ORIGINAL ARTICLE

Systemic Free Fatty Acid Disposal Into Very Low-Density Lipoprotein Triglycerides

Christina Koutsari,[1] Manpreet S. Mundi,[1] Asem H. Ali,[1] Bruce W. Patterson,[2] and Michael D. Jensen[1]

We measured the incorporation of systemic free fatty acids (FFA) into circulating very low-density lipoprotein triglycerides (VLDL-TGs) under postabsorptive, postprandial, and walking conditions in humans. Fifty-five men and 85 premenopausal women with BMI 18–24 (lean) and 27–36 kg/m^2 (overweight/obese) received an intravenous bolus injection of [1,1,2,3,3-$^2H_5$]glycerol (to measure VLDL-TG kinetics) and either [1-^{14}C]palmitate or [9,10-^{3}H] palmitate to determine the proportion of systemic FFA that is converted to VLDL-TG. Experiments started at 0630 h after a 12-h overnight fast. In the postabsorptive protocol, participants rested and remained fasted until 1330 h. In the postprandial protocol, volunteers ingested frequent portions of a fat-free smoothie. In the walking protocol, participants walked on a treadmill for 5.5 h at ~3× resting energy expenditure. Approximately 7% of circulating FFA was converted into VLDL-TG. VLDL-TG secretion rates (SRs) were not statistically different among protocols. Visceral fat mass was the only independent predictor of VLDL-TG secretion, explaining 33–57% of the variance. The small proportion of systemic FFA that is converted to VLDL-TG can confound the expected relationship between plasma FFA concentration and VLDL-TG SRs. Regulation of VLDL-TG secretion is complex in that, despite a broad spectrum of physiological FFA concentrations, VLDL-TG SRs did not vary based on different acute substrate availability. ***Diabetes* 62:2386–2395, 2013**

The splanchnic region is a major site of plasma free fatty acid (FFA) removal from the systemic circulation, accounting for 30–45% of systemic FFA uptake in the postabsorptive state (1,2). Hepatic uptake of FFA accounts for ~80% of the uptake of the entire splanchnic region, making liver a central organ for systemic FFA disposal (3). In healthy postabsorptive adults, the majority (65–95%) of secreted very low-density lipoprotein triglyceride (VLDL-TG) fatty acids are derived from plasma FFA (1,4–6). Postprandially, systemic FFA remains the major fatty acid source, accounting for 45–55% of VLDL-TG fatty acids (7,8). Because of the quantitative importance of liver in plasma FFA uptake and the quantitative importance of plasma FFA for VLDL-TG production, an intuitive expectation may be that shunting of plasma FFA into VLDL-TG is a major pathway of plasma FFA disposal in humans.

Few studies have specifically assessed what proportion of circulating FFA is converted to VLDL-TG. Small studies in the postabsorptive state have found that ~4–7% (9,10) and ~6% (1,11,12) of systemic FFA enters the VLDL-TG pathway in women and men, respectively. In postprandial participants consuming fat-free meals, ~15% of plasma FFAs were directed toward VLDL-TG secretion (13,14). There is no information regarding other conditions typically encountered in normal human physiology, such as during physical activity. Furthermore, it is unknown whether adiposity and sex affect the proportion of systemic FFA that is directed toward VLDL-TG production.

There is conflicting information regarding whether obesity and sex affect VLDL-TG secretion rates (SR). Previous studies reported that adiposity does not relate to plasma VLDL-TG SRs (1,15,16), whereas other reports showed that greater adiposity is associated with greater VLDL-TG SRs (14–20). Similarly, some studies have found no sex differences in VLDL-TG SRs (19,21,22), whereas other studies reported greater VLDL-TG secretion in nonobese women than in nonobese men, and lower VLDL-TG secretion in obese women than in obese men (15,23,24). Furthermore, although VLDL-TG production is clearly a substrate-driven process when plasma FFA concentrations are experimentally altered (25), some cross-sectional studies have not found a relationship between plasma FFA concentration or turnover and VLDL-TG SRs (14,19,21). Because most cross-sectional studies have included a relatively small number of volunteers, it has been challenging to reliably assess independent predictors of VLDL-TG SRs. Understanding the interplay between adipose tissue lipolysis and FFA conversion into VLDL-TG will increase our understanding of FFA metabolism in health and disease.

The goals of the current study were to determine the quantitative importance of VLDL-TG as a pathway of systemic FFA disposal in lean and obese individuals under postabsorptive, postprandial, and walking conditions, to provide a comprehensive evaluation of VLDL-TG kinetics under different physiological conditions, and to identify independent predictors of VLDL-TG SRs.

From the [1]Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota; and the [2]Center for Human Nutrition, Washington University School of Medicine, St. Louis, Missouri.
Corresponding author: Michael D. Jensen, jensen.michael@mayo.edu.
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RESEARCH DESIGN AND METHODS

Participants. After approval from the Mayo Clinic Institutional Review Board, a total of 55 men and 85 premenopausal women gave informed written consent to participate in the study. Volunteers had a BMI of 18–24 (lean) or 27–36 kg/m^2 (overweight/obese) and were healthy nonsmokers who were weight-stable for at least 2 months before the study and were using no medications known to influence lipid metabolism. The majority of volunteers participated in one protocol, whereas ~13% of volunteers participated in two protocols. Volunteers in the three protocols and within each sex were matched for age and BMI. Data related to adipose tissue fatty acid storage and plasma FFA oxidation in some of these volunteers have been previously published (26–28).

Experimental Design

Procedures common to all protocols. Volunteers received a standardized weight-maintenance diet for 5 days before the study. They were then admitted to the Clinical Research Unit (CRU) at 1700 h and given a standardized meal at 1800 h. The next day (study day 1), at 0630 h a continuous infusion of [U-^{13}C]palmitate was started (Cambridge Isotope Laboratories, Andover, MA) bounded to human albumin at 0.6–1.2 nmol·kg fat-free mass (FFM)$^{-1}$·min^{-1} (postprandial protocol) and 2–4 nmol·kg FFM^{-1}·min^{-1} (resting postabsorptive and walking protocols) until 1230 h. After 30 min for isotopic equilibration, arterialized blood samples were collected to measure plasma palmitate kinetics.

At 0730 h, an intravenous bolus of [1,1,2,3,3-$^{2}H_5$]glycerol (50 μmol/kg; Cambridge Isotope Laboratories) was administered. At 0800 h, participants received an intravenous bolus of either [1-^{14}C]palmitate (~20 μCi or ~60 μCi) or [9,10-^{3}H]palmitate (~75 μCi or ~200 μCi) bound to human albumin. The bolus injections of [1,1,2,3,3-$^{2}H_5$]glycerol and palmitate radiotracer were separated by 30 min to allow for some of the blood sample time points to coincide. The variation in the dose of each palmitate radiotracer was attributable to the fact that the initial low doses were not sufficient for studying the disposal of FFA into skeletal muscle (unpublished data). All radioactive tracers were purchased from NEN Life Science Products (PerkinElmer, Boston, MA).

Arterialized blood samples were collected for measurement of plasma free glycerol and VLDL-TG glycerol tracer-to-tracee ratio (TTR), as well as VLDL-TG fatty acid (VLDL-TGFA) specific activity (SA) (10).

