Enhanced dietary fat clearance in postobese women

May Faraj,* Peter Jones,† Allan D. Sniderman,* and Katherine Cianflone[1],*

Mike Rosenbloom Laboratory for Cardiovascular Research* and School of Dietetics and Human Nutrition,† McGill University, Montreal, Quebec, Canada H3A 1A1

Abstract **The objective of this study was to examine the postprandial response to an exogenous fat source in eight weight-stable postobese subjects (2–3 years after gastric bypass) and eight matched control women, using a stable isotope, [^{13}C]oleate. After a high fat breakfast meal (1,062 cal, 67% fat), [^{13}C]oleate in triglyceride (TG)-rich lipoproteins (S_f >400 and S_f 20–400) and nonesterified fatty acids (NEFA), and ^{13}C in breath CO_2, were monitored over 8 h. There were no differences in resting energy expenditure, thermic effect of food, carbohydrate/fat oxidation ratio, breath $^{13}CO_2$ enrichment, or fecal fat content between postobese and control subjects. Postprandially, there was no difference in S_f 20–400 TG or NEFA, but postobese subjects had lower S_f >400 incremental area under the curve (AUC) (−33%, $P < 0.0025$) and glucose [$P < 0.01$ by repeated measures analysis of variance (RM ANOVA)]. Postprandial ^{13}C in S_f >400 TG returned to fasting levels 4 h earlier in postobese subjects and was lower than in control subjects at 4 and 6 h ($P < 0.05$ by RM ANOVA). The greatest difference was in the [^{13}C]NEFA profiles. In control subjects [^{13}C]NEFA increased markedly over 8 h; postobese subject [^{13}C]NEFA remained close to fasting nonenriched values, and was strikingly lower than in control subjects (72% lower by AUC, $P < 0.0001$ by RM ANOVA). Finally, postobese subjects tended to have lower postprandial insulin ($P < 0.01$, 4 h), lower postprandial acylation-stimulating protein, and lower fasting leptin (−46%, $P < 0.02$).** **This study demonstrates clear metabolic differences in exogenous dietary fat partitioning in postobese women. These findings are compatible with an increased efficiency of dietary fat storage and suggest one possible mechanism for promotion of weight regain in postobese individuals.**—Faraj, M., P. Jones, A. D. Sniderman, and K. Cianflone. **Enhanced dietary fat clearance in postobese women.** ***J. Lipid Res.*** **2001.** 42**: 571–580.**

Supplementary key words triglyceride • C3adesArg • stable isotope • [^{13}C]oleate • nonesterified fatty acid • insulin • leptin

Obesity, defined as a body mass index (BMI, weight divided by height squared) exceeding 30 kg/m^2, is common in Western countries and is associated with decreased longevity and increased morbidity (1). Weight loss decreases morbidity and mortality in the obese (2), but most obese individuals who lose a significant amount of weight tend to regain it (3). There are two schools of thought regarding weight regain. On the one hand, there are those who propose that genetically determined lower setpoints in basal metabolic rate (BMR), thermic effect of food (TEF), decreased fat/carbohydrate oxidation rate, or increased insulin sensitivity predispose postobese individuals to regain weight (4–8).

On the other hand, there are those who believe that obesity and regaining lost weight result, quite simply, from overeating and decreased physical activity (9, 10). In a prospective study of premenopausal obese women, mean BMR, TEF, and fasting and postprandial substrate oxidation decreased after weight loss to normal BMI. Nevertheless, the subjects were not significantly different from matched never-obese subjects (10). In other studies, neither the 24-h energy expenditure, carbohydrate/fat oxidation rate (11), TEF, nor energy expenditure during various controlled daily activities was different from those of matched never-obese subjects (12). Discrepancies among studies may be attributed to differences in body composition, meal size and composition, and length of study (13, 14).

Clearly, there are opposing beliefs regarding the causes underlying the pronounced tendency of the postobese to regain weight. We have investigated this question from the perspective of postprandial fat metabolism and three specific hormones that influence this process: acylation-stimulating protein (ASP), insulin, and leptin.

ASP is a 76-amino acid basic protein, with a mass of 8,933 Da, which has been identified in human plasma. ASP markedly increases triglyceride (TG) synthesis through enhanced fatty acid esterification and glucose transport in human adipocytes and skin fibroblasts (15–20). ASP is identical to complement C3-derived activation peptide C3adesArg, and is generated through the interaction of complement factors B and D (adipsin) with C3, all of which are synthesized and secreted by adipose tissue (21,

Abbreviations: ASP, acylation-stimulating protein; AUC, area under the curve; BMI, body mass index; BMR, basal metabolic rate; BSA, body surface area; NEFA, nonesterified fatty acids; REE, resting energy expenditure; RM ANOVA, repeated measures analysis of variance; TEF, thermic effect of food; TG, triglyceride; TRL, triglyceride-rich lipoproteins; W/H, waist-to-hip ratio.

