Storage Rates of Circulating Free Fatty Acid Into Adipose Tissue During Eating or Walking in Humans

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We measured subcutaneous adipose tissue free fatty acid (FFA) storage rates in postprandial and walking conditions to better understand the contributions of this pathway to body fat distribution. Palmitate tracers were infused intravenously and fat biopsies collected to measure palmitate storage in upper- (UBSQ) and lower-body subcutaneous (LBSQ) fat in 41 (17 men) and 40 (16 men) volunteers under postprandial and under postabsorptive walking conditions, respectively. Postprandial palmitate storage was greater in women than men in UBSQ (0.50 ± 0.25 vs. 0.33 ± 0.37 μmol · kg fat^{-1} · min^{-1}; P = 0.007) and LBSQ fat (0.37 ± 0.25 vs. 0.22 ± 0.20 μmol · kg fat^{-1} · min^{-1}; P = 0.005); storage rates were significantly greater in UBSQ than LBSQ fat in both sexes. During walking, UBSQ palmitate storage did not differ between sexes, whereas LBSQ storage was greater in women than men (0.40 ± 0.22 vs. 0.25 ± 0.15 μmol · kg fat^{-1} · min^{-1}; P = 0.01). In women only, walking palmitate storage was significantly greater in LBSQ than UBSQ fat. Adipocyte CD36 and diacylglycerol acyltransferase (DGAT) correlated with LBSQ palmitate storage in the postprandial and walking condition, respectively. We conclude that UBSQ fat is the preferred postprandial FFA storage depot for both sexes, whereas walking favors storage in LBSQ fat in women. Transmembrane transport (CD36) and esterification into triglycerides (DGAT) may be rate-limiting steps for LBSQ FFA storage during feeding and exercise. ***Diabetes*** **61:329–338, 2012**

Adipose tissue buffers the daily flux of fatty acids in circulation (1). The major fuel functions of adipose tissue are storage of dietary fatty acids postprandially and release of free fatty acids (FFAs) in the postabsorptive state and during physical activity. Despite active lipolysis, adipose tissue directly takes up and stores circulating FFAs in postabsorptive humans (2–4). Although lesser in magnitude than dietary fat storage, the regional patterns of direct FFA storage in the postabsorptive state match the well-known sex-specific body fat distribution (5), whereas dietary fatty acid storage does not (6). Specifically, postabsorptive, direct FFA storage favors redistribution of FFAs to lower-body subcutaneous fat (LBSQ) in women and to upper-body subcutaneous (UBSQ) fat in men (5).

The FFA storage pathway has been easier to detect in the postprandial state, due to net fat storage in adipose tissue combined with suppressed lipolysis (7,8). In a mixed group of women and men, no regional differences were observed in postprandial FFA uptake between abdominal and femoral subcutaneous fat (8). It is unknown whether there are sex differences in regional FFA storage rates in subcutaneous fat postprandially. If there is preferential FFA storage in one fat depot over another in either sex, this would suggest that postprandial FFA storage can contribute to sex-specific regulation of body fat distribution.

The other major condition that alters adipose tissue fatty acid balance is physical activity. During physical activity, adipose tissue lipolysis increases its supply of FFAs to systemic circulation and working muscles. Whether circulating FFAs can be taken up and stored in adipose tissue via the direct pathway under conditions of stimulated lipolysis is unknown.

In the current study, we quantitatively measured FFA storage in subcutaneous fat in humans during feeding or walking to assess the potential contribution of this pathway to regulating body fat distribution in adults. Furthermore, we attempted to identify regulatory factors that may play a role in FFA storage by examining three proteins/enzymes involved in adipocyte FFA storage (collectively termed FFA storage factors): CD36, which is implicated in the transmembrane transport of FFAs (9), acyl-CoA synthetase (ACS) activity, which is involved in rapid activation of imported FFAs (10,11), and diacylglycerol acyltransferase (DGAT) activity, which catalyzes the final, committed step in triglyceride (TG) synthesis, the conversion of diacylglycerol to TGs (12). Lastly, we investigated whether obesity downregulates FFA storage in adipose tissue under postprandial and walking conditions, as it does for postabsorptive lipolysis and dietary fat storage (13–16).

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 receiving no medications, including oral contraceptives, participated in the study. Twenty-four premenopausal women (8 lean) and 17 men (9 lean) participated in the postprandial protocol; 24 premenopausal women (9 lean) and 16 men (9 lean) participated in the walking protocol. They were weight stable for >3 months prior to the study and exhibited a wide range of adiposity (20–36 kg/m^2).

Study protocol. Participants received their meals from the Mayo Clinic Clinical Research Unit (CRU) for 5 days prior to the study to ensure stable energy intake and macronutrient composition (50% carbohydrates, 35% fat, and 15% protein). Volunteers were then admitted to the CRU 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, at 0630 h we started a continuous infusion of [U-^{13}C]palmitate (Cambridge Isotope Laboratories, Andover, MA) at rates of 0.6–1.2 nmol · kg fat-free mass (FFM)$^{-1}$ · min^{-1} (postprandially) and 2–4 nmol · kg FFM^{-1} · min^{-1} (during walking). After 30 min for isotopic equilibration, blood samples were collected to measure plasma palmitate turnover.

In the postprandial protocol, at 0620 h, the participants began consuming small portions of a fat-free "smoothie" (fat-free frozen yogurt, skim milk,

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Beneprotein [Nestlé Nutrition, Florham Park, NJ], Polycose [Abbott Nutrition, Columbus, OH], and frozen unsweetened strawberries) at 20-min intervals until 0900 h. Overall, the smoothie portions covered 15% of each individual's daily resting energy expenditure and provided 30% of energy as protein and 70% as carbohydrate. The goal of this feeding paradigm was to have a relatively continuous nutrient intake to allow steady state of FFA kinetics. The lack of dietary fat avoided the entry of chylomicron-derived fatty acids into the plasma FFA pool. The volunteers rested throughout this protocol.

In the walking protocol, the volunteers remained fasting and began walking on the treadmill at ~2 mph at 0700 h. They continued walking until 0815 h. Everything else was timed identically with the postprandial protocol.