Study protocols. In the postabsorptive protocol, volunteers remained fasted until 1330 h on day 1. They received a fat-free lunch at 1330 h (70% of energy requirements). The volunteers rested throughout this protocol until the last blood draw at 1730 h. Resting energy expenditure (REE) in the postabsorptive condition was 1,522 ± 218 kcal/day for women and 1,897 ± 314 kcal/day for men as determined with indirect calorimetry.

In the postprandial protocol, at 0620 h on day 1, the participants began consuming small portions of a fat-free smoothie (fat-free frozen yogurt, skim milk, Beneprotein [Nestlé Nutrition], Polycose [Abbott Nutrition], and frozen unsweetened strawberries) at 20-min intervals until 1720 h. Overall, the smoothie portions (30% of energy as protein, 70% as carbohydrate) provided 70% of each individual's daily resting energy requirements. The goal of this feeding paradigm was to have a relatively continuous nutrient intake to allow steady-state FFA kinetics. Volunteers rested throughout the study. Energy expenditure (EE) in the postprandial state (average for 0700–1230 h) was 1,571 ± 238 kcal/day for women and 1,966 ± 305 kcal/day for men.

In the walking protocol, volunteers began walking on the treadmill at ~2 mph at 0700 h on day 1. They continued walking until 1230 h. By design, EE was approximately three fold greater in the walking state than in the postabsorptive state. Volunteers remained fasted until 1330, and they received a fat-free lunch at 1330 h. The last blood sample was drawn at 1730 h. EE during walking (average for 0700–1230 h) was 4,678 ± 659 kcal/day for women and 5,567 ± 987 kcal/day for men.

Body composition measurements. Total and regional fat masses were assessed as previously described (10).

Assays

Plasma palmitate concentration and SA. Plasma palmitate SA (29), plasma palmitate concentration and enrichment at steady state, and [U-^{13}C] palmitate infusate concentration and enrichment were measured as previously described (30). All other metabolic parameters were measured as previously described (31).

Plasma VLDL-TG concentrations and SA and TTR of plasma and VLDL-TG glycerol. VLDL were isolated (10) and VLDL-TG concentrations were corrected for free glycerol (32). VLDL-TG lipid radioactivity (dpm/μmol TG), plasma glycerol, and VLDL-TG glycerol TTR were determined as previously described (10).

Calculations. The fractional catabolic rate (FCR) of VLDL-TG (pools/h) was determined by compartmental modeling using a bolus of [1,1,2,3,3-$^{2}H_5$]glycerol (33). This single VLDL compartment model incorporates the plasma [1,1,2,3,3-$^{2}H_5$]glycerol TTR time course as measured for each volunteer, thus accounting for all sources of systemic tracer recycling, and resolves VLDL-TG turnover kinetics from tracer recycling of [1,1,2,3,3-$^{2}H_5$]glycerol through hepatic or splanchnic lipid stores. VLDL-TG FCR was used for the calculation of VLDL-TG SR as follows:

$$\text{VLDL-TG SR } (\mu\text{mol/min}) = (\text{VLDL-TG FCR } \times \text{Pool}_{\text{VLDL-TG}})/60$$

$$\text{Pool}_{\text{VLDL-TG}} = C_{\text{VLDL-TG}} \times \text{PV} \times 1.4$$

where $\text{Pool}_{\text{VLDL-TG}}$ is the VLDL-TG pool size, $C_{\text{VLDL-TG}}$ is the average concentration of VLDL-TG glycerol (μmol/L) over the 9.5-h time course, and PV is the plasma volume estimated assuming 0.055 L/kg FFM. The 1.4 correction factor is based on published data of the difference between measured and estimated $\text{Pool}_{\text{VLDL-TG}}$ (34). Because we used the time-averaged VLDL-TG concentration, the VLDL-TG SR reported corresponds to the mean SR over the time course.

To determine the amount (dpm) of ^{14}C or ^{3}H palmitate that traversed the VLDL-TGFA pool, the area under the VLDL-TGFA SA compared with the time curve (dpm×min×μmol^{-1} VLDL-TGFA) was multiplied by the VLDL-TGFA SR (μmol/min). By dividing this product (dpm that traversed the VLDL-TGFA pool) by the dose (dpm) of ^{14}C or ^{3}H palmitate administered, the fraction of palmitate tracer disposal into VLDL-TGFA was assessed.

Systemic palmitate turnover (rate of appearance = rate of disappearance) was calculated by dividing the [U-^{13}C]palmitate infusion rate by the steady-state plasma [U-^{13}C]palmitate enrichment throughout the infusion after the steady state was achieved at 60 min. The rate at which plasma palmitate appeared in VLDL-TGFA was calculated by multiplying the fraction of plasma palmitate that traversed the VLDL-TG pool by the rate of disappearance of palmitate.

Homeostasis model assessment of insulin resistance was calculated as fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L) ÷ 22.5.

Statistics. Values are expressed as means ± SD. Statistical comparisons between groups were performed using two-factor ANOVA to assess the main effects of protocol, obesity, and their interaction. Tukey test was used for post hoc analysis. Repeated-measures ANOVA was used to assess the effect of time on plasma VLDL-TG and palmitate concentrations. The Pearson test was used to assess bivariate relationships. $P < 0.05$ was considered statistically significant.

Stepwise multivariate analyses were performed to evaluate the independent contributions of body composition and other clinical variables to the variance in the VLDL-TG SR and the percent of systemic palmitate that was converted to VLDL-TG. We accepted variables with a variance inflation factor of <10 into the initial model and $P < 0.15$ in univariate regressions. The variables that entered the final model had a significance level of $P < 0.05$. Each of the stepwise analyses was performed in forward, backward, and mixed directions. The results that were consistent among the three directions are presented. Statistical analyses were performed with JMP 9.0.1 (SAS Institute, Cary, NC) and Statistica 7.0 (StatSoft, Tusla, OK).

RESULTS

Volunteer characteristics. Tables 1 and 2 provide the clinical characteristics of the women and men, respectively, who participated in the study protocols. Volunteers were well-matched for age and BMI across the three protocols. For all studies together, participant average age was ~35 years old. We observed the expected differences in body composition and metabolic characteristics between lean and obese individuals. Namely, overweight or obese women (Table 1) and overweight or obese men (Table 2) had significantly greater body fatness, FFM, homeostasis model assessment of insulin resistance, and plasma TG concentrations than the lean individuals across all three protocols. No significant differences were observed in any of these variables among protocols. Overall, in both sexes, insulin concentrations were significantly greater in the overweight or obese than in the lean participants (ANOVA $P < 0.0001$ in women; ANOVA $P = 0.01$ in men).

As expected, insulin concentrations were significantly greater in the postprandial protocol than in the postabsorptive resting and walking protocols (all post hoc $P < 0.0001$ in both sexes).

Plasma palmitate concentration, kinetics, and conversion to plasma VLDL-TG. Figure 1 depicts the decay in plasma palmitate SA after the [1-^{14}C]palmitate or [9,10-^{3}H]palmitate intravenous bolus. SA was maximum at 1 min after the bolus and decreased to ~1% of maximum by 30 min.