[1] To whom correspondence should be addressed.
e-mail: mdkc@musica.mcgill.ca

22). Although circulating ASP levels in the general circulation tend to drop postprandially (23, 24), ASP production increases locally in the adipose tissue bed, with maximal production at 3–5 h (24). There is a positive correlation between venoarterial ASP difference and calculated nonesterified fatty acid (NEFA) uptake into adipose tissue (24). On average, fasting plasma ASP is elevated in obesity (females more than males), decreasing proportionally with moderate weight loss (25–27).

Circulating insulin increases postprandially and fasting plasma insulin and leptin levels increase in obesity. It has been suggested that in postobese subjects, insulin sensitivity may be modified, but the results are inconsistent. Although Ranneries and colleagues (7) showed a greater insulin sensitivity (lower insulin/glucose ratio) in postobese women as compared with matched never-obese control subjects, the same group concluded in earlier studies that no significant difference existed (4, 28).

The objective of the present study was to examine whether postobese women (2–3 years postgastric bypass, weight stable), compared with never-obese matched control subjects, demonstrate differences in postprandial fat metabolism, resting energy expenditure (REE), TEF, carbohydrate/fat oxidation rate, and metabolic hormones that have been implicated in the pathogenesis of obesity.

MATERIALS AND METHODS

Study population inclusion criteria

Subjects for the control (never-obese) and postobese groups were selected according to the following inclusion criteria: *i*) 30–50-year-old women, with regular menstruation; *ii*) BMI between 20 and 27 kg/m^2; *iii*) normal fasting plasma concentration of total plasma TG, plasma NEFA, total plasma cholesterol, plasma high density lipoprotein (HDL) cholesterol, plasma low density lipoprotein (LDL) cholesterol, plasma apolipoprotein B (apoB), serum insulin, and serum glucose as defined elsewhere (29); *iv*) no reported history of cardiovascular disease, diabetes, hypertension, or gastrointestinal, respiratory, or any hormonal disorders; *v*) not taking any medication that affects lipid metabolism within 6 months of the study; and *vi*) weight stable for 6 months before the study (no loss or gain >4.5 kg). In addition, the never-obese subjects had no reported history of obesity or weight problems, and the obese subjects had no reported postoperational gastrointestinal complications for at least 6 months before the study.

Postobese subjects were recruited through the Obesity Clinic of the Royal Victoria Hospital (Montreal, Quebec, Canada). All subjects had been morbidly obese (BMI >40) and had gastric bypass surgery 2–3 years before the study and were presently weight stable. Control subjects were recruited through posted advertisement. All subjects signed an informed consent before enrollment, and the study was approved by the Ethics Review Committee of the Royal Victoria Hospital. The control subjects were selected to match the postobese group for age, BMI, body surface area (BSA), percent body fat, waist/hip ratio, plasma TG, and habitual level of physical activity. Three postobese were smokers; the same was true of the control group.

Body fat was measured by electric conductivity through fat-free mass, and can be influenced by the hydration state of the body (30). So as not to create an artifact due to dehydration, the subjects were therefore instructed not to exercise, engage in intense cardiovascular activity, or consume alcoholic beverages for 3 days before the assessment of percent body fat.

All the subjects were between 2 and 7 days after menstruation (i.e., follicular stage). At this stage, sex hormones such as estrogen and progesterone are at their lowest levels, and energy expenditure is at its minimum (31, 32). Therefore this time period was selected to eliminate possible effects of intersubject variation in sex hormones on plasma ASP, lipid metabolism, and energy expenditure in both groups.

Study protocol

On the study day, the subjects came in at 8 AM after an overnight 12-h fast. Fasting baseline weight, height, BMI, BSA, percent body fat, fasting REE, and substrate oxidation rate were measured. Fasting blood and breath samples were collected. All the subjects were given the same meal, equivalent to approximately 60% of the daily caloric requirements. This high fat/high energy meal was selected to provide an equal acute challenge, above the normal fat and energy intake per meal of both groups, where average North American daily fat intake is 35–40% (33). The high fat breakfast meal consisted of an omelet with cheese and 35% cream, bacon, margarine, banana, peanut butter, sugar-free raspberry jam, and white bread. Total meal weight was 384 g and the homogenized volume was about 250 ml. Nutrient analysis was calculated on the basis of the manufacturer nutrient information available on the food labels combined with a nutritional software (Food Processor for Windows, version 6.0; ESHA Research, Salem, OR), with a Canadian database. The high fat meal consisted of 1,062 kcal. The macronutrient energy distribution was 12% protein (31.9 g), 21% carbohydrate (55.8 g), and 67% fat (80.0 g) that was 25% saturated, 26% monounsaturated, and 10% polyunsaturated plus 6% other sources. The high fat meal was labeled with 1.430 ml (1.280 g) of [^{13}C]oleate (99% enrichment) (Cambridge Isotopes, Xenia, OH). Oleate was chosen as the dietary fat label because it is the most abundant fatty acid in dietary fat (34), plasma TG (40.7%), and adipose tissue (46.2%) (35), and its metabolism is representative of other long-chain fatty acids (36). The [^{13}C]oleate was spread over the two slices of toast. All the subjects were instructed to eat the labeled toast slowly throughout the meal. The breakfast meal was free of simple carbohydrates and consisted of solid food only, with no beverage allowed during food intake or directly after in order to avoid potential rapid gastric emptying in the postobese group (37, 38). All the subjects were instructed to eat slowly but to be finished within a 20-min period, and to lie down on a sofa bed after food consumption. All the subjects from both groups were monitored during and after food consumption. After the meal, the subjects were sedentary to eliminate effects of physical activity on REE; thus any postprandial increase in energy expenditure could be attributed solely to the TEF.

