSQ region ($\sim 631 \text{ g FFA/year}$ assuming a 12-h postabsorptive state/day) in postabsorptive women. Using the same approach to estimate FFA redistribution in men, if palmitate storage in subcutaneous fat ($\sim 3.6 \text{ mol/min}$) was distributed in proportion to release rates, $\sim 3.3$ and $\sim 1.3 \text{ mol/min}$ palmitate would be stored into UBSQ and LBSQ fat, respectively. Compared with the actual storage rates (3.0 and 1.6 $\text{mol/min}$, respectively), the FFA storage pathway would redistribute only $\sim 0.3 \text{ mol/min}$ palmitate from UBSQ to LBSQ fat ($\sim 92 \text{ g FFA/year}$).

**DISCUSSION**

We assessed the quantitative importance of direct FFA storage into subcutaneous adipose tissue in postabsorptive humans and its potential role in body fat distribution. We further examined the effect of obesity and key lipogenic

---

**TABLE 3**

Regional ACS and DGAT activities and CD36 content in subcutaneous adipose tissue

|                  | UBSQ fat | LBSQ fat | UBSQ fat | LBSQ fat |
|------------------|----------|----------|----------|----------|
| CD36 (relative units · mg lipid$^{-1}$) | $17 \pm 7 (37)$ | $20 \pm 7 (50)^\dagger$ | $21 \pm 9 (14)$ | $20 \pm 11 (15)$ |
| ACS (pmol · mg lipid$^{-1}$ · min$^{-1}$) | $53 \pm 22 (37)$ | $68 \pm 23 (50)^\ddagger$ | $61 \pm 36 (16)$ | $51 \pm 22 (16)$ |
| DGAT (pmol · mg lipid$^{-1}$ · min$^{-1}$) | $5.1 \pm 3.1 (37)$ | $5.0 \pm 2.8 (50)^*\ddagger$ | $3.7 \pm 1.5 (15)$ | $3.1 \pm 1.1 (16)$ |
| CD36 (relative units · 1,000 adipocytes$^{-1}$) | $10 \pm 9 (34)$ | $15 \pm 6 (44)^\dagger$ | $14 \pm 6 (12)$ | $15 \pm 10 (15)$ |
| ACS (pmol · 1,000 adipocytes$^{-1}$ · min$^{-1}$) | $31 \pm 18 (34)$ | $52 \pm 22 (44)^\ddagger$ | $35 \pm 22 (14)$ | $36 \pm 18 (16)$ |
| DGAT (pmol · 1,000 adipocytes$^{-1}$ · min$^{-1}$) | $2.8 \pm 1.6 (34)$ | $3.6 \pm 2.5 (44)^\dagger$ | $2.3 \pm 1.4 (13)$ | $2.3 \pm 1.2 (16)$ |

Data are means $\pm$ SD ($n$). Statistics were performed on log-transformed data. $^*P < 0.05$ between sexes $^\dagger P < 0.05$ between depots within sex.
factors involved in distinct steps of FFA handling by the adipose tissue. The major findings are 1) women stored more circulating FFA (as represented by palmitate) in their subcutaneous fat depots than men; 2) women preferentially stored FFA in their LBSQ region, whereas men preferentially stored FFA in their UBSQ fat depot; 3) circulating FFA concentration was an independent predictor of FFA storage in both depots; in addition, CD36 predicted storage in UBSQ fat and sex-predicted storage in LBSQ fat, and 4) as opposed to lipolysis and meal fat storage (10–13), obesity did not affect the magnitude of FFA storage per unit fat mass.

The finding that women store greater amounts of circulating FFA in their subcutaneous fat than men agrees with indirect observations from previous work (6). The sex difference was noted in both depots and at any given plasma FFA concentration (Fig. 1). This observation indicates that, as in the case of dietary fat storage (32,33), women store greater amounts of fatty acids in subcutaneous adipose tissue than men. The most striking observation was that palmitate storage (per unit fat mass) exhibited preferential lower body fat accumulation in women and preferential upper body fat accumulation in men. Given that regional in vivo differences in the storage of dietary fat (34,35) or adipose tissue lipolytic rates (36,37) do not seem to account for variations in human body fat distribution under isoenergic conditions, this is the first direct evidence for a pathway whose pattern matches sex-specific patterns of fat distribution.