In both sexes, plasma palmitate concentrations were suppressed during the postprandial protocol compared with the resting postabsorptive concentrations (post hoc $P < 0.0001$ in both sexes) (Tables 1 and 2 and Fig. 2). The

TABLE 1
Clinical characteristics, plasma palmitate, and VLDL-TG kinetics of the women who participated in the studies

	Women								
	Postabsorptive		Postprandial		Walking		ANOVA P		
	Lean (n = 12)	OW/Obese (n = 25)	Lean (n = 8)	OW/Obese (n = 16)	Lean (n = 8)	OW/Obese (n = 16)	Protocol	Obesity	Interaction
Age (years)	35 ± 9	36 ± 8	35 ± 7	35 ± 9	30 ± 8	33 ± 8	*	*	*
BMI (kg/m^2)	23.0 ± 1.3	31.7 ± 2.9	23.0 ± 1.6	33.0 ± 3.4	22.0 ± 1.6	31.8 ± 2.9	*	*	*
Weight (kg)	64 ± 6	85 ± 9	63 ± 6	88 ± 10	59 ± 4	86 ± 10	0.35	<0.0001	0.42
Body fat (%)	31 ± 4	45 ± 5	32 ± 5	46 ± 3	28 ± 3	45 ± 3	0.13	<0.0001	0.39
UBSQ fat (kg)	10.8 ± 1.8	19.8 ± 2.8	11.0 ± 2.8	21.5 ± 4.1	8.6 ± 1.4	21.0 ± 3.6	0.45	<0.0001	0.36
Visceral fat (kg)	1.0 ± 0.7	3.1 ± 1.9	1.2 ± 0.6	2.9 ± 1.9	0.8 ± 0.2	2.6 ± 1.1	0.73	<0.0001	0.87
FFM (kg)	44 ± 5	46 ± 6	42 ± 3	47 ± 6	41 ± 3	47 ± 5	0.80	<0.0001	0.48
HOMA-IR	0.7 ± 0.3	1.6 ± 0.7	1.2 ± 0.5	1.9 ± 0.9	0.9 ± 0.4	1.9 ± 1.0	0.24	<0.0001	0.80
Glucose (mmol/L)†	4.72 ± 0.28	4.94 ± 0.33	5.00 ± 0.17	5.27 ± 0.39	4.66 ± 0.44	5.44 ± 0.39	0.02	<0.0001	0.02
Plasma TG (μmol/L)†	502 ± 92	897 ± 377	797 ± 346	851 ± 441	829 ± 217	951 ± 380	0.12	0.02	0.20
Insulin (pmol/L)‡	26 ± 11	49 ± 20	96 ± 50	141 ± 90	28 ± 8	46 ± 24	<0.0001¶	0.01	0.55
Palmitate (μmol/L)‡	104 ± 22	108 ± 33	29 ± 13	32 ± 25	148 ± 36	160 ± 21	<0.0001‖	0.35	0.85
Palmitate R_a (μmol/min)	102 ± 21	123 ± 39	45 ± 20	68 ± 39	236 ± 74	255 ± 46	<0.0001‖	0.04	0.98
Tracer into VLDL-TG (%)	6 ± 3	9 ± 6	5 ± 2	8 ± 5	6 ± 3	7 ± 2	0.46	0.006	0.64
Palm R_a in VLDL-TG	6 ± 2	11 ± 8	2 ± 2	7 ± 8	12 ± 6	19 ± 6	<0.0001**	0.0005	0.86
VLDL-TG (μmol/L)§	176 ± 70	529 ± 412	332 ± 262	492 ± 330	292 ± 203	372 ± 205	0.66	0.007	0.23
VLDL-TG FCR (pools/h)	0.9 ± 0.3	1.0 ± 0.5	0.5 ± 0.2	0.5 ± 0.2	2.6 ± 3.9	1.2 ± 0.4	<0.0001‖	0.16	0.07
VLDL-TG SR (μmol/min)	8 ± 2	24 ± 13	7 ± 3	17 ± 16	16 ± 10	25 ± 10	0.07	<0.0001	0.40
VLDL-TG clearance (mL/min)	49 ± 17	55 ± 27	25 ± 9	33 ± 11	64 ± 33	72 ± 22	<0.0001**	0.17	0.98

Values are mean ± SD. Palm R_a in VLDL-TG is expressed in μmol/min. OW, overweight; HOMA-IR, homeostasis model assessment of insulin resistance; R_a, rate of appearance. *Selected to be similar, not subject to statistical testing. †Postabsorptive state. ‡Average 0700–1330 h. §Average 0700–1730 h of experiment. ¶Postabsorptive vs. postprandial ($P < 0.0001$); postprandial vs. walking ($P < 0.0001$). ‖Postabsorptive vs. postprandial ($P < 0.001$); postprandial vs. walking ($P < 0.001$); postabsorptive vs. walking ($P < 0.001$). **Postabsorptive vs. postprandial ($P < 0.05$); postprandial vs. walking ($P < 0.05$); postabsorptive vs. walking ($P < 0.05$).

walking protocol resulted in the expected increase in plasma palmitate concentrations (post hoc $P < 0.0001$ compared with resting in both sexes).

Adipose tissue lipolysis (as represented by plasma palmitate rate of appearance) was significantly greater in the overweight or obese individuals (ANOVA $P < 0.05$ in both sexes) (Tables 1 and 2). Compared with the resting postabsorptive state, postprandial lipolysis rates were ~50% lower (post hoc $P < 0.0001$ in both sexes) and lipolysis during walking was ~1.5- to 2-fold greater (post hoc $P < 0.0001$ in both sexes).

On average, ~6 and ~9% of the palmitate tracer appeared in the VLDL-TG pool 9.5 h after the tracer bolus in lean and obese women, respectively (effect of obesity $P = 0.006$) (Table 1). There were no significant differences between fasting, feeding, and walking in women (ANOVA $P = 0.46$). Similarly, in men, ~7% of the palmitate tracer was converted to VLDL-TG (Table 2). The only exception was the overweight or obese men in the postprandial protocol, in whom 15% of circulating FFA entered the VLDL-TG pool (post hoc $P \leq 0.001$ vs. all subgroups in men) (Table 2).

The rate of appearance of plasma palmitate into VLDL-TG was approximately two fold greater in overweight or obese women than in lean women (ANOVA $P = 0.0005$) (Table 1). In women, the rate of appearance of plasma palmitate into VLDL-TG was the greatest during the walking protocol, the least during the postprandial protocol, and intermediate during the resting postabsorptive protocol (all post hoc comparisons $P < 0.05$) (Table 1). In men, the rate of appearance of plasma palmitate into the VLDL-TG pool was not significantly different between lean and overweight or obese individuals (ANOVA $P = 0.11$) (Table 2). The rate of appearance was greater during the walking protocol than in either the resting postabsorptive (post hoc $P = 0.02$) or the postprandial protocol (post hoc $P = 0.01$) (Table 2).