After the initiation of food intake, postprandial blood samples, breath samples, energy expenditure, and substrate oxidation rate measurements were collected at 2, 4, 6, and 8 h, in addition to the previous fasting measurements. During the 8-h study period, the subjects were not allowed to smoke or to consume any food or beverage (except for water and one cup of decaffeinated tea or coffee with no sugar or milk added). To verify fat absorption, random fecal samples were obtained at a separate time from postobese women and analyzed for fecal fat content (acid steatocrit method).

Body fat and energy measurements

Percent body fat was determined at fasting state with bioelectric impedance analysis (Tanita, Skokie, IL). Waist circumference was measured as the horizontal circumference midway be-

tween the lowest rib margin and the iliac crest, whereas the hip circumference was measured at the point yielding the maximum circumference over the buttocks (39). The waist/hip (W/H) ratio was obtained by dividing waist circumference over hip circumference. Subject BSA was calculated on the basis of weight and height, using the DuBois approximation (40).

REE was assessed through open circuit indirect calorimetry (Deltatrac Sensor Medics, Anaheim, CA) calibrated for each subject, using a reference gas with 96% O_2 and 4% CO_2. After a 20-min rest, measurements were taken over 15 min at 0, 2, 4, 6, and 8 h. The Weir equation was used to automatically calculate REE (41). Urinary nitrogen excretion was assumed to be an average of 12 g/day (42). Substrate oxidation of carbohydrate, fat, and protein were automatically calculated by Deltatrac instrumentation and expressed as the carbohydrate/fat oxidation rate. The coefficient of variation of the 15-min measurements of REE and substrate oxidation was <6%. TEF was measured as the area under the curve (AUC) of REE with the basal level subtracted.

Blood measurements

Venous blood samples (40 ml) were collected at 0, 2, 4, 6, and 8 h. Blood was immediately centrifuged at 1,500 rpm, 4°C for 15 min and aliquots were frozen at −80°C for ASP, lipids, insulin, and glucose measurement. The remaining plasma was used immediately for the isolation of TG-rich lipoprotein (TRL) (S_f > 400 and S_f 20–400) and plasma NEFA fractions.

Plasma ASP was assayed by in-house enzyme-linked immunosorbent assay, using a monoclonal antibody as capture antibody and a polyclonal antibody as detecting antibody as described in detail elsewhere (24, 26), with a coefficient of variation of <5%. Insulin was assayed in serum samples by commercial radioimmunosorbent assay for human insulin (Medicorp, Montreal, Quebec, Canada). Leptin was measured by radioimmunoassay (Linco, St. Charles, MO). Glucose was assayed in serum samples by a commercial enzymatic colorimetric kit (Sigma, St. Louis, MO).

TRL (S_f >400 and S_f 20–400) were isolated by sequential ultracentrifugation (rotor Ti50, L8-80 ultracentrifuge; Beckman, Palo Alto, CA) at 30,000 rpm, 4°C for 30 min (S_f >400) and at 40,000 rpm, 4°C for 18 h (S_f 20–400) (43). The remaining solution (infranate) was kept for NEFA analysis. Plasma total TG, S_f >400 TG, and S_f 20–400 TG were assayed by a commercial enzymatic colorimetric kit (Roche Diagnostics, Laval, Quebec, Canada). Total plasma NEFA was measured by a commercial enzymatic colorimetric kit (Roche Diagnostics). Total cholesterol and HDL cholesterol concentrations were measured in fasting samples by a commercial enzymatic colorimetric kit (Roche Diagnostics). Plasma HDL cholesterol was separated according to Gidez et al. (44) by heparin/manganese chloride precipitation (Sigma). Plasma LDL cholesterol was calculated according to the Friedewald equation as evaluated by Schectman, Patsches, and Sasse (45). Fasting plasma apoB concentration was measured by commercial nephelometric assay.

Stable isotope measurements of breath samples

Immediately after terminating the indirect calorimetry measurements, breath samples were collected into collection bags. CO_2 was trapped by slowly bubbling into a 100-cm spiral glass trap containing 10 ml of 1 N NaOH solution (46). NaOH solutions were frozen at −80°C for later determination of ^{13}C enrichment in breath CO_2 (within 2 months). For analysis, trapped CO_2 (in NaOH solution) was released by addition of 2 ml of 85% *o*-phosphoric acid per 2 ml of NaOH solution in a Vacutainer (46). Vacutainers were directly transferred to a dual inlet stable isotope ratio mass spectrometer (IR/MS) (Vacuum Generators, Cheshire, UK) for ^{13}C enrichment analysis. In the dual inlet IR/MS system, each sample is compared with a reference standard under identical instrumental conditions. The reference limestone standard, Pee Dee Belemnite (PDB), is defined to be 0 ppm (47). The instrument was calibrated with CO_2 gas of known isotopic enrichment of $^{13}C/^{12}C = 0.0107403$ [$\delta^{13}C$ (‰) = −44.221 ± 0.296 ppm].