In both protocols, ~60 μCi intravenous bolus of [1-^{14}C]palmitate or ~200 μCi [9,10-^{3}H]palmitate (NEN Life Science Products; PerkinElmer, Boston, MA) was given at 0800 h. Abdominal and femoral subcutaneous fat 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-TGs to accumulate in adipose tissue via VLDL (4). Participants were dismissed from the CRU after completion of the study.

Body composition measurements. Total and regional fat masses were assessed with dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI). Leg fat mass was considered LBSQ fat. Visceral fat mass was estimated using a combination of single-slice CT (L_2–L_3 interspace) and dual-energy X-ray absorptiometry–measured abdominal fat (17). Total body fat minus visceral fat mass and LBSQ fat mass was UBSQ fat mass.

Adipose tissue biopsies and tissue handling. Subcutaneous adipose tissue biopsies were collected from the abdominal and femoral regions as previously described (3,4).

Assays

Measurement of adipocyte size and adipose tissue lipid SA. Adipocyte size (18) and adipocyte lipid SA (disintegrations per minute per gram lipid) (4) were assessed after digestion of adipose tissue with collagenase and separation of adipocytes as previously described.

Plasma palmitate concentration and SA. Aliquots of the radioactive palmitate infusates were counted to assess the exact amount of radiotracer administered. Plasma palmitate SA was measured using high-performance liquid chromatography (19). All other metabolic parameters were measured as previously described (20). Plasma palmitate concentration and [U-^{13}C]palmitate enrichment, and infusate enrichment and concentration were measured using a liquid chromatography/mass spectrometry method (21).

Measurement of adipose tissue storage factors. We obtained sufficient tissue from a subset of participants to measure adipose tissue CD36 content and ACS and DGAT activities. Approximately 250 mg of flash-frozen adipose tissue was homogenized in 2 mL of standard homogenization buffer (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 255 mM sucrose) with antiprotease 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 milligram lipid.

CD36 protein content. We used a sandwich ELISA to measure adipose tissue CD36 content as previously described (5,22).

ACS activity. We measured the conversion of [^{3}H]palmitate to its CoA derivative using a modification of the method of Hall et al. (23) as previously described (5).

DGAT activity. We used the method of Coleman (24) modified to use the cytosolic fraction (25) as previously described (5).

Calculations. Systemic palmitate turnover was calculated by dividing the [U-^{13}C]palmitate infusion rate by steady-state plasma [U-^{13}C]palmitate enrichment. At steady state, the rate of appearance (Ra; μmol/min) and rate of disappearance (Rd) of palmitate are equal (Ra = Rd = turnover rate). The regional adipocyte lipid SA (dpm/g lipid) was used to calculate the fraction of injected radiolabeled palmitate stored per kilogram adipose tissue lipid (fat). The abdominal biopsy was considered representative of the UBSQ depot, whereas the femoral fat biopsy was considered representative of the LBSQ depot. Palmitate storage rates into each fat depot (μmol · kg fat^{-1} · min^{-1}) were measured as the product of steady-state palmitate Rd (μmol/min^{-1}) and the corresponding fraction of injected radiotracer stored per kilogram fat in each depot (% tracer · kg fat^{-1}).

Different means of data expression can have a significant impact on data interpretation. When the goal is to compare FFA storage efficiencies between depots as a way to understand whether one depot is more likely to gain fat than another, expressing the data per unit fat mass is most likely the preferred way. When attempting to understand the regulatory factors for the functioning unit, the adipocyte, expressing the data per adipocyte is probably preferred. Likewise, in the current study, 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 FFAs than another depot. When examining the regulation of fatty acid storage to understand which storage factors may play a critical role within a depot and a condition, we focused on the per 1,000 adipocytes expression.

Statistics. Data are represented as means ± SD for normally distributed data or median with interquartile intervals (25th–75th) for nonnormally distributed data. Nonnormally distributed data were logarithmically transformed to ensure normal distribution. Statistical analyses were performed using unpaired and paired Student *t* test for sex and depot comparisons, respectively. *P* values of <0.05 were considered statistically significant. The Pearson test was used to assess bivariate relationships. Multiple linear regression analysis was used to assess independent predictors of regional palmitate storage rates within a depot and condition.

We recently reported palmitate storage rates into subcutaneous fat depots in the postabsorptive state (5). The measurements of palmitate storage rates and adipose tissue storage factors in the postprandial and walking (current manuscript), as well as in the postabsorptive, condition (5) were performed concurrently using identical methods in age- and BMI-matched individuals. Therefore, we assessed whether palmitate storage rates and storage factors vary between the postabsorptive, postprandial, and walking condition. This was done using ANOVA followed by Tukey post hoc test, when ANOVA revealed statistically significant effects.

RESULTS

Subject characteristics. Table 1 provides the volunteer characteristics. Women and men in the postprandial and walking protocols were matched for age and BMI. We observed the expected sex differences in body fatness. Specifically, women had a significantly greater percentage of body fat, total fat mass, and subcutaneous fat masses than men. In both sex groups and in both protocols, femoral adipocytes were significantly larger than abdominal adipocytes. By design, energy expenditure was approximately threefold greater in the walking than postprandial state. The postprandial protocol resulted in the expected suppression of plasma palmitate concentration (average ~34 μmol/L) compared with usual postabsorptive concentrations (~90 μmol/L) (5,26). In contrast, the walking protocol resulted in the expected increase in plasma palmitate concentrations (average ~140 μmol/L). In the postprandial protocol, women had a significantly lower palmitate concentration than men, whereas in the walking protocol, palmitate concentrations were significantly greater in women (Table 1). Adipose tissue lipolysis rates (as represented by plasma palmitate turnover) did not differ between sexes in the postprandial protocol; they were significantly greater in women than men in the walking protocol. Insulin concentrations averaged ~19 and ~5 μIU/mL in the postprandial and walking conditions, respectively.

Regional palmitate storage rates into subcutaneous adipose tissue

Postprandial protocol. Plasma palmitate storage rates in both depots were significantly greater in women than men, whether expressed per kilogram fat or per 1,000 adipocytes (Table 2). The percentage of palmitate tracer stored in UBSQ or LBSQ fat at 30 min after the tracer bolus was significantly greater in women than in men (Table 2). In both sexes, palmitate storage rates per kilogram fat as well as the percentage of tracer stored were significantly greater in UBSQ than LBSQ fat.