Plasma VLDL-TG concentration and kinetics. Figure 3 depicts the average plasma VLDL-TG concentrations (μmol/L) and VLDL-TGFA SA (dpm/μmol) after the [1-^{14}C] palmitate or [9,10-^{3}H]palmitate bolus in women and men. Plasma VLDL-TG concentrations changed modestly over time during the 9.5-h observation interval (effect of time repeated measures ANOVA $P < 0.0001$ in each sex and protocol). Specifically, the average VLDL-TG concentration ranged from 430 to 510 μmol/L (women) and from 566 to 650 μmol/L (men) in the postabsorptive protocol; from 360 to 532 μmol/L (women) and from 612 to 802 μmol/L (men) in the postprandial protocol; and from 300 to 400 μmol/L (women) and from 580 to 690 μmol/L (men) in the walking protocol (Fig. 2). Because in most cases the absolute changes in VLDL-TG concentrations deviated by ± 7–14% from the mean values, we considered that steady-state VLDL-TG kinetic approaches could be used. The only exception was women in the postprandial protocol who deviated on average by ±19%. In both sexes,

TABLE 2
Clinical characteristics, plasma palmitate, and VLDL-TG kinetics of the men who participated in the studies

	Men								
	Postabsorptive		Postprandial		Walking		ANOVA P		
	Lean (n = 12)	OW/Obese (n = 9)	Lean (n = 9)	OW/Obese (n = 8)	Lean (n = 11)	OW/Obese (n = 6)	Protocol	Obesity	Interaction
Age (years)	31 ± 9	35 ± 6	31 ± 10	34 ± 9	30 ± 7	34 ± 9	*	*	*
BMI (kg/m^2)	23.2 ± 1.7	33.0 ± 2.4	23.1 ± 2.0	32.8 ± 2.7	23.4 ± 1.3	33.3 ± 2.4	*	*	*
Weight (kg)	76 ± 5	105 ± 14	77 ± 9	101 ± 7	74 ± 8	109 ± 14	0.75	<0.0001	0.39
Body fat (%)	18 ± 5	32 ± 3	18 ± 4	34 ± 6	18 ± 5	33 ± 4	0.86	<0.0001	0.82
UBSQ fat (kg)	7.3 ± 2.4	17.7 ± 4.1	7.1 ± 2.0	18.2 ± 5.0	6.8 ± 2.5	18.6 ± 4.6	0.54	<0.0001	0.67
Visceral fat (kg)	1.5 ± 0.6	5.1 ± 2.0	1.5 ± 0.8	4.8 ± 1.9	1.6 ± 0.9	5.1 ± 1.9	0.92	<0.0001	0.95
FFM (kg)	61 ± 6	69 ± 7	62 ± 8	66 ± 6	60 ± 4	71 ± 8	0.81	<0.0001	0.38
HOMA-IR	0.7 ± 0.3	2.2 ± 1.4	0.8 ± 0.3	2.5 ± 1.0	0.9 ± 0.3	2.2 ± 1.4	0.79	<0.0001	0.80
Glucose (mmol/L)†	4.94 ± 0.33	5.22 ± 0.5	5.27 ± 0.56	5.49 ± 0.50	5.11 ± 0.33	5.72 ± 0.28	0.04	0.003	0.50
Plasma TG (μmol/L)†	829 ± 318	1,400 ± 512	826 ± 466	1,120 ± 397	795 ± 264	1,361 ± 394	0.53	<0.0001	0.49
Insulin (pmol/L)‡	23 ± 8	62 ± 35	74 ± 25	200 ± 100	18 ± 3	41 ± 15	<0.0001¶	<0.0001	0.003
Palmitate (μmol/L)‡	106 ± 24	112 ± 40	33 ± 10	40 ± 15	130 ± 23	134 ± 25	<0.0001‖	0.44	0.99
Palmitate R_a (μmol/min)	126 ± 47	146 ± 53	60 ± 21	72 ± 29	187 ± 49	248 ± 49	<0.0001‖	0.01	0.23
Tracer into VLDL-TG (%)	8 ± 3	6 ± 2	7 ± 3	15 ± 7	6 ± 3	8 ± 2	<0.0001	0.12	<0.0001**
Palm R_a in VLDL-TG	11 ± 7	6 ± 5	5 ± 3	11 ± 8	12 ± 6	20 ± 8	0.001††	0.11	0.01
VLDL-TG (μmol/L)§	430 ± 233	763 ± 408	438 ± 289	972 ± 534	346 ± 286	1,042 ± 515	0.61	<0.0001	0.35
VLDL-TG FCR (pools/h)	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.9 ± 0.3	0.4 ± 0.1	0.002	0.02	0.004‡‡
VLDL-TG SR (μmol/min)	17 ± 7	31 ± 11	11 ± 3	32 ± 7	19 ± 7	37 ± 10	0.08	<0.0001	0.35
VLDL-TG clearance (mL/min)	44 ± 13	46 ± 16	28 ± 9	41 ± 24	67 ± 25	39 ± 10	0.02	0.38	0.006§§

Values are mean ± SD. Palm R_a in VLDL-TG is expressed in μmol/min. OW, overweight; HOMA-IR, homeostasis model assessment of insulin resistance; R_a, rate of appearance. *Selected to be similar, not subject to statistical testing. †Postabsorptive state. ‡Average 0700–1330 h. §Average 0700–1730 h of experiment. ¶Postabsorptive vs. postprandial ($P < 0.0001$); postprandial vs. walking ($P < 0.0001$). ‖Postabsorptive vs. postprandial ($P < 0.0001$); postprandial vs. walking ($P < 0.0001$); postabsorptive vs. walking ($P < 0.0001$). **Overweight/obese men in postprandial protocol vs. all subgroups ($P < 0.0001$). ††Postabsorptive vs. walking ($P = 0.02$); postprandial vs. walking ($P = 0.01$). ‡‡Lean men in walking protocol vs. all subgroups ($P < 0.0001$). §§Lean men: postabsorptive vs. postprandial ($P < 0.05$); postprandial vs. walking ($P < 0.05$); postabsorptive vs. walking ($P < 0.05$); overweight/obese men: no significant differences among protocols.

VLDL-TG concentrations were significantly greater in overweight or obese than in lean individuals (ANOVA $P = 0.007$ in women; $P < 0.0001$ in men), but they did not differ significantly by protocol (Tables 1 and 2).

The pattern of VLDL-TG SR mirrored the pattern of VLDL-TG concentrations. Particularly, VLDL-TG SRs were significantly greater in overweight or obese than in lean individuals, but they did not differ significantly by protocol (Tables 1 and 2).

VLDL-TG clearance (mL/min) was not significantly different between lean and overweight or obese individuals (ANOVA $P = 0.17$ in women; $P = 0.38$ in men) (Tables 1 and 2). In women, VLDL-TG clearance was the greatest during the walking protocol, the least during the postprandial protocol, and intermediate during the resting postabsorptive protocol (all post hoc $P < 0.05$) (Table 1). In men, a significant protocol by obesity interaction effect was observed ($P = 0.006$) (Table 2). Specifically, in lean men, the VLDL-TG clearance pattern was similar to that observed for women (all post hoc $P < 0.05$) (Table 2). In overweight or obese men, VLDL-TG clearance was similar among protocols ($P = 0.98$) (Table 1).

Predictors of VLDL-TG metabolism

Univariate regression analyses. We performed exploratory univariate analyses to identify significant predictors of VLDL-TG SR. The variables with the strongest correlation coefficients were BMI, upper body subcutaneous (UBSQ) fat mass, homeostasis model assessment of insulin resistance, palmitate concentration (both sexes, postabsorptive and postprandial protocols), and visceral fat mass (both sexes, all protocols) (Table 3). Predictors of the percentage of palmitate converted to VLDL-TG were BMI and percent body fat (mostly in men), UBSQ fat mass, and visceral fat mass (in both sexes) (Table 3). The correlations between visceral fat mass and VLDL-TG SR are depicted in Fig. 4.