^{13}C enrichment was calculated according to the following formula (48):

$$\delta^{13}C(‰)_{t=i} = [(R_{S,\,t=i} - R_{PDB}) / R_{PDB}] \times 10^3$$

where

$$\delta^{13}C(‰)_{t=i} = \delta \text{ at time } = i \text{ h in parts per million}$$

$$R_{S,\,t=i} = {}^{13}C/{}^{12}C \text{ of the sample at time } = i \text{ h}$$

$$R_{PDB} = {}^{13}C/{}^{12}C \text{ of PDB } = 0.0112372$$

^{13}C enrichment in breath samples was expressed as a percentage of the administered ^{13}C dose recovered in breath CO_2 per hour, and was calculated according to Schoeller et al. (49):

$$\%\,{}^{13}C_{rec/h} = \frac{\text{mM excess } {}^{13}C/\text{mM } CO_{2,\,t=i}}{\text{mM } {}^{13}C_{administered}} \times \text{mM } CO_{2\,excreted/h} \times 1.25 \times 100$$

where

$$\text{mM excess } {}^{13}C/\text{mM } CO_{2,\,t=i} = [\delta^{13}C(‰)_{t=i} - \delta^{13}C(‰)_{t=0}]R_{PDB} \times 10^{-3}$$

$$\text{mM } {}^{13}C_{administered} = \left(\frac{\text{mg } [{}^{13}C]\text{oleate}}{M}\right)\left(\frac{P \times n}{100}\right)$$

where mg [^{13}C]oleate is the mass of administered [^{13}C]oleate (1.280 g); *M* is the molecular weight of [^{13}C]oleate (283.45); *P* is ^{13}C isotope purity (99%); *n* is the number of the labeled carbon position; mM $CO_{2\,excreted/h}$ = BSA(300 mM/m^2·h), and 1.25 is a correction factor to adjust for the uptake of label into the HCO_3 pool for the same age group (50).

Stable isotope measurement in blood samples

^{13}C enrichment was measured in plasma infranate NEFA, S_f >400 TG, and S_f 20–400 TG. Lipids (0.25–0.9 mg) were extracted with 5 volumes of chloroform–methanol 2:1 (v/v). The organic extract was evaporated under N_2 gas and redissolved in chloroform, and NEFA and TG were separated by thin-layer chromatography on LK5 silica gel plates 150Å (Whatman, Clifton, NJ), using hexane–ether–acetic acid 75:25:1 (v/v/v) as mobile phase. Lipids were stained with iodine vapor and identified by using known standards. Spots were scraped, lipids were extracted with hexane–chloroform–diethyl ether 5:2:1 (v/v/v), and samples were transferred into 18-cm combustion tubes (Vycor; Corning Glass Works, Corning, NY) and solvent evaporated. CuO (0.6 g) and 2-cm silver wire were added and tubes were flame sealed under vacuum at a pressure of less than 20 matm. TG and NEFA samples were fully combusted at 520°C for 4 h. The generated CO_2 and H_2O were separated by trapping H_2O with a −95°C methanol slurry. CO_2 was then collected in a Vacutainer submerged in a −198°C liquid N_2 bath (46). Vacutainers containing the CO_2 samples were analyzed (as described above) in duplicate for ^{13}C enrichment within 12 h. Enrichment was calculated as described above for breath samples (48). ^{13}C concentration in each of the three plasma pools (S_f <400 TG, S_f 20–400 TG, and plasma NEFA was calculated as (^{13}C enrichment) × (molar concentration of TG or NEFA in that pool).

Fecal fat measurement

Random stool samples were collected from postobese women on a separate date and analyzed for fecal fat content by the acid steatocrit method. The random acid steatocrit method is a quick and accurate way to quantitatively estimate fecal fat with a sensitivity of 100% and specificity of 95% when compared with the 72-h stool fecal fat method (51). In brief, the stool samples ($\geq$0.5 g) were diluted in water (1:3 by volume), homogenized, and then mixed with 5 N perchloric acid (pH $\leq$1). The mixture was centrifuged and the fatty and solid layers were measured. The normal range is 0–31% (51–53).

Statistical analysis

All results are expressed as means ± SEM. Fasting baseline measurements were analyzed by unpaired *t*-test. Data were analyzed by two-way repeated measures analysis of variance (two-way RM ANOVA), with all pairwise multiple comparisons (including interactions) using Bonferroni *t*-tests. Statistical analysis was performed with SigmaStat (Jandel, San Rafael, CA). When the normality or equal variance test failed, data were transformed to the trapezoid AUC, and the mean difference was tested by unpaired *t*-test. Correlation between fasting values and AUC of selected parameters was conducted by Pearson product moment correlation. Significance was set at $P < 0.05$.