By comparing the observed rates of regional FFA storage against what would occur if FFA storage took place in proportion to release, we estimated the postabsorptive FFA storage pathway would redistribute ~631 g fatty acids/year from the UBSQ to LBSQ fat depot in women but only ~92 g fatty acids/year in men. These results suggest that under postabsorptive conditions, the FFA storage pathway is quantitatively significant in regulating and/or maintaining the sex-specific body fat distribution patterns by redistributing fatty acids between fat depots. This pathway is easier to detect in the postprandial state, when there is net fat storage into adipose tissue (38,39). FFA uptake into abdominal subcutaneous fat in men during the early and midpostprandial period was 82–183 nmol · 100 g−1 · min−1 (corresponding to ~0.21–0.46 μmol palmitate · kg−1 · min−1) (38). In addition, in a mixed group of women and men, no difference was observed in postprandial FFA uptake between abdominal and femoral subcutaneous fat (39).

Another approach to evaluate the quantitative significance of the FFA storage pathway in adipose tissue is by comparing its magnitude with that of FFA storage into nonadipose tissues, such as muscle. Muscles rely on the supply of FFA from plasma. It was shown that in the noncontracting skeletal muscle, approximately half of the plasma FFAs entering the muscle are used for esterification into intramyocellular triglycerides (40). The incorporation of plasma palmitate into intramyocellular triglycerides was reported to be 0.28 μmol · kg wet muscle−1 · min−1 in men and 0.50 μmol · kg wet muscle−1 · min−1 in women (41). Palmitate storage in the current study (average of UBSQ and LBSQ fat) was 0.24 μmol · kg fat−1 · min−1 in men and 0.39 μmol · kg fat−1 · min−1 in women. Thus, FFA storage (as represented by palmitate) into adipose tissue lipid appears to be ~80% of the corresponding rate of FFA incorporation into intramyocellular triglycerides in the resting postabsorptive muscle. Other studies (40) have found somewhat lower rates of palmitate incorporation into intramyocellular triglycerides in men (~0.18 μmol · kg wet muscle−1 · min−1 calculated using the same precursor pool as in the study by Kanaley et al. [41]). Collectively, these findings indicate that postabsorptive FFA storage into subcutaneous fat is not substantially different in magnitude compared with the incorporation of FFAs into intramyocellular triglycerides.

A surprising discovery was that plasma palmitate concentrations were positively correlated with adipocyte palmitate storage rates in both depots and sexes. Whereas we might logically expect such a relationship in nonadipose tissues such as muscle or liver, this finding for adipose tissue seemed counterintuitive. Because increased FFA concentrations are most often secondary to increased lipolysis, we anticipated the latter would result in an unfavorable concentration gradient, thereby reducing FFA uptake and storage. The implication of this observation is that, as adipose tissue lipolysis increases, the reuptake and storage of circulating FFAs also increases, yet in a depot- and sex-specific manner.

In our attempts to identify regulatory factors that may play a role in FFA storage within a fat depot, we examined three proteins/enzymes involved in FFA handling by the adipocyte: CD36 content, ACS activity, and DGAT activity (collectively termed “lipogenic factors”). Although significant univariate correlations were observed between certain lipogenic factors and palmitate storage rates (Fig. 3), only CD36 (along with plasma palmitate concentration) independently predicted FFA storage in UBSQ fat. None of

### TABLE 4

Univariate correlations between CD36 content and ACS and DGAT activities in subcutaneous adipose tissue

|               | Women     | Men       |
|---------------|-----------|-----------|
|               | CD36      | ACS       | CD36      | ACS       |
| UBSQ fat      |           |           |           |           |
| CD36 (relative units · 1,000 adipocytes−1) | r = 0.70, P < 0.0001 | r = 0.91, P < 0.0001 |
| ACS (pmol · 1,000 adipocytes−1 · min−1) | r = 0.52, P = 0.0017 | r = 0.37, P = 0.23 |
| DGAT (pmol · 1,000 adipocytes−1 · min−1) | r = 0.77, P < 0.0001 | r = 0.50, P = 0.081 |
| LBSQ fat      |           |           |           |           |
| CD36 (relative units · 1,000 adipocytes−1) | r = 0.62, P < 0.0001 | r = 0.72, P = 0.0026 |
| ACS (pmol · 1,000 adipocytes−1 · min−1) | r = 0.31, P = 0.043 | r = 0.49, P = 0.061 |
| DGAT (pmol · 1,000 adipocytes−1 · min−1) | r = 0.62, P < 0.0001 | r = 0.74, P = 0.0009 |