Plasma palmitate concentration did not predict palmitate storage rates per kilogram fat independently of sex (effect of palmitate concentration: UBSQ, $P = 0.17$; LBSQ, $P = 0.12$). In men, but not women, LBSQ palmitate storage rates decreased as a function of LBSQ fat mass in a nonlinear fashion ($r = -0.57$, $P = 0.021$) (Fig. 1). However, UBSQ fat mass did not significantly correlate with palmitate storage rates per kilogram UBSQ fat in either group (women: $r = 0.12$, $P = 0.59$; men: $r = 0.33$, $P = 0.19$) (Fig. 1).

TABLE 1
Anthropometrics and fat distribution of study participants

	Postprandial protocol			Walking protocol		
	Women $n = 24$	Men $n = 17$	P	Women $n = 24$	Men $n = 16$	P
Age (years)	35 ± 8	32 ± 10	*	32 ± 8	30 ± 9	*
BMI (kg/m^2)	29.7 ± 5.6	27.6 ± 5.5	*	28.6 ± 5.3	26.9 ± 5.2	*
Wt (kg)	79.8 ± 14.8	88.2 ± 15.1	0.08	76.6 ± 15.6	86.5 ± 19.6	0.09
Total body fat (%)	41.1 ± 7.5	25.5 ± 9.2	<0.0001	36.9 ± 8.4	23.4 ± 8.5	<0.0001
Total body fat (kg)	33.1 ± 10.5	23.2 ± 11.6	0.009	30.7 ± 11.6	21.1 ± 11.6	0.013
UBSQ fat (kg)	18.0 ± 6.3	12.3 ± 6.7	0.009	16.9 ± 6.7	10.9 ± 6.6	0.008
LBSQ fat (kg)	12.8 ± 4.0	7.9 ± 3.2	0.0001	11.8 ± 4.6	7.3 ± 3.4	0.001
Visceral fat (kg)	2.3 ± 1.8	3.3 ± 2.2	0.27	2.0 ± 1.2	2.8 ± 2.1	0.18
Abdominal adipocyte size (μg lipid/cell)	0.61 ± 0.25	0.50 ± 0.24	0.18	0.61 ± 0.25	0.45 ± 0.22	0.035
Femoral adipocyte size (μg lipid/cell)	0.77 ± 0.18†	0.65 ± 0.31†	0.17	0.80 ± 0.26†	0.64 ± 0.30†	0.11
Plasma palmitate (μmol/L)§	26 (18–34)	35 (27–45)	0.049	164 (134–175)	125 (112–150)	0.004
Plasma palmitate turnover (μmol/min)§	55 (38–77)	68 (42–80)	0.39	247 (212–289)	213 (152–239)	0.03
Energy expenditure (kcal/min)	1.1 ± 0.2	1.4 ± 0.2	0.0004	3.1 ± 0.6	3.9 ± 0.7	0.001
Glucose (mg/dL)	107 ± 10	115 ± 13	0.06	96 ± 8	95 ± 5	0.72
Insulin (μIU/mL)	18 ± 14	19 ± 15	0.87	6 ± 3	4 ± 2	0.016
Growth hormone (ng/mL)	1.1 ± 1.8	0.2 ± 0.4	0.03	4.0 ± 3.3	1.1 ± 1.6	0.007
Epinephrine (pg/mL)	21 ± 8	33 ± 14	0.003	32 ± 16	34 ± 21	0.71

Values are means ± SD for normally distributed data or medians (25th–75th quantiles) for nonnormally distributed data. *Selected to be similar, not subject to statistical testing. †$P < 0.01$ between depots within sex. §Statistics performed on log-transformed data.

Walking protocol. Plasma palmitate storage rates per kilogram UBSQ fat did not differ between sexes, whereas storage rates per kilogram LBSQ fat were significantly greater in women than men (Table 2). In women, palmitate storage rates per kilogram fat were significantly greater in LBSQ than UBSQ depot, but did not significantly differ between the two fat depots in men.

As in the postprandial protocol, plasma palmitate concentration did not significantly predict palmitate storage rates per kilogram fat independently of sex (effect of palmitate concentration: UBSQ, $P = 0.12$; LBSQ, $P = 0.27$). Neither UBSQ fat mass (women: $r = 0.33$, $P = 0.11$; men: $r = 0.33$, $P = 0.20$) nor LBSQ fat mass (women: $r = 0.07$, $P = 0.73$; men: $r = 0.26$, $P = 0.32$) were correlated with palmitate storage rates in the corresponding depots.

Women stored a greater percentage of palmitate tracer in their UBSQ and LBSQ depots compared with men (Table 2). Fractional storage in adipose tissue during walking was much lower than during the postprandial state, particularly in women. The percentage of palmitate tracer stored did not differ significantly between UBSQ and LBSQ depots in either sex (Table 2).

Regional CD36 content and ACS and DGAT activity. Average values of CD36 content and ACS and DGAT activities for the participants from whom we were able to collect adequate adipose tissue are provided in Table 3 (postprandial protocol) and Table 4 (walking protocol).

Postprandial protocol. No significant sex differences were observed in the three fatty acid storage factors in either depot regardless of the unit of expression (per milligram lipid or per 1,000 adipocytes) (Table 3). In women, CD36 content was significantly greater in the LBSQ than UBSQ depot (per milligram lipid and per 1,000 adipocytes). In both sexes, ACS activity was significantly greater, whereas DGAT activity was significantly lower in LBSQ than UBSQ fat (per 1,000 adipocytes).

Walking protocol. As in the postprandial protocol, no significant sex differences were observed in the three FFA storage factors in either depot (Table 4). However, significant depot differences were observed. CD36 content was significantly greater in the LBSQ than UBSQ fat in both sexes (per milligram lipid and per 1,000 adipocytes). ACS activity was significantly greater in the LBSQ than UBSQ depot in women (per milligram lipid and per 1,000 adipocytes) and men (per 1,000 adipocytes). In contrast, DGAT activity was significantly lower in LBSQ than UBSQ fat in men (per milligram lipid).

Relationship between regional fatty acid storage factors and regional palmitate storage rates. When expressed per milligram lipid, no significant correlations were observed between regional storage factors and palmitate storage rates in either depot, the only exception being a weak relationship between ACS activity and palmitate storage rates in UBSQ fat in women during walking ($r = 0.49$, $P = 0.043$).