RESULTS

Baseline characteristics of the postobese and control subjects are given in **Table 1**. Postobese subjects had undergone gastric bypass within the past 2–3 years and had been weight stable for at least 6 months before the study. None of the fasting plasma values measured were significantly different between the two groups except for fasting leptin, which was significantly reduced in the postobese ($P <$ 0.02). The fat load meal was well tolerated, and neither the postobese women nor the control subjects reported any feelings of dizziness, flushing, or sweating, which are symptoms associated with fast or delayed gastric emptying (37, 38).

TABLE 1. Baseline characteristics of the control and postobese women

	Control (n = 8)	Postobese (n = 8)
Age (years)	37.5 ± 1.9	40.3 ± 2.1
Weight (kg)	63.3 ± 2.2	63.6 ± 2.8
Height (cm)	166.5 ± 1.6	163.7 ± 1.5
BMI (kg/m^2)	22.8 ± 0.7	23.7 ± 0.9
BSA (m^2)	1.67 ± 0.03	1.78 ± 0.04
Waist-to-hip ratio	0.74 ± 0.01	0.78 ± 0.02
% body fat	32.6 ± 1.7	30.9 ± 2.6
FFM (kg)	42.5 ± 1.2	43.5 ± 1.2
Fat mass (kg)	20.8 ± 1.6	20.0 ± 2.4
TG (mM)	0.80 ± 0.11	0.78 ± 0.08
NEFA (mM)	0.42 ± 0.08	0.38 ± 0.05
Cholesterol (mM)	4.15 ± 0.19	3.78 ± 0.20
HDL chol (mM)	1.51 ± 0.12	1.45 ± 0.13
LDL chol (mM)	2.29 ± 0.21	2.00 ± 0.14
apoB (mg/dl)	71.4 ± 5.1	69.0 ± 5.8
Glucose (mM)	4.33 ± 0.15	4.38 ± 0.21
Insulin (pM)	117.5 ± 8.3	126.9 ± 5.9
ASP (nM)	23.4 ± 4.9	21.3 ± 3.4
Leptin (ng/ml)	9.5 ± 1.4	5.1 ± 0.8[a]

Fasting plasma values were measured for control and postobese women. ASP, acylation-stimulating protein; BMI, body mass index; BSA, body surface area; FFM, fat-free mass; TG, triglyceride; NEFA, nonesterified fatty acids; HDL, high density lipoproteins; LDL, low density lipoproteins; chol, cholesterol; apoB, apolipoprotein B. Values are given as averages ± SEM.

[a] $P < 0.02$ postobese versus control subjects.

Energy metabolism was first examined in the two groups. As shown in **Fig. 1** (top), there was no difference in basal REE (kcal/h) or TEF (over the 8 h) between postobese and control groups. The carbohydrate/fat oxidation ratio is also shown in Fig. 1 (bottom). Data were transformed to the trapezoid AUC of the carbohydrate/fat oxidation ratio as the normality test failed. There was no significant difference in the mean AUC of the carbohydrate/fat oxidation ratio between the postobese subjects (18.5 ± 5.3 g/g) and the control group (20.2 ± 3.0 g/g).

We then looked at changes in concentration of plasma lipids, lipoproteins, NEFA, and glucose over the course of the fat load. As shown in **Fig. 2** (top), there was an increase in plasma TG by 2–4 h (average increase of 74.1 ± 11.9% postobese and 60.9 ± 11.9% control), which decreased rapidly to fasting levels. When the plasma was subdivided into the two fractions of TRL, S_f >400 and S_f 20–400, the greatest differences between the two groups are seen in the S_f >400 fraction (Fig. 2, middle). Although there was no mean group difference, the S_f >400 TG returned to baseline values earlier in the postobese (6 vs. 8 h) with significantly lower plasma S_f >400 TG at 6 h (P =

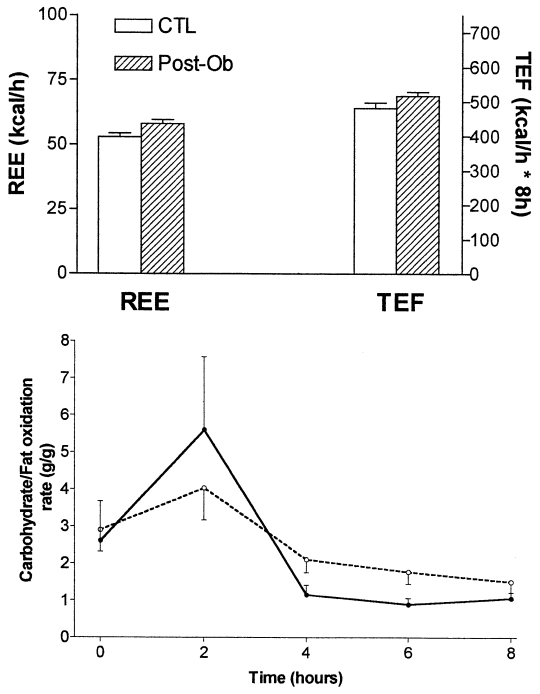

Fig. 1. Resting energy expenditure (REE), thermic effect of food (TEF), and carbohydrate/fat oxidation ratio in control and postobese subjects. After the fat load, REE (top), TEF, and carbohydrate/fat oxidation ratio (bottom) were measured over a 15-min sampling time for eight control subjects (CTL, open columns and open circles) and eight postobese women (Post-Ob, hatched columns and closed circles). Results are expressed as average ± SEM.