Stepwise multivariate regression analyses. Table 4 presents the results from the stepwise multivariate regression analyses. Sex, BMI, UBSQ fat mass, visceral fat mass, plasma palmitate concentration, REE (or EE), and homeostasis model assessment of insulin resistance were included in the initial analyses. FFM did not qualify as a possible independent predictor because it was used for the calculation of VLDL-TG SR.

Visceral fat was the only independent predictor of VLDL-TG SR explaining 33% (walking protocol) and 57% (postabsorptive and postprandial protocol) of the variance. When all three conditions were analyzed together, visceral fat and plasma palmitate concentration explained 55% of the variance in VLDL-TG SRs contributing 46 and 9%, respectively. Because visceral fat was the only independent predictor of VLDL-TG secretion that we could identify, we

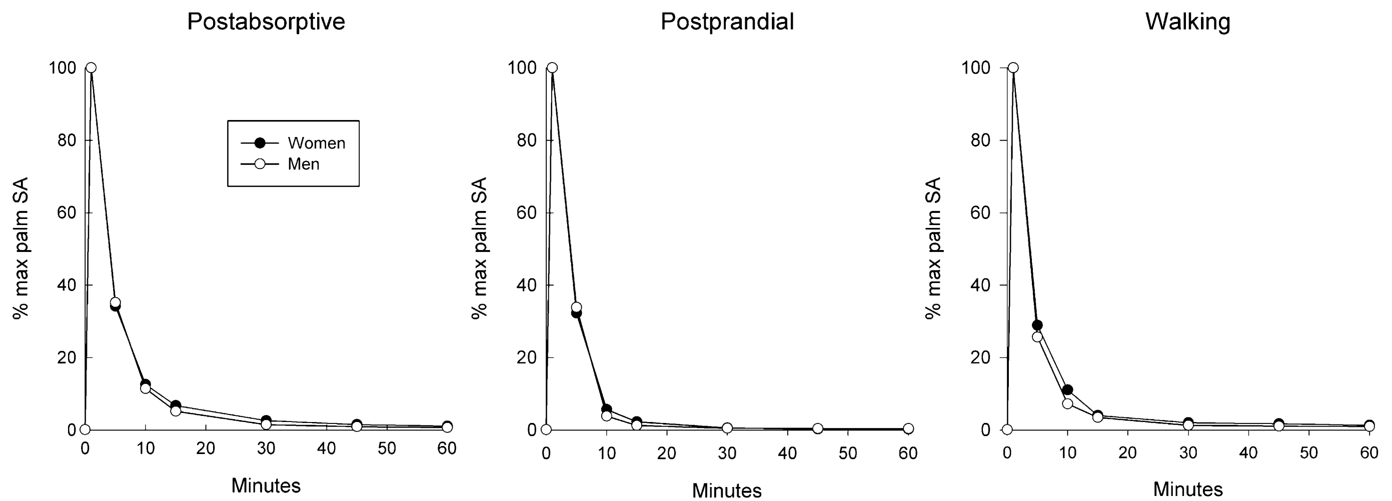

FIG. 1. The decay of plasma palmitate radioactivity (% of maximum SA) after the intravenous bolus administration of [1-^{14}C]palmitate or [9,10-^{3}H]palmitate in all women (filled circles [●]) and all men (open circles [○]) who participated in the postabsorptive (*left*), postprandial (*center*), and walking protocols (*right*). The bolus injection was administered at 0 min. All values are mean values. SD values have been omitted for the sake of clarity. Max palm, maximum palmitate.

investigated what the results would be if visceral fat was not included as a variable (Table 4). An entirely different picture appeared. Homeostasis model assessment of insulin resistance and REE emerged as significant predictors of VLDL-TG SRs in the postabsorptive protocol, palmitate concentration, BMI, and sex emerged as significant predictors of VLDL-TG SRs in the postprandial protocol, and EE emerged as a significant predictor of VLDL-TG SRs in the walking protocol.

No independent predictors were identified for the percent of palmitate that was converted to VLDL-TG in the postabsorptive and walking conditions. In the postprandial protocol, visceral fat and palmitate concentration predicted 39% of the variance, contributing 31 and 8%, respectively. When all protocols were analyzed together, visceral fat was the only independent predictor accounting for 5% of the variance.

DISCUSSION

These studies provide quantitative information on the conversion of systemic FFA (as represented by palmitate) to VLDL-TG in a large number of volunteers of both sexes, of different adiposity levels, and in different physiological states. We also provide a comprehensive assessment of plasma VLDL-TG kinetics and independent predictors. There were some novel findings in these studies. Only ~7% of circulating FFA was directed toward VLDL-TG secretion in lean or overweight or obese healthy individuals under postabsorptive, postprandial, and walking conditions. The only exception was that 15% of circulating FFAs are converted to plasma VLDL-TG in overweight or obese men in the postprandial condition. VLDL-TG SRs were not significantly different between the postabsorptive, postprandial, and walking conditions. Visceral fat mass was a strong (and the only independent) predictor of VLDL-TG SRs,

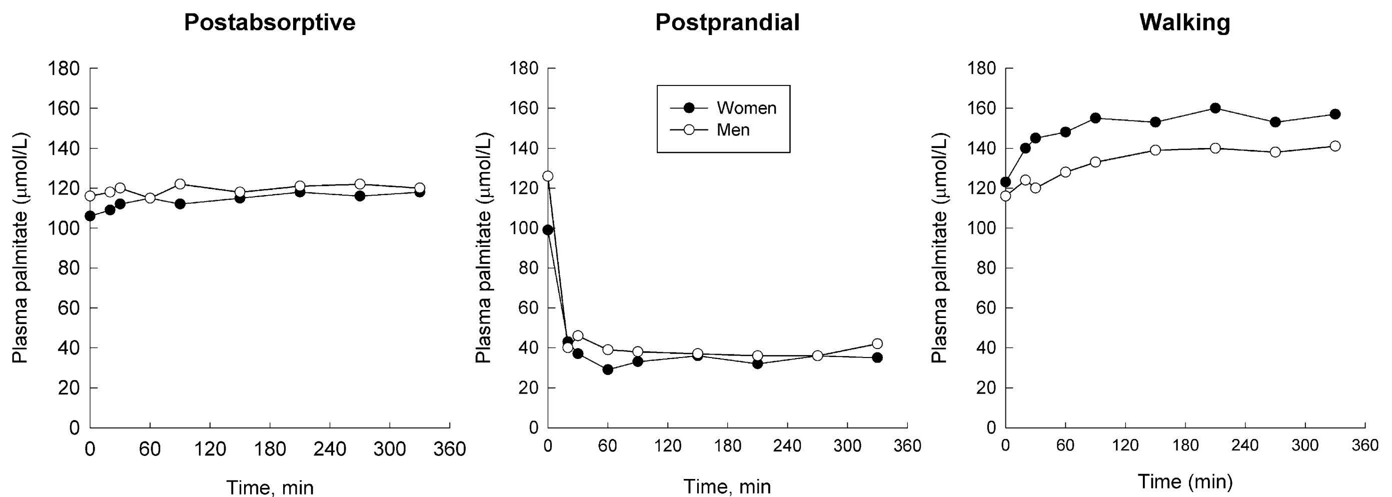

FIG. 2. The time course of plasma palmitate concentration in all women (filled circles [●]) and all men (open circles [○]) who participated in the postabsorptive (*left*), postprandial (*center*), and walking protocols (*right*). All values are mean values. SD values have been omitted for the sake of clarity. In the postprandial protocol, volunteers received small portions of a fat-free smoothie every 20 min throughout the time course of the study. In the walking protocol, volunteers walked on the treadmill from 0 min to 330 min (i.e., 0–5.5 h).