Postprandial protocol. Palmitate storage rates per 1,000 UBSQ adipocytes were significantly and positively correlated with CD36 content in women ($r = 0.52$, $P = 0.033$) and DGAT activity in men ($r = 0.68$, $P = 0.045$) (Fig. 2). Palmitate storage rates per 1,000 LBSQ adipocytes were significantly and positively correlated with CD36 content (women: $r = 0.45$, $P = 0.044$; men: $r = 0.63$, $P = 0.016$) and ACS activity (men: $r = 0.62$, $P = 0.023$) (Fig. 2).

Walking protocol. Palmitate storage rates per 1,000 UBSQ adipocytes were significantly and positively correlated with ACS activity (women: $r = 0.50$, $P = 0.049$; men: $r = 0.57$, $P = 0.041$) and DGAT activity (men: $r = 0.52$, $P = 0.037$) (Fig. 3). Palmitate storage rates per 1,000 LBSQ adipocytes were significantly and positively correlated with DGAT activity in men ($r = 0.73$, $P = 0.0040$) (Fig. 3).

Independent predictors of regional palmitate storage rates per 1,000 adipocytes

Postprandial protocol. When sex, CD36, and DGAT activity were included in a multivariate regression analysis, sex ($P < 0.0001$) was the only significant independent predictor of palmitate storage in UBSQ adipocytes. In LBSQ adipocytes, sex, CD36 content, and ACS activity were included in the analysis. Sex ($P = 0.001$) and CD36

TABLE 2
Palmitate tracer fractional storage and palmitate storage rates in subcutaneous adipose tissue depots

	Postprandial protocol			Walking protocol		
Palmitate storage	Women $n = 23$	Men $n = 17$	P	Women $n = 24$	Men $n = 16$	P
Storage in UBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.382 (0.305–0.653)	0.193 (0.106–0.282)	0.007	0.245 (0.186–0.477)	0.215 (0.159–0.271)	0.211
Storage in LBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.349 (0.227–0.416)*	0.160 (0.116–0.219)*	0.005	0.363 (0.261–0.457)*	0.194 (0.140–0.270)	0.011
Storage in UBSQ fat (pmol · 1,000 adipocytes^{-1} · min^{-1})	0.236 (0.164–0.383)	0.086 (0.060–0.188)	0.002	0.177 (0.116–0.244)	0.074 (0.050–0.136)	0.010
Storage in LBSQ fat (pmol · 1,000 adipocytes^{-1} · min^{-1})	0.232 (0.156–0.342)	0.086 (0.071–0.146)	0.0004	0.303 (0.248–0.433)†	0.157 (0.084–0.214)*	0.003
Palmitate tracer stored in UBSQ fat (%)	14.1 (9.8–19.2)	3.6 (2.2–6.7)	<0.0001	1.8 (1.1–2.3)	1.0 (0.6–1.3)	0.002
Palmitate tracer stored in LBSQ fat (%)	6.2 (4.8–10.0)†	2.3 (1.2–3.4)*	<0.0001	1.8 (1.1–2.4)	0.7 (0.5–1.2)	0.0002

Values are medians (25th–75th quantiles). Percent of palmitate tracer stored was measured at 30 min after the tracer bolus. Statistics performed on log-transformed data. *$P < 0.05$ vs. UBSQ fat within sex. †$P < 0.0001$ vs. UBSQ fat within sex.

content ($P = 0.047$) remained significant, independent predictors of LBSQ palmitate storage.

Walking protocol. In UBSQ adipocytes, neither sex nor any of the three fatty acid handling factors predicted palmitate storage rates. In LBSQ adipocytes, sex ($P = 0.013$) and DGAT activity ($P = 0.004$) predicted palmitate storage rates.

Comparison of palmitate storage rates and storage factors among the postabsorptive, postprandial, and walking conditions. Overall, palmitate storage rates per kilogram UBSQ, LBSQ, and whole-body subcutaneous (WBSQ) fat were not significantly different among the three states in either women or men (Table 5). The only exception was a significantly greater storage rate into UBSQ fat between the postprandial and walking condition in women (Table 5). When expressed per milligram lipid, ACS activity in men (both depots) and DGAT activity in both sexes (both depots) were significantly greater in the walking than the postabsorptive state (Supplementary Table 1). In addition, DGAT activity in men (both depots) was significantly greater in the walking than the postprandial state. Because fat cell size did not differ between the three states, the comparisons of palmitate storage rates and storage factors between the three conditions were similar when expressed per 1,000 adipocytes, as with the per unit fat mass expression (data not shown).

DISCUSSION

We measured circulating FFA storage rates into UBSQ and LBSQ fat under postprandial and physical activity conditions. Unexpectedly, the absolute rates of palmitate storage per kilogram fat were relatively similar between postabsorptive, postprandial, and walking conditions (Table 5). This was surprising because the postprandial state favors fat storage, whereas the postabsorptive and walking conditions do not. We also found that both sexes preferentially stored FFAs in UBSQ relative to LBSQ fat in the postprandial state, whereas the walking condition favored LBSQ storage in women only. Lastly, in LBSQ adipocytes, CD36 content independently predicted postprandial palmitate storage, whereas DGAT activity independently predicted palmitate storage during walking.

Using arterio-venous balance/isotopic tracer methodology, Bickerton et al. (7) found FFA uptake rates into abdominal subcutaneous fat in men in the early and midpostprandial period of 82–183 nmol · 100 g fat^{-1} · min^{-1} (corresponding to ~0.21–0.46 μmol palmitate · kg fat^{-1} · min^{-1}). These values agree with our measures of palmitate storage in UBSQ fat in men postprandially (~0.33 μmol palmitate · kg fat^{-1} · min^{-1}). Although the absolute rates of palmitate storage were comparable between the postabsorptive, postprandial, and walking conditions, the relative importance of subcutaneous fat as a storage site for plasma palmitate differed remarkably between the three states. Approximately 4 and 8% of circulating FFAs were stored in WBSQ fat in postabsorptive men and women, respectively (4). Corresponding values were ~7 and ~24% in the postprandial state and ~2 and ~4% in the walking condition (Table 2). The absolute rates of FFA storage per kilogram fat remained relatively stable among the three states mainly due to "counterbalancing" variations in systemic FFA turnover. The only exception was in women who had greater storage rates in UBSQ in the postprandial than the walking condition.