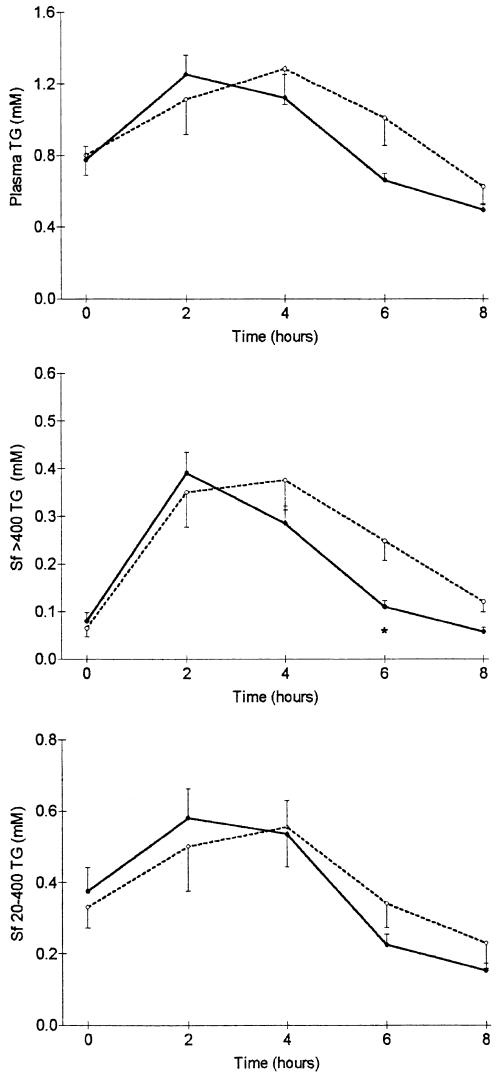

Fig. 2. Total plasma, S_f >400, and S_f 20–400 triglyceride (TG) in control and postobese subjects. After the fat load, plasma total TG (top), S_f >400 TG (middle), and S_f 20–400 TG (bottom) were measured at each time point for eight control subjects (open circles) and eight postobese women (closed circles). Results are expressed as the average ± SEM at each time point. * $P = 0.02$ for postobese versus control subjects at 6 h, by two-way repeated measures analysis of variance (RM ANOVA).

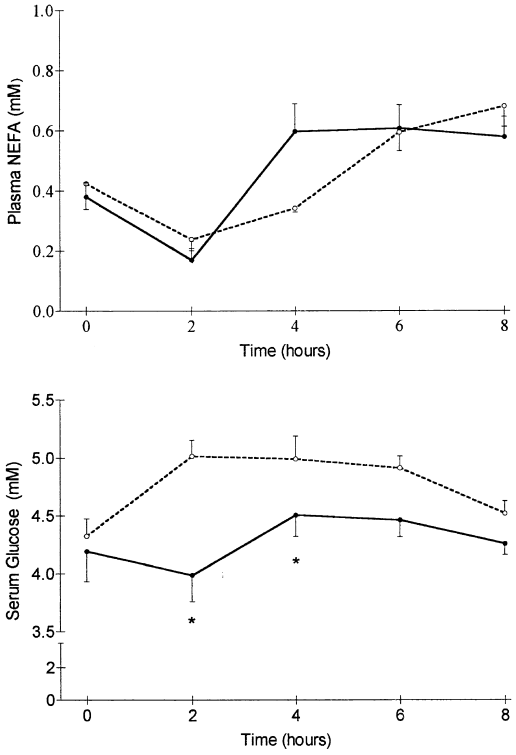

Fig. 3. Total plasma nonesterified fatty acids (NEFA) and serum glucose in control and postobese subjects. After the fat load had been administered, plasma total NEFA (top) and serum glucose (bottom) were measured at each time point for eight control subjects (open circles) and eight postobese women (closed circles). Results are expressed as the average ± SEM at each time point where $P = 0.006$ for postobese versus control subjects and * $P < 0.05$ for postobese versus control subjects at 2 and 4 h, respectively, for serum glucose by two-way RM ANOVA.

0.02). Overall, S_f >400 incremental AUC was 35% lower in the postobese group (1.61 ± 0.2 mM·8 h in control vs. 1.05 ± 0.08 mM·8 h in postobese, $P < 0.025$). The profiles for S_f 20–400 were similar between the two groups (Fig. 2, bottom).

Plasma changes in NEFA and glucose are shown in **Fig. 3**. In both postobese and control groups there was a drop in NEFA at 2 h ($P < 0.05$ vs. fasting for both groups). NEFA increases thereafter over the remaining time course, but there was no significant difference between postobese and control subjects. On the other hand, there was a group difference in postprandial plasma glucose between postobese and control subjects over the time of the study ($P = 0.006$, two-way RM ANOVA), where the postobese had consistently lower plasma glucose as shown in Fig. 3 (bottom), particularly at 2 and 4 h ($P < 0.05$).