Statistics were performed on log-transformed data.
the three proteins/enzymes predicted FFA storage in LBSQ fat independently of sex and palmitate concentration. These results suggest that transmembrane transport of FFA (as represented by CD36 content) may be a limiting step in FFA storage in UBSQ fat. The findings are in line with previous work where, compared with wild-type adipocytes, the incorporation of radiolabeled fatty acid into triacylglycerol was decreased in adipocytes from
FAT/CD36-null mice (42). We were unable to identify any of the lipogenic factors that we assessed as rate-limiting for the storage of FFAs in the LBSQ depot. Therefore, women exhibit greater FFA storage rates in LBSQ fat than men (Fig. 1); however, none of the three lipogenic factors were implicated in that effect. A limitation that has to be noted.

FIG. 3. Relationship between palmitate (Palm) storage in UBSQ fat (μmol/1,000 adipocytes/min) and abdominal CD36 content (units per 1,000 adipocytes) (A), abdominal ACS activity (pmol/1,000 adipocytes/min) (B), and abdominal DGAT activity (pmol/1,000 adipocytes/min) (C) and between palmitate storage in LBSQ fat (μmol/1,000 adipocytes/min) and femoral CD36 content (units per 1,000 adipocytes) (D), femoral ACS activity (pmol/1,000 adipocytes/min) (E), and femoral DGAT activity (pmol/1,000 adipocytes/min) (F) in men (●, solid line) and women (○, dashed line). Log values were used to achieve normal distribution.
is that because of the limited CD36, ACS, and DGAT data in men compared with women, our ability to detect significant relationships was probably greater in women than in men. We also note that CD36 is expressed on adipose tissue macrophages as well as adipocytes. It seems unlikely that this source of CD36 affects our conclusions, however. Macrophages represent a small fraction (≤5%) of stromovascular cells in adipose tissue of individuals with the BMI range of the current study, and the proportions do not differ between UBQ and LBSQ fat (43). Stromovascular cells represent ~64% of total adipose tissue cells (44); thus, macrophages contribute only ~3% (0.64 × 0.05) of adipose tissue cells. Furthermore, CD36 mRNA is more highly expressed in adipocytes than SV cells in humans (45). Collectively, these findings suggest that macrophage CD36 protein does not substantially affect the relationships we observed.

In the context of obesity, a fascinating observation was that obese individuals maintained the ability to store FFAs from plasma to the same extent as normal-weight individuals (Fig. 4). This is in stark contrast with the well-known downregulation that occurs in obesity in lipolysis (10–13) and dietary fat storage (13). This is a biologically remarkable observation, because it allows obese people to dispose more fatty acids in their subcutaneous fat than lean individuals. In individuals with >40 kg subcutaneous fat, one-third of the fatty acids that were released in the postabsorptive state were stored back in subcutaneous fat.

In summary, this is the first study to directly show that the FFA storage pathway, which had remained undetected in postabsorptive humans until recently (6–8), can have considerable, long-term, and sex-specific effects on body fat distribution. Furthermore, as opposed to the “inappropriate” downregulation that occurs in meal fat storage (13), the subcutaneous adipose tissue of obese individuals maintains its ability to store FFA from plasma to the same degree as normal-weight individuals. This suggests that, along with reduced lipolysis (10–13), the FFA storage pathway offers another way of protecting the body from excessive amounts of circulating FFAs in obesity.

ACKNOWLEDGMENTS

This work was supported by grants DK40484, DK45343, DK50456, and RO0585 from the U.S. Public Health Service and by the Mayo Foundation.

No potential conflicts of interest relevant to this article were reported.

C.K. researched the data and wrote the manuscript. A.H.A. and M.S.M. researched the data and reviewed and edited the manuscript. M.D.J. designed the study and reviewed and edited the manuscript.