Postprandially, both sexes had greater palmitate storage rates in the UBSQ than LBSQ depot. This observation

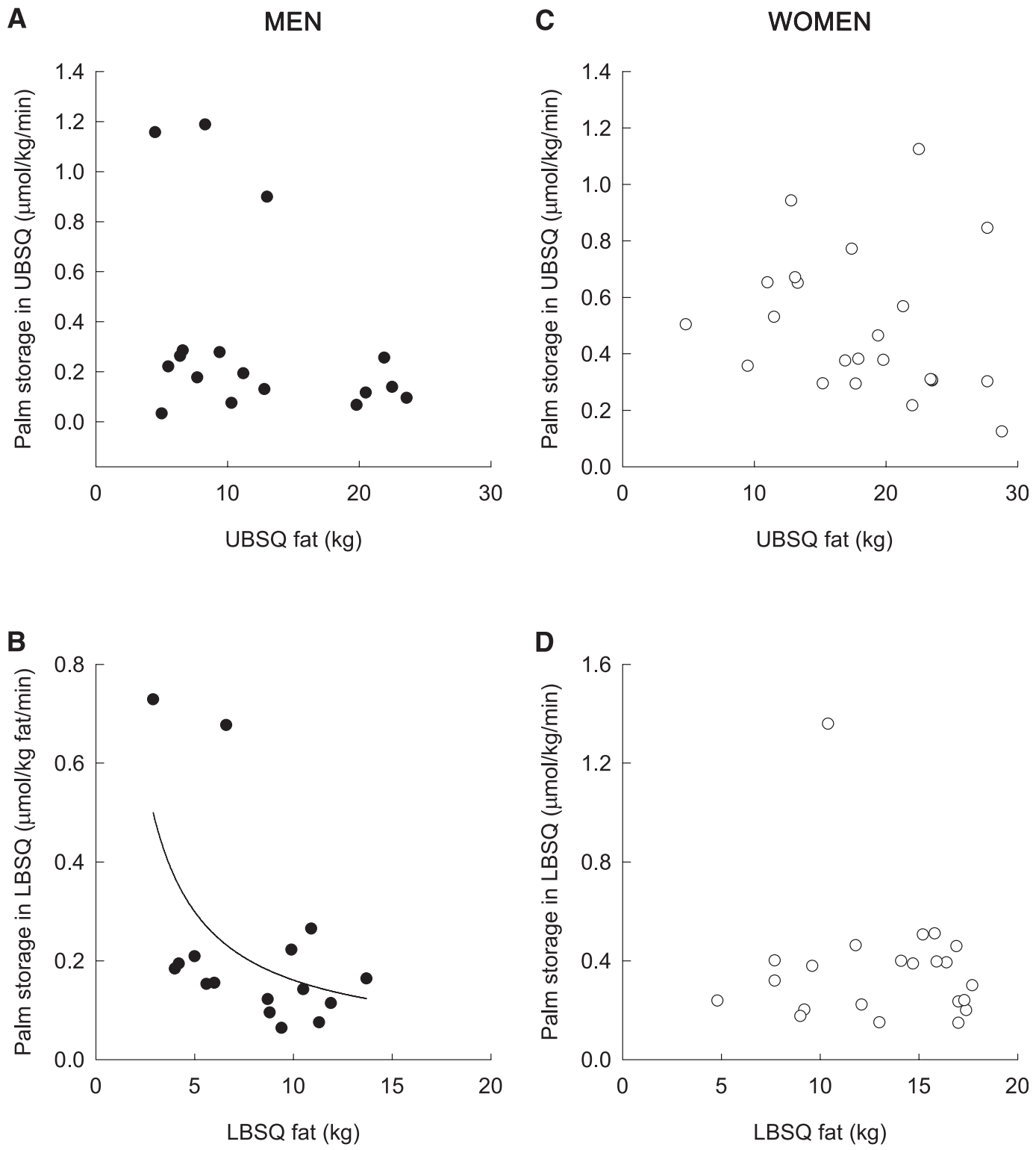

FIG. 1. Relationship between palmitate (palm) storage (μmol · kg fat^{-1} · min^{-1}) in UBSQ fat and UBSQ fat mass in men (*A*), between palmitate storage in LBSQ fat and LBSQ fat mass in men (*B*), between palmitate storage in UBSQ fat and UBSQ fat mass in women (*C*), and between palmitate storage in LBSQ fat and LBSQ fat mass in women (*D*) in the postprandial protocol.

differs from our recent findings in the postabsorptive state, where women showed preferential FFA storage in LBSQ fat, whereas men exhibited preferential storage in UBSQ fat (5). The preferential postprandial accumulation of circulating FFAs in the UBSQ depot is in line with the regional pattern of uptake/storage of dietary fat (16). Although the feeding protocol in the current study was fat free, we have previously shown that both sexes exhibited preferential storage of palmitate tracer in the UBSQ depot, when test meals containing average or high amounts of fat were ingested (27). Thus, regardless of the presence/absence of dietary fat, plasma FFAs are preferentially stored in the

TABLE 3
Regional ACS and DGAT activities and CD36 content in subcutaneous adipose tissue in the postprandial protocol

	Women		Men	
	UBSQ fat	LBSQ fat	UBSQ fat	LBSQ fat
CD36 (relative units · mg lipid^{-1})	14 (13–16) (n = 17)	19 (17–23) (n = 22)*	15 (13–15) (n = 12)	15 (11–21) (n = 15)
ACS (pmol · mg lipid^{-1} · min^{-1})	66 (46–80) (n = 19)	72 (54–90) (n = 23)	42 (40–75) (n = 11)	61 (52–74) (n = 14)
DGAT (pmol · mg lipid^{-1} · min^{-1})	6.1 (4.4–8.9) (n = 18)	5.5 (4.2–7.6) (n = 23)	5.3 (5.0–6.3) (n = 9)	4.9 (3.6–5.5) (n = 14)
CD36 (relative units · 1,000 adipocytes^{-1})	8 (6–10) (n = 17)	15 (12–17) (n = 22)*	8 (4–10) (n = 12)	7 (5–22) (n = 15)
ACS (pmol · 1,000 adipocytes^{-1} · min^{-1})	37 (24–40) (n = 19)	57 (40–69) (n = 23)*	27 (21–38) (n = 11)	41 (26–60) (n = 14)*
DGAT (pmol · 1,000 adipocytes^{-1} · min^{-1})	3.4 (2.7–4.8) (n = 18)	2.5 (1.9–3.5) (n = 23)*	3.5 (3.0–4.5) (n = 9)	2.2 (1.7–2.6) (n = 14)*

Values are medians (25th–75th quantiles). Statistics were performed on log-transformed data. *$P < 0.05$ between depots within sex.