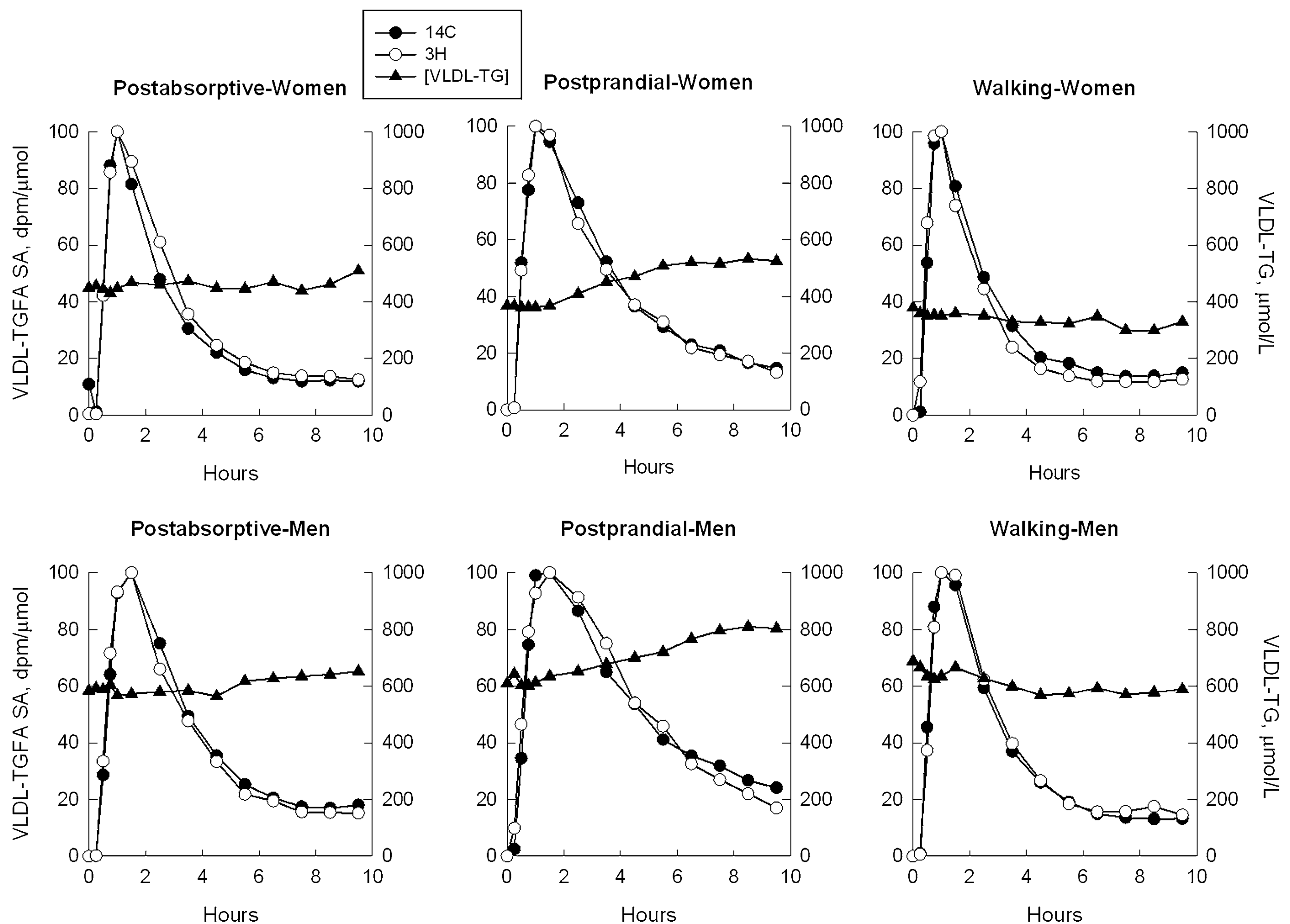

FIG. 3. The time course of the percent of peak plasma radioactivity in VLDL-TGFA after the intravenous bolus injection of [1-^{14}C]palmitate (filled circles [●], *left y*-axis) or [9,10-^{3}H]palmitate and VLDL-TG concentration (filled triangles [▲], *right y*-axis) in all women (*top panels*) and all men (*bottom panels*) who participated in the postabsorptive (*left*), postprandial (*center*), and walking protocols (*right*). The bolus was administered at 0 h. All values are mean values. SD values have been omitted for the sake of clarity. In the postprandial protocol, volunteers received small portions of a fat-free smoothie every 20 min throughout the time course of the study. In the walking protocol, volunteers walked on the treadmill from 0 to 5.5 h.

explaining 33% (walking) and 57% (postabsorptive and postprandial) of the variance.

Previous studies that included smaller numbers of participants also indicated that the VLDL-TG pathway accounts for 4–7% of plasma FFA disposal under postabsorptive conditions (1,9–12,14). The current study extends the previous findings to overweight or obese adults, indicating that an additional ~2% of FFA are directed toward VLDL-TG in women. We also found similar proportions of systemic FFA are trafficked into VLDL-TG in the postabsorptive, postprandial, and the walking states despite considerable differences in systemic FFA turnover and availability. The reason why overweight or obese men directed double the proportion of systemic FFA (~15%) toward VLDL-TG in the postprandial protocol is not apparent. It is possible that hepatic fatty acid oxidation in the overweight/obese men was reduced in the postprandial protocol, diverting plasma FFA toward hepatic esterification/VLDL-TG secretion to a much greater extent than in the other groups and protocols. Hepatic fatty acid oxidation has been shown to be upregulated in obese compared with nonobese individuals in the postabsorptive state (35); however, we are unaware of available data in the postprandial state. In addition, evidence of greater hepatic partitioning of fatty acids between oxidation and VLDL-TG production has been reported recently in postabsorptive lean women than in men (36).

Another approach for evaluating the quantitative importance of VLDL-TG as a route of nonoxidative FFA disposal would be by comparing its magnitude with that of other nonoxidative pathways, such as storage into skeletal muscle or adipose tissue. It has been estimated that ~11% and ~10% of systemic FFAs are stored in intramyocellular TGs in postabsorptive women and men, respectively (37). Storage in subcutaneous fat corresponds to 8–12% of systemic FFA turnover in postabsorptive women and to ~4% in men (26,38,39). Collectively, these observations demonstrate that, at least in the postabsorptive state, the VLDL-TG pathway is of somewhat lower quantitative importance than FFA storage in muscle and subcutaneous adipose tissue in women. In men, conversion of systemic FFA into VLDL-TG is of intermediate importance compared with FFA storage into muscle (highest importance) and storage into subcutaneous adipose tissue (lowest importance). Only ~1–2% of systemic FFA is stored into visceral adipose tissue in postabsorptive women and men (10,40).