The current address for A.H.A. is the National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Child Health and Human Development, Bethesda, Maryland.

The authors are indebted to the research volunteers for their participation. We are also grateful to Barbara Norby, Carley Vrieze, Christy Alired, Debra Harteneck, Darlene Lucas, Lendia Zhou, Maksym Karpyak, Rebekah Herrmann, Jessica Eastman, and Carol Silverling, as well as the Mayo Clinic Clinical Research Unit nursing staff and the Center for Translational Science Activities Mass Spectrometry Core Laboratory for technical assistance and help with data collection.

REFERENCES

1. Kim S, Popkin BM. Commentary: understanding the epidemiology of overweight and obesity: a real global public health concern (discussion 81–82). Int J Epidemiol 2006;35:60–67
2. Pischon T, Boeing H, Hoffmann K, et al. General and abdominal adiposity and risk of death in Europe. N Engl J Med 2008;359:2105–2120
3. Vague J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculus disease. Am J Clin Nutr 1956;4:20–34
4. Basu A, Basu R, Shah P, Vella A, Rizza RA, Jensen MD. Systemic and regional free fatty acid metabolism in type 2 diabetes. Am J Physiol Endocrinol Metab 2001;280:E1000–E1006
5. Nielsen S, Guo ZK, Johnson CM, Hensrud DD, Jensen MD. Splanchic lipolysis in human obesity. J Clin Invest 2004;113:1582–1588
6. Shaidi S, Koutsari C, Jensen MD. Direct free fatty acid uptake into human adipocytes in vivo: relation to body fat distribution. Diabetes 2007;56:1369–1375
7. Koutsari C, Dunesci DA, Patterson BW, Votruba SB, Jensen MD. Plasma free fatty acid storage in subcutaneous and visceral adipose tissue in postabsorptive women. Diabetes 2008;57:1186–1194
8. Hannukainen JC, Kallikoski KK, Borra RJ, et al. Higher free fatty acid uptake in visceral than in abdominal subcutaneous fat tissue in men. Obesity (Silver Spring) 2010;18:261–265

9. Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia 2002; 45:1201–1210

10. Birkenhager JC, Tjabbes T. Turnover rate of plasma FFA and rate of esterification of plasma FFA to plasma triglycerides in obese humans before and after weight reduction. Metabolism 1969;18:18–32

11. Campbell PJ, Carlson MG, Nurjan N. Fat metabolism in human obesity. Am J Physiol 1994;266:E690–E695

12. Horowitz JF, Coppack SW, Paramore D, Cryer PE, Zhao G, Klein S. Effect of short-term fasting on lipid kinetics in lean and obese women. Am J Physiol 1999;276:E278–E284

13. McQuaile SE, Hodson L, Neville MJ, et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetologia 2011;60:47–55

14. Hamilton JA, Kamp F. How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? Diabetes 1999;48:2255–2260

15. Frohme J, Stahl A. Protein-mediated fatty acid uptake: novel insights from mammalian cells. Prog Lipid Res 2000;39:83–107

16. Hajri T, Abumrad NA. Fatty acid transport across membranes: relevance to nutrition and metabolic pathology. Annu Rev Nutr 2002;22:383–415

17. Doege H, Stahl A. Protein-mediated fatty acid uptake: novel insights from in vivo models. Physiology (Bethesda) 2006;21:259–268

18. Kampf JP, Parmley D, Kleinfield AM. Free fatty acid transport across adipocytes is mediated by an unknown membrane protein pump. Am J Physiol Endocrinol Metab 2007;293:E1207–E1214

19. Gargiulo CE, Stuhlatsz-Kropser SM, Schaffer JE. Localization of adipocyte long-chain fatty acyl-CoA synthetase at the plasma membrane. J Lipid Res 1999;40:881–892

20. Lobo S, Wiczer BM, Smith AJ, Bernlohr DA. Fatty acid metabolism in adipocytes: functional analysis of fatty acid transport proteins 1 and 4. J Lipid Res 2007;48:609–620

21. Coleman RA, Lee DP. Enzymes of triacylglycerol synthesis and their regulation. Prog Lipid Res 2004;43:134–176

22. Jensen MD, Kanaley JA, Reed JE, Sheedy PF. Measurement of abdominal and visceral fat with computed tomography and dual-energy x-ray absorptiometry. Am J Clin Nutr 1995;61:274–278