UBSQ fat depot postprandially. The recent study by McQuaid et al. (8) reported no regional difference in postprandial FFA uptake between abdominal and femoral subcutaneous fat. The conflicting results between McQuaid et al. (8) and our study may be due to the different study populations and/or methodologies used. Because the present quantitative findings are consistent with our previous qualitative findings (27), we suggest that the postprandial regional FFA storage patterns are similar between sexes (favoring upper-body fat accumulation) and, thus, do not contribute to the sex-specific regulation of body fat distribution. Of interest, we estimated postprandial regional lipolysis rates per kilogram fat using group mean averages (28) and found UBSQ rates were two to three times those of LBSQ rates in both men and women.

In contrast to the postprandial state, palmitate storage rates per kilogram fat during walking were 40% greater in the LBSQ than UBSQ depot in women. In addition, LBSQ palmitate storage rates were significantly greater in women than men. We previously reported that during exercise, LBSQ FFA release rates per kilogram fat were approximately one-third less in women than men (P = NS) (29). No regional differences in palmitate storage rates during walking were observed in men. Thus, both physical activity and the resting, postabsorptive state (5) favor FFA storage in the LBSQ region in women. Collectively, our findings indicate that FFA storage in subcutaneous fat exhibits regional- and sex-specific patterns. Specifically, under postabsorptive and walking conditions, the FFA storage pathway favors the sex differences in body fat distribution, whereas under postprandial conditions, it does not.

Although significant univariate correlations were observed between certain storage factors and regional palmitate storage rates (Figs. 2 and 3), in LBSQ adipocytes, CD36 content independently predicted postprandial palmitate storage, whereas DGAT activity independently predicted palmitate storage during walking. We hypothesize that, at times of low circulating FFA concentrations, LBSQ adipocyte FFA storage is largely dependent upon the availability of proteins that facilitate inward fatty acid transport (such as CD36). In contrast, during high FFA concentrations, such as during walking, fatty acid entry into the adipocyte is less dependent on transport proteins and exceeds the capacity of DGAT to esterify fatty acids into triglycerides via DGAT, rendering DGAT a possible limiting step in this process.

With the exception of palmitate storage in UBSQ fat during walking, women consistently had greater palmitate storage rates per kilogram fat than men. Therefore, as with dietary fat (6,30), women store greater amounts of fatty

TABLE 4
Regional ACS and DGAT activities and CD36 content in subcutaneous adipose tissue in the walking protocol

	Women		Men	
	UBSQ fat	LBSQ fat	UBSQ fat	LBSQ fat
CD36 (relative units · mg lipid^{-1})	12 (10–19) (n = 16)	15 (12–20) (n = 20)*	16 (13–21) (n = 12)	19 (16–26) (n = 12)*
ACS (pmol · mg lipid^{-1} · min^{-1})	65 (49–80) (n = 17)	79 (57–91) (n = 22)*	89 (61–117) (n = 14)	80 (51–102) (n = 14)
DGAT (pmol · mg lipid^{-1} · min^{-1})	7.0 (5.5–8.0) (n = 20)	6.2 (4.2–7.7) (n = 23)	8.1 (5.6–12.2) (n = 17)	6.1 (4.7–9.6) (n = 16)*
CD36 (relative units · 1,000 adipocytes^{-1})	8 (5–14) (n = 15)	13 (9–18) (n = 16)*	7 (6–8) (n = 12)	12 (9–18) (n = 11)*
ACS (pmol · 1,000 adipocytes^{-1} · min^{-1})	44 (29–60) (n = 16)	61 (41–70) (n = 19)*	38 (24–45) (n = 13)	39 (29–76) (n = 13)*
DGAT (pmol · 1,000 adipocytes^{-1} · min^{-1})	4.7 (3.2–7.3) (n = 20)	5.2 (3.5–6.0) (n = 19)	3.6 (2.5–4.7) (n = 16)	3.5 (2.3–5.7) (n = 15)

Values are medians (25th–75th quantiles). Statistics were performed on log-transformed data. *$P < 0.05$ between depots within sex.