TABLE 3
Exploratory univariate regression analyses for the prediction of VLDL-TG SRs in women and in men

Independent variables	VLDL-TG SRs (µmol/min)						% of palmitate converted into VLDL-TG					
	Postabsorptive		Postprandial		Walking		Postabsorptive		Postprandial		Walking	
	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P
BMI (kg/m^2), women	0.41	<0.0001	0.27	0.007	0.12	NS	0.04	NS	0.40	0.001	0.04	NS
BMI (kg/m^2), men	0.35	0.005	0.64	0.0001	0.37	0.009	0.37	0.003	0.20	NS	0.01	NS
Body fat (%), women	0.26	0.0013	0.09	NS	0.13	NS	0.11	0.04	0.14	NS	0.07	NS
Body fat (%), men	0.26	0.02	0.43	0.004	0.29	0.02	0.35	0.004	0.24	0.04	0.01	NS
UBSQ fat (kg), women	0.34	<0.0001	0.25	0.01	0.20	0.03	0.12	0.04	0.35	0.002	0.09	NS
UBSQ fat (kg), men	0.27	0.02	0.37	0.01	0.28	0.03	0.42	0.001	0.14	NS	0.02	NS
Visceral fat (kg), women	0.65	<0.0001	0.58	<0.0001	0.39	0.001	0.07	NS	0.66	<0.0001	0.20	0.03
Visceral fat (kg), men	0.41	0.0018	0.55	0.0006	0.30	0.02	0.31	0.04	0.16	NS	0.01	NS
HOMA-IR, women	0.50	<0.0001	0.34	0.005	0.04	NS	0.14	0.03	0.24	0.02	0.01	NS
HOMA-IR, men	0.36	0.005	0.28	0.03	0.18	NS	0.12	NS	0.10	NS	0.01	NS
REE (or EE) (kcal/day), women	0.13	0.03	0.20	0.03	0.03	NS	0.08	NS	0.25	0.01	0.03	NS
REE (or EE) (kcal/day), men	0.20	0.04	0.18	NS	0.17	NS	0.08	NS	0.03	NS	0.01	NS
Palmitate (µmol/L), women	0.30	0.0004	0.50	0.0001	0.08	NS	0.04	NS	0.35	0.003	0.03	NS
Palmitate (µmol/L), men	0.26	0.02	0.15	NS	0.14	NS	0.05	NS	0.01	NS	0.30	0.01
Insulin (pmol/L 0–330 min), women	0.44	<0.0001	0.04	NS	0.10	NS	0.23	0.003	0.15	NS	0.02	NS
Insulin (pmol/L 0–330 min), men	0.39	0.002	0.16	0.12	0.30	0.03	0.03	NS	0.05	NS	0.03	NS

EE during feeding or walking (0700–1230 h). HOMA-IR, homeostasis model assessment of insulin resistance; NS, not significant.

An interesting observation was that VLDL-TG SRs did not differ significantly among the postabsorptive, postprandial, and walking protocols. This was despite the ~50% lower systemic FFA concentrations in the postprandial state and ~50% greater FFA concentrations during walking compared with the postabsorptive resting state. Experimental changes in plasma FFA concentrations are well-known to lead to changes in VLDL-TG SRs in humans (25). However, there is also evidence from cross-sectional studies that insulin-mediated suppression of plasma FFA concentrations is a poor predictor of postabsorptive VLDL-TG SRs (19). In the present cross-sectional study, plasma palmitate concentration was a poor predictor of VLDL-TG secretion, explaining only 9% of the variance when all protocols were analyzed together. The poor or absent association between plasma FFA concentration and VLDL-TG secretion that has been observed in the present and previous cross-sectional studies (14,19,21) may reflect the fact that only a small proportion of plasma FFA crosses the VLDL-TG pool.

Insulin plays a pivotal role in controlling systemic FFA availability, but it also controls multiple steps in hepatic VLDL assembly and secretion (41). Experimental hyperinsulinemia (plasma insulin ~250–600 pmol/L) reduces VLDL-TG SRs in healthy individuals (25,42,43). In the only study that investigated the effect of meal ingestion (fat-free meal covering 40% of REE) on VLDL-TG kinetics, VLDL-TG SR was reduced by ~50% in healthy men (plasma insulin peaked at ~300 pmol/L) (44). In our postprandial protocol, plasma insulin concentrations averaged 74 (lean men) to 200 pmol/L (obese men), which is substantially lower than insulin concentrations achieved by Sondergaard et al. (44). Despite the suppression of adipose tissue lipolysis, the insulin concentrations achieved during the postprandial protocol may have been insufficient to suppress VLDL-TG secretion.

VLDL-TG SRs in the walking protocol did not differ significantly from those in the two resting protocols despite the two fold greater plasma FFA turnover and ~50% greater FFA concentration than in the postabsorptive resting protocol. There is limited and conflicting information on the effects of acute exercise on VLDL-TG kinetics. Morio et al. (45) suggested that VLDL SRs increased in response to 45 min of exercise at 40% Vo_{2max}. In contrast, Sondergaard et al. (22) found reduced VLDL-TG SRs during 90 min of exercise at 50% Vo_{2max}. Hepatic partitioning of FFA between esterification and oxidation may be particularly important during exercise, and it may depend on exercise intensity and duration. For instance, 4 h of low-intensity exercise (30% Vo_{2max}) increased splanchnic uptake of FFA by three fold compared with the resting state (46). However, rates of hepatic gluconeogenesis also increased (46), so whether the excess FFAs reaching the liver are used for providing ATP for gluconeogenesis compared with serving as a substrate for VLDL-TG synthesis is unknown.

An important strength of our study was the large number of participants, which allowed us to investigate independent predictors of VLDL-TG secretion. Visceral fat mass was a strong, and the only, independent predictor of VLDL-TG secretion in any of the individual protocols, accounting for one-third to one-half of the variance. One way that greater visceral adiposity may increase VLDL-TG SRs is through a greater proportion of hepatic FFA delivery originating from visceral adipose tissue lipolysis, which can vary from 5 to 50% as a function of visceral adiposity