The data for stable isotope enrichment in the plasma and breath pools are shown in **Figs. 4** and **5**. In the ^{13}C S_f >400 curves (Fig. 4, top), there was an earlier return of postprandial labeled TG to fasting nonenriched levels in the postobese women (at 8 vs. 4 h, control vs. postobese), with the postobese having lower plasma [^{13}C]TG at both 4 and 6 h ($P < 0.05$ by RM ANOVA). However, there was less difference in the ^{13}C S_f 20–400 curves (bottom) between postobese and control subjects: both increased to a maximum at 4 h and decreased toward basal thereafter, with a trend for lower AUC in the postobese.

The most striking difference was in the appearance of labeled [^{13}C]oleate in the NEFA plasma pool. As shown in Fig. 5 (top), whereas the [^{13}C]oleate increased in the control group, reaching a maximum at 6–8 h, there was little change over baseline enrichment in the appearance of [^{13}C]oleate in the postobese group, indicating little spillover of the lipolyzed dietary TG into the plasma NEFA pool ($P < 0.0001$, two-way RM ANOVA, postobese vs. control). In fact, AUC [^{13}C]NEFA was reduced by 72% in the postobese versus the control subjects (0.21 ± 0.07 μM·8 h in postobese vs. 0.73 ± 0.17 μM·8 h in control, $P =$

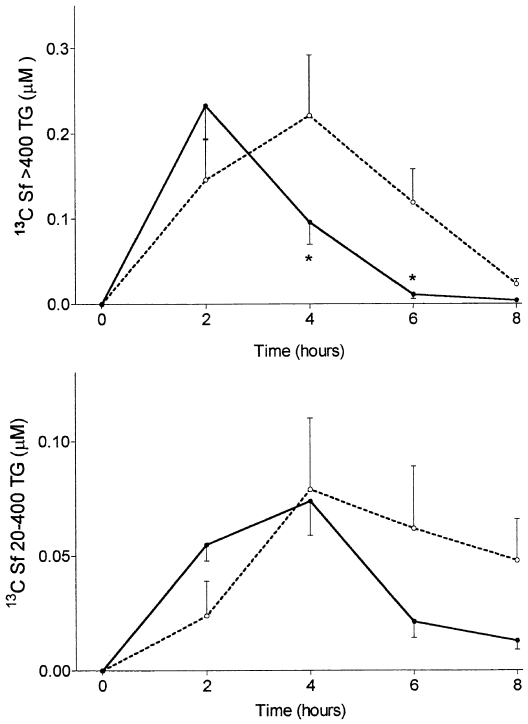

Fig. 4. S_f >400 [^{13}C]TG and S_f 20–400 [^{13}C]TG in control and postobese subjects. After the fat load, [^{13}C]oleate in S_f >400 TG (top) and [^{13}C]oleate in S_f 20–400 TG (bottom) were measured at each time point for eight control subjects (open circles) and eight postobese women (closed circles). Results are expressed as the average ± SEM at each time point. * $P < 0.05$ for postobese versus control subjects at 4 and 6 h for S_f >400 [^{13}C]TG by two-way RM ANOVA.

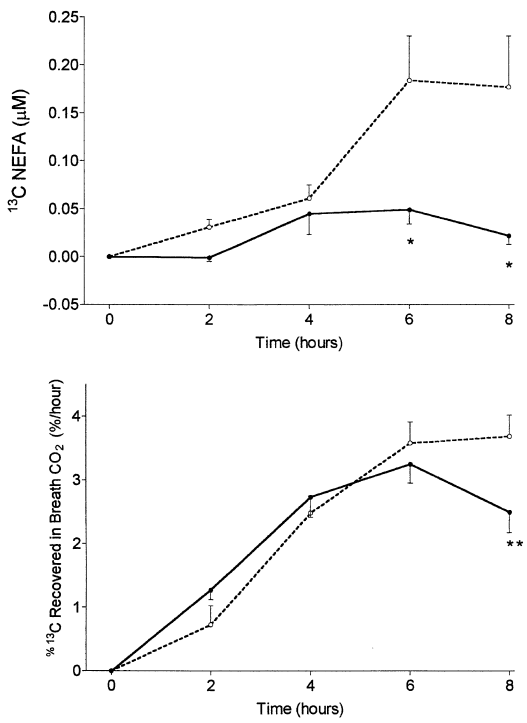

Fig. 5. [^{13}C]NEFA and ^{13}C recovered in breath CO_2 in control and postobese subjects. After the fat load had been administered, [^{13}C]oleate in NEFA ([^{13}C]NEFA, top) and ^{13}C recovered in breath CO_2 (bottom) were measured at each time point for eight control subjects (open circles) and eight postobese women (closed circles). Results are expressed as the average ± SEM at each time point. * $P <$ 0.0001 for postobese versus control subjects overall and at 6 and 8 h for [^{13}C]NEFA by two-way RM ANOVA; ** $P < 0.01$ for postobese versus control subjects at 8 h for ^{13}C recovered in breath CO_2.