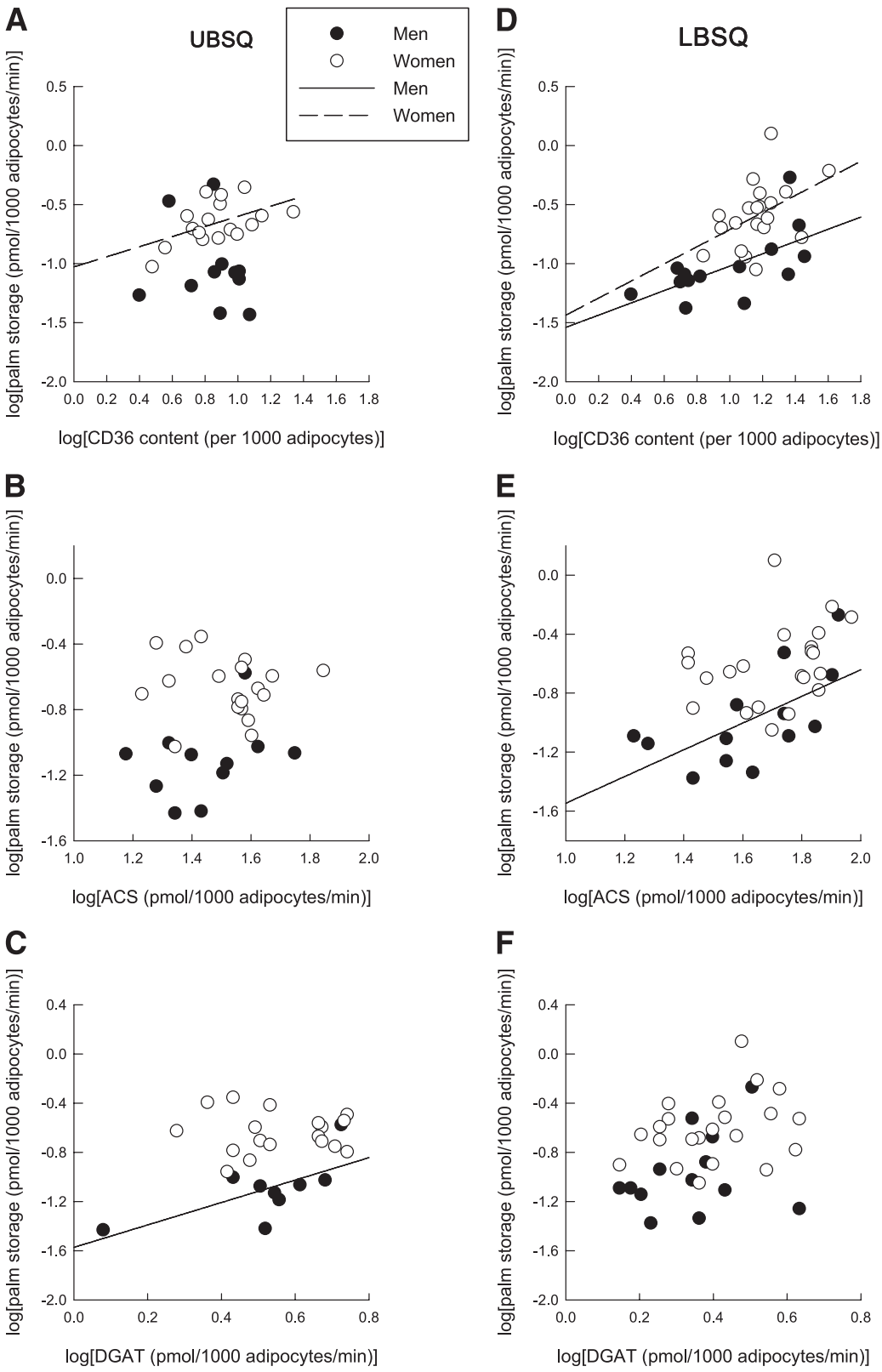

FIG. 2. Relationship between postprandial palmitate (palm) storage in UBSQ fat (μmol · 1,000 adipocytes^{-1} · min^{-1}) and abdominal CD36 content (units · 1,000 adipocytes^{-1} · min^{-1}) (*A*), abdominal ACS activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*B*), and abdominal DGAT activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*C*), and between palmitate storage in LBSQ fat (μmol · 1,000 adipocytes^{-1} · min^{-1}) and femoral CD36 content (units · 1,000 adipocytes^{-1} · min^{-1}) (*D*), femoral ACS activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*E*), and femoral DGAT activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*F*) in men and women. Log-transformed values were used to achieve normal distribution.