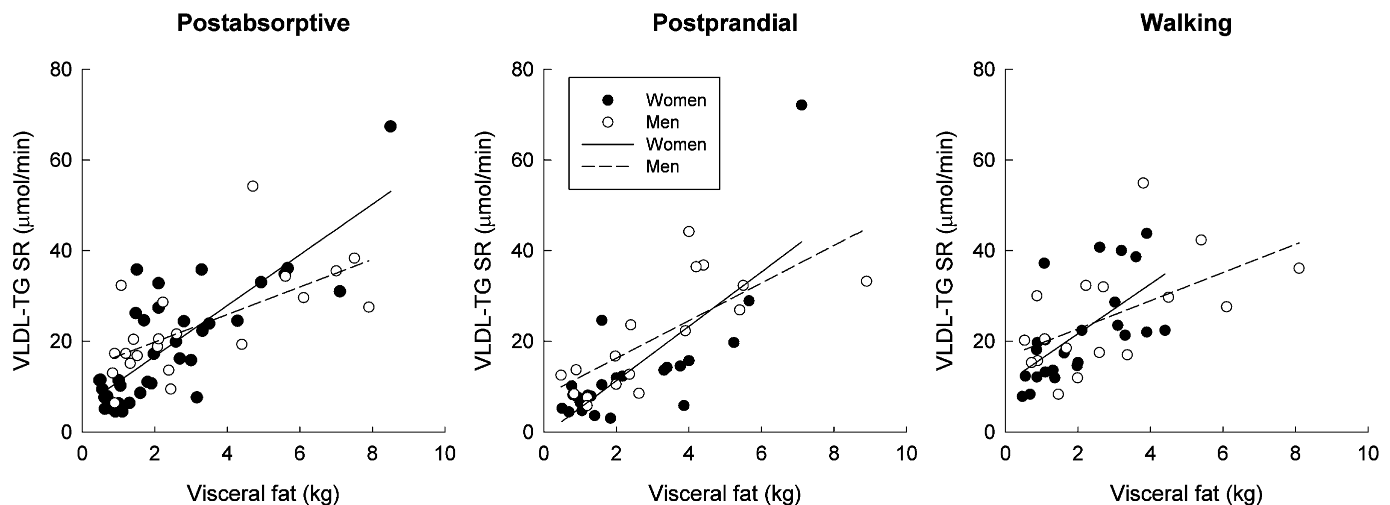

FIG. 4. Univariate correlations between visceral fat mass and VLDL-TG SR in all women (filled circles [●]) and all men (open circles [○]) who participated in the postabsorptive (*left*), postprandial (*center*), and walking protocols (*right*).

(2). In addition, greater amounts of visceral fat secrete more interleukin-6 into the portal vein (47), and interleukin-6 increases VLDL secretion (48). The combination of greater hepatic FFA and interleukin-6 delivery may be particularly important in driving VLDL-TG SRs. Visceral fat mass eliminated all other factors that have been found to be significant predictors of VLDL secretion in other studies, such as BMI (19), REE (21), homeostasis model assessment of insulin of resistance (49). However, these studies did not include measurement of visceral fat mass, which may have confounded the results. Notably, after accounting for visceral fat, sex was not an independent predictor of VLDL-TG SRs in any of the three protocols.

Some limitations of the current study should be acknowledged. First, studies using the VLDL-TG tracer dilution or the splanchnic arteriovenous balance technique tend to report greater VLDL-TG SRs compared with techniques using a VLDL-TG precursor, mathematical modeling, and calculation of the VLDL-TG pool (1,34,50). However, our VLDL-TG kinetics protocol takes into account tracer recycling, which may eliminate a factor that contributes to the discrepancy among techniques (33). Another potential limitation is the application of a steady-state VLDL-TG kinetic model to the nonsteady-state VLDL-TG concentrations during the postprandial protocol (Fig. 3). To understand the potential error that might occur from applying steady-state models to the data, we explored

TABLE 4
Stepwise multivariate regression analyses for the prediction of VLDL-TG SRs

	Including visceral fat		Excluding visceral fat	
	Adjusted R^2 ×100	P	Adjusted R^2 ×100	P
Postabsorptive (n = 58)				
Total variance in VLDL-TG SRs	58	<0.0001	45	<0.0001
Visceral fat mass (kg)	58	<0.0001		
HOMA-IR			39	<0.0001
REE (kcal/day)			6	0.03
Postprandial (n = 41)				
Total variance in VLDL-TG SRs	57	<0.0001	62	<0.0001
Visceral fat mass (kg)	57	<0.0001		
Palmitate (μmol/L)			23	<0.0001
BMI (kg/m^2)			22	<0.0001
Sex			17	0.06
Walking (n = 41)				
Total variance in VLDL-TG SRs	33	<0.0001	20	<0.0001
Visceral fat mass (kg)	33	<0.0001		
EE (during walking) (kcal/day)			20	0.01
All protocols (n = 140)				
Total variance in VLDL-TG SRs	55	<0.0001	48	<0.0001
Visceral fat mass (kg)	46	<0.0001		
Plasma palmitate (μmol/L)	9	<0.0001	15	<0.0001
BMI (kg/m^2)			21	<0.0001
Sex			10	<0.0001
HOMA-IR			2	0.05

VLDL-TG SRs presented as μmol/min. HOMA-IR, homeostasis model assessment of insulin resistance.

three nonsteady-state models that tested the fit of the observed data to changes in synthesis, secretion, or FCR of VLDL-TG. The models tested whether the increases in VLDL-TG concentration could be explained by decreases in FCR, increases in synthesis and secretion with no change in FCR, and increases in secretion with no changes in synthesis or FCR. The first two models could not adequately fit the VLDL-TG concentration and glycerol tracer time course data without systematic errors. However, the third model provided an excellent fit to both the concentration and enrichment time course data and was considered to be physiologically plausible because it is known that a sizeable fraction of VLDL–apolipoprotein B synthesized in the liver is not secreted but undergoes intracellular degradation in a manner dependent on the availability of lipid substrates (hence, some of the TG associated with apolipoprotein B is not secreted). Of note, the mean nonsteady-state SR obtained with this model differed by only ~3% from the steady-state model, which is well within the errors associated with measuring VLDL-TG turnover rates and concentrations. We therefore suggest that our results represent a realistic description of VLDL-TG kinetics in the postprandial state, although we acknowledge that our nonsteady-state model for VLDL-TG kinetics needs to be validated against other approaches for measuring VLDL-TG kinetics. The fact that we obtained similar estimates of the percentage of plasma palmitate being converted to VLDL-TG with estimates from other studies using entirely different techniques (including the arteriovenous balance technique) (1) and similar nutritional states (postabsorptive and postprandial) suggests that our estimates are most likely correct. Another limitation is that our postprandial protocol was not as "natural" as would be consuming a normal-size meal in a single setting. However, a single-meal approach would create gross nonsteady-state circumstances that would make it even more difficult to measure VLDL-TG FCR and SRs. Finally, we did not measure intrahepatic fat content, which is the best independent determinant of VLDL-TG SRs and is superior even to visceral fat in class II obese patients (average BMI, ~36 kg/m^2) (51) and type 2 diabetic patients (42). It is unknown whether intrahepatic fat is a strong and unique independent predictor of VLDL-TG SRs in healthy adults with BMI <36 kg/m^2.

In conclusion, we observed a disconnect between plasma FFA concentrations and turnover and VLDL-TG SRs in a wide range of physiological conditions. Despite major differences in FFA concentrations, VLDL-TG SRs did not vary based on different substrate availability. This observation agrees with the view of Nielsen and Karpe (52) that regulation of VLDL-TG secretion is complex in that it involves interplay between hepatic FFA delivery, hepatic fatty acid partitioning, hormonal, and nutritional factors. The small proportion of systemic FFA that is converted to VLDL-TG can confound the expected relationship between plasma FFA concentration and VLDL-TG SRs. Finally, visceral fat was a strong, and the only, independent predictor of VLDL-TG secretion identified in the current study.

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C.K. performed experiments, analyzed data, interpreted the results of experiments, prepared figures and tables, drafted the manuscript, edited and revised the manuscript, and approved the final version of the manuscript. M.S.M. and A.H.A. performed experiments, edited and revised the manuscript, and approved the final version of the manuscript. B.W.P. performed VLDL-TG modeling analyses, edited and revised the manuscript, and approved the final version of the manuscript. M.D.J. conceived and designed the research, interpreted the results of experiments, edited and revised the manuscript, and approved the final version of the manuscript. M.D.J. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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