23. Tchoukalova YD, Harteneck DA, Karwoski RA, Tarara J, Jensen MD. A quick, reliable, and automated method for fat cell sizing. J Lipid Res 2003;44:1795–1801

24. Miles JM, Ellman MG, McClean KL, Jensen MD. Validation of a new method for determination of free fatty acid turnover. Am J Physiol 1987;252:E431–E438

25. Nielsen S, Guo Z, Abu JB, Klein S, O’Brien PC, Jensen MD. Energy expenditure, sex, and endogenous fuel availability in humans. J Clin Invest 2003;111:981–988

26. Persson X-MT, Blachnio-Zabielska AU, Jensen MD. Rapid measurement of plasma free fatty acid concentration and isotopic enrichment using LC/MS. J Lipid Res 2010;51:2761–2765

27. Allred CC, Kremenay T, Koutsari C, Zhou L, Ali AH, Jensen MD. A novel ELISA for measuring CD36 protein in human adipose tissue. J Lipid Res 2011;52:408–415

28. Hall AM, Smith AJ, Bernlohr DA. Characterization of the acyl-CoA synthetase activity of purified murine fatty acid transport protein 1. J Biol Chem 2003;278:43008–43013

29. Coleman RA. Diacylglycerol acyltransferase and monoacylglycerol acyltransferase from liver and intestine. Methods Enzymol 1992;209:98–104

30. Hou XG, Moser S, Sarr MG, Thompson GB, Que FG, Jensen MD. Visceral and subcutaneous adipose tissue diacylglycerol acyltransferase activity in humans. Obesity (Silver Spring) 2009;17:1129–1134

31. Koutsari C, Basu R, Rizza RA, Nair KS, Khosla S, Jensen MD. Nonoxidative free fatty acid disposal is greater in young women than men. J Clin Endocrinol Metab 2011;96:541–547

32. Romanski SA, Nelson RM, Jensen MD. Meal fatty acid uptake in adipose tissue: gender effects in nonobese humans. Am J Physiol Endocrinol Metab 2000;279:E455–E462

33. Votrub SB, Jensen MD. Sex-specific differences in leg fat uptake are revealed with a high-fat meal. Am J Physiol Endocrinol Metab 2006;291: E1115–E1123

34. Márín P, Rebuffé-Schivre M, Björntorp P. Uptake of triglyceride fatty acids in adipose tissue in vivo in man. Eur J Clin Invest 1990;20:158–165

35. Márín P, Lönö L, Andersson B, et al. Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues in vivo in men: effects of testosterone. J Clin Endocrinol Metab 1996;81:1018–1022

36. Martin ML, Jensen MD. Effects of body fat distribution on regional lipolysis in obesity. J Clin Invest 1991;88:609–613

37. Guo ZK, Hensrud DD, Johnson CM, Jensen MD. Regional postprandial fatty acid metabolism in different obesity phenotypes. Diabetes 1999;48:1586–1592

38. Bickerton AS, Roberts R, Fielding BA, et al. Preferential uptake of dietary fatty acids in adipose tissue and muscle in the postprandial period. Diabetes 2007;56:168–170

39. McQuaile SE, Humphreys SM, Hodson L, Fielding BA, Karpe F, Frayn KN. Femoral adipose tissue may accumulate the fat that has been recycled as VLDDL and nonesterified fatty acids. Diabetes 2010;59:2465–2473

40. Sacchetti M, Saltin B, Osada T, van Hall G. Intramuscular fatty acid metabolism in contracting and non-contracting human skeletal muscle. Diabetes 2002;51:87–95

41. Kanaley JA, Shadid S, Sheehan MT, Guo ZK, Jensen MD. Relationship between plasma FFA, intramyocellular triglycerides and long-chain acylcarnitines in resting humans. J Physiol 2009;587:5039–5050

42. Febbraio M, Abumrad NA, Hajjar DP, et al. A null mutation in murine CD36 receptors in adipose tissue with insulin resistance in nondiabetic humans. Arterioscler Thromb Vasc Biol 2009;29:1328–1335