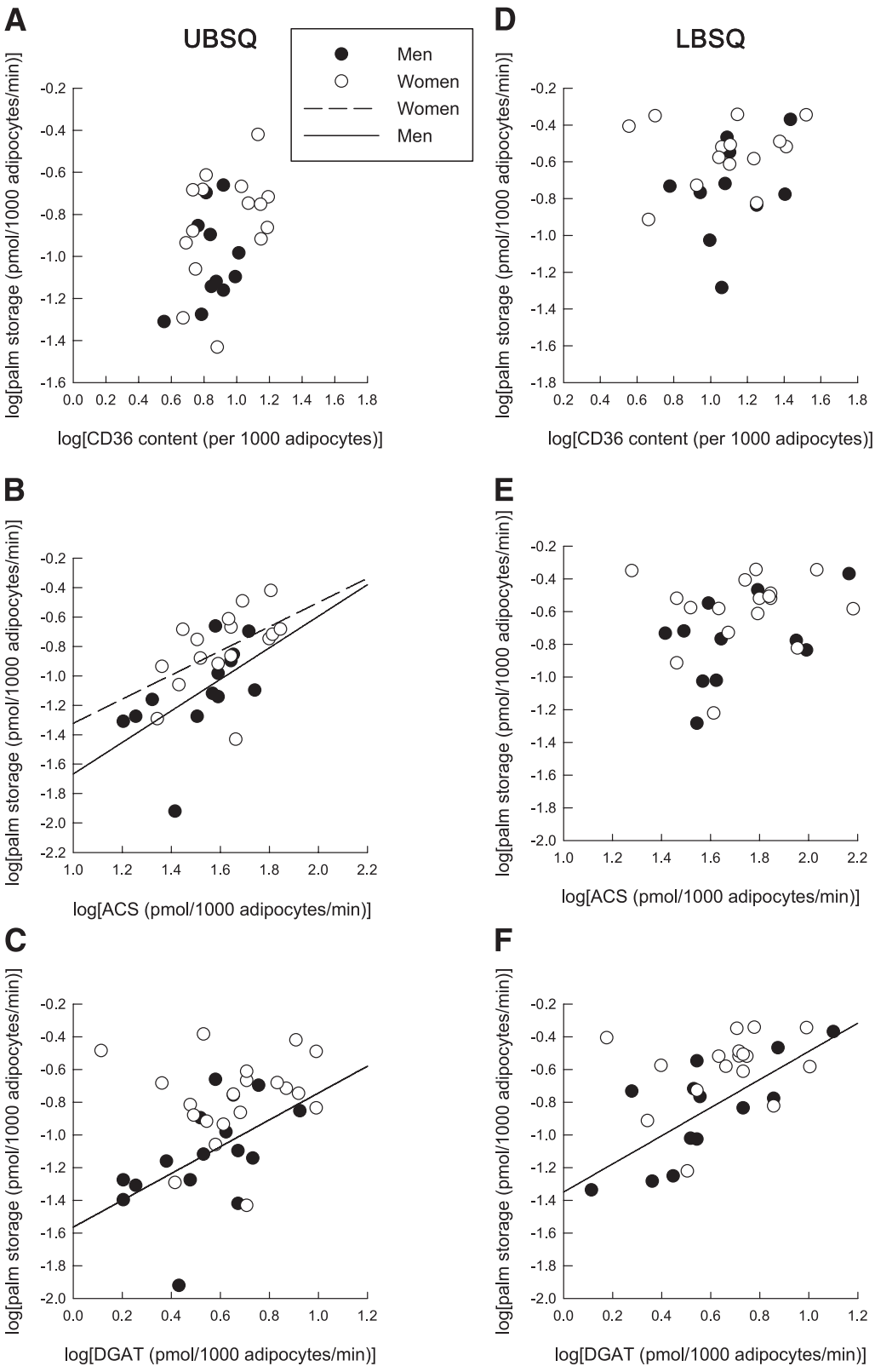

FIG. 3. Relationship between palmitate (palm) storage during walking in UBSQ fat (μmol · 1,000 adipocytes^{-1} · min^{-1}) and abdominal CD36 content (units · 1,000 adipocytes^{-1} · min^{-1}) (*A*), abdominal ACS activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*B*), and abdominal DGAT activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*C*), and between palmitate storage in LBSQ fat (μmol · 1,000 adipocytes^{-1} · min^{-1}) and femoral CD36 content (units · 1,000 adipocytes^{-1} · min^{-1}) (*D*), femoral ACS activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*E*), and femoral DGAT activity (pmol · 1,000 adipocytes^{-1} · min^{-1}) (*F*) in men and women. Log values were used to achieve normal distribution.

TABLE 5
Palmitate storage rates in subcutaneous adipose tissue in postabsorptive, postprandial, and walking conditions

	Postabsorptive	Postprandial	Walking	ANOVA, P
Women				
Palmitate storage in UBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.322 (0.254–0.469) (n = 46)	0.382 (0.305–0.653) (n = 23)	0.245 (0.186–0.477) (n = 24)*	0.0016
Palmitate storage in LBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.405 (0.256–0.529) (n = 49)	0.349 (0.227–0.416) (n = 22)	0.363 (0.261–0.457) (n = 24)	0.30
Palmitate storage in WBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.718 (0.550–1.056) (n = 46)	0.787 (0.606–1.006) (n = 22)	0.589 (0.465–0.943) (n = 23)	0.21
Men				
Palmitate storage in UBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.211 (0.148–0.374) (n = 25)	0.193 (0.106–0.282) (n = 17)	0.222 (0.169–0.342) (n = 16)	0.72
Palmitate storage in LBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.206 (0.112–0.287) (n = 25)	0.160 (0.116–0.219) (n = 16)	0.233 (0.154–0.454) (n = 16)	0.62
Palmitate storage in WBSQ fat (μmol · kg fat^{-1} · min^{-1})	0.420 (0.275–0.620) (n = 24)	0.374 (0.237–0.514) (n = 16)	0.433 (0.348–0.900) (n = 15)	0.99

Values are medians (25th–75th quantiles). Statistics were performed on log-transformed data. Data in the postabsorptive state are from a previously published article (5). They are presented here for comparison. *$P < 0.05$ vs. postprandial state by Tukey post hoc test.

acids in subcutaneous fat than men. No significant sex differences were observed in any of the storage factors that we examined (Table 3). This suggests that other factors account for the male/female differences found in direct FFA storage. From the proteins that have been consistently implicated in the transmembrane transport of FFAs (CD36, FABP(pm) [plasma membrane-associated fatty acid-binding protein], and FATP1 [fatty acid transport protein 1]), we only assessed CD36. Although there are limited data on the role of FABP(pm) in adipocytes (31), FATP1 has been shown to play a major role in insulin-stimulated fatty acid uptake (11,32). Perhaps, sex differences in the translocation of adipocyte FATP1 to the plasma membrane after meals account for the male/female differences in postprandial FFA storage.

None of the fatty acid storage factors that we assessed independently predicted palmitate storage in UBSQ fat. Thus, although women exhibited greater palmitate storage in UBSQ fat than men in the postprandial protocol, none of the three FFA storage factors were implicated in that effect. Similarly, the greater ACS and/or DGAT activities that we observed in the walking versus the postabsorptive condition (Supplementary Table 1) did not translate into greater palmitate storage rates during walking. This suggests that the direct FFA storage pathway may not be regulated between different nutritional states merely through changes in FFA storage factor quantities/activities. However, the cross-sectional design of our study does not allow us to make firm conclusions. Understanding the role of fatty acid storage factors in adipocyte FFA storage in different nutritional states could allow us to further refine our understanding of how different body fat distributions develop in vivo and, thereby, develop strategies to prevent or reduce unhealthy fat deposition.

Finally, we investigated whether obesity downregulates FFA storage in subcutaneous fat during eating or walking, as it does for postabsorptive lipolysis and meal fat storage (13–16). Because regional palmitate storage per kilogram fat was generally similar in individuals with lesser and greater regional adiposity in postprandial (Fig. 1 *A*, *C*, and *D*) and walking conditions, the FFA storage pathway allows obese individuals to clear more FFAs into WBSQ fat than lean people. The only exception was a reduction in postprandial palmitate storage as LBSQ fat increased in men (Fig. 1). This pattern matches the reduction in dietary fat storage in LBSQ fat that we have previously observed in men (33), suggesting an overall diminished capacity for fatty acid storage in this depot postprandially, as adiposity increases.

A limitation of the current study is the use of palmitate as fatty acid tracer to assess direct FFA storage in adipose tissue. Palmitate was used because it comprises one-fourth of circulating fatty acids and it is a reliable tracer for systemic lipolysis (34). Although the validity of using palmitate as an index of total plasma FFA storage has not been systematically examined, we note that the postprandial patterns we report here are very similar to those obtained using an oleate FFA tracer (27). Another limitation is that the present design provides only a single snapshot of the direct FFA storage pathway. Long-term intervention studies involving weight gain/loss would help provide a more complete understanding of the role of direct FFA storage as it relates to body fat distribution.

In summary, WBSQ fat stored circulating palmitate at relatively constant rates in the postabsorptive, postprandial, and walking states. This is despite the fact that the relative contribution of subcutaneous fat as a site of FFA storage varied dramatically from one state to the other. The postprandial condition favored FFA storage in the UBSQ region in both sexes, whereas walking, similar to the postabsorptive state (5), favored FFA storage in LBSQ fat in women. The present findings extend our observations in the postabsorptive state (5) and reinforce the suggestion that the FFA storage pathway offers a way of protecting the body from excessive amounts of circulating FFAs in obesity, especially in women.

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