0.011). This suggests that the NEFA released by lipoprotein lipase activity are taken up efficiently into a tissue compartment for storage or oxidation. $^{13}CO_2$ released into the breath is a measure of that oxidation as shown in Fig. 5 (bottom). There was a consistent increase in breath CO_2 in both groups over the 8 h. However, there was no difference between the two groups except at 8 h, at which time the percent $^{13}CO_2$ recovered in breath was lower in the postobese women ($P < 0.05$).

The analysis of the TG mass and the [^{13}C]TG curves assumes that there was no major difference in entry of the labeled fat into the plasma compartment between the two groups. Fecal fat analysis in the postobese women revealed that they had normal acid steatocrit values, with an average of 15.1 ± 6.4% (where the normal range is 0–31%). This indicated normal fat absorption in the postobese women.

The plasma [^{13}C]TG is hydrolyzed by lipoprotein lipase to produce [^{13}C]oleate, which enters either the plasma NEFA pool or enters peripheral tissues. The two exit pools necessarily compete with each other because a fatty acid that has entered the plasma NEFA pool, by definition, has not been taken up by peripheral tissues. In this study, only the two plasma components are measured ([^{13}C]TG and [^{13}C]NEFA) and tissue uptake is inferred. **Figure 6** illustrates the AUC for plasma [^{13}C]TG and [^{13}C]NEFA in the control and postobese subjects. The results for the two groups are significantly different. In the control subjects, the [^{13}C]NEFA AUC is 74.1% of the [^{13}C]TG AUC whereas in the postobese subjects, it is only 23.5% ($P =$ 0.019). The differences point to a smaller proportion of [^{13}C]TG entering the plasma NEFA pool and thus proportionally more entering the peripheral tissues of the postobese group compared with the control group.

Finally, we also measured postprandial responses of hormones known to be involved in adipose tissue TG metabolism, namely insulin and ASP (**Fig. 7**). Fasting plasma levels of both hormones were not different between the two groups (Table 1). Postprandially, plasma ASP levels decreased over the 8-h time course to a maximum decrease at 8 h (AUC drop −36 ± 13 nM·8 h in postobese vs. −18 ± 11 nM·8 h in control). Although there was a trend for a lower postprandial ASP in the postobese the difference was not significant between the two groups. With respect to insulin, there was an increase in plasma insulin at 2 h in both groups ($P < 0.05$ vs. fasting). However, there was an earlier return to basal levels at 4 h in the postobese, at which point it was significantly lower than in the control group ($P = 0.003$).

Correlations between ASP and insulin (postprandial) and fasting leptin were analyzed against fasting and postprandial parameters and the significant results are shown

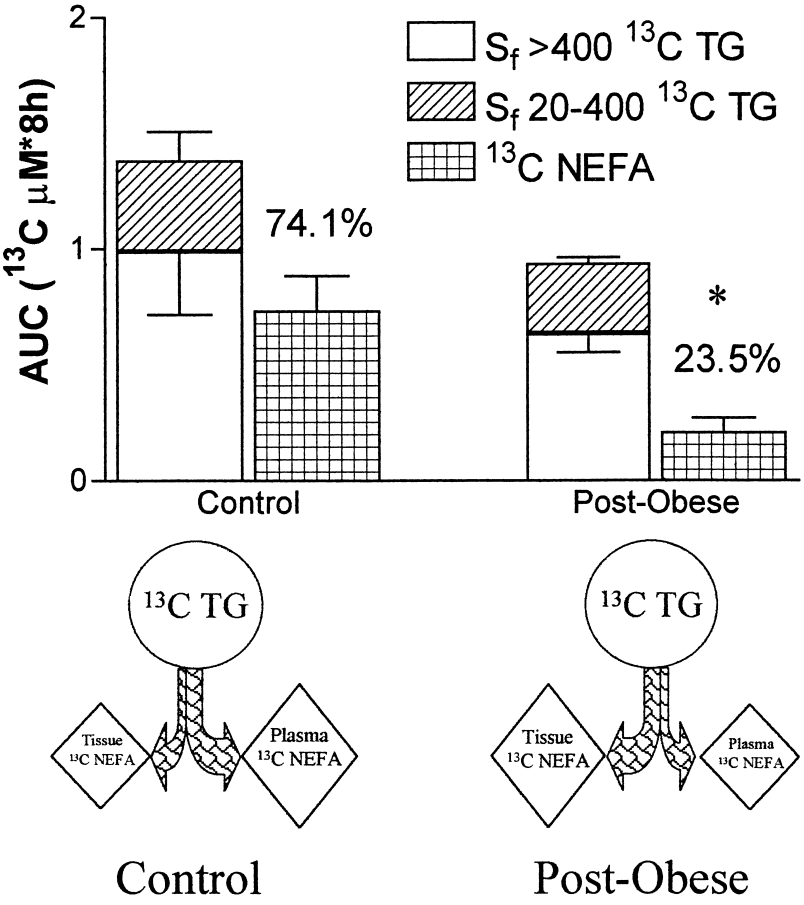

Fig. 6. Area under the curve (AUC) for S_f >400 [^{13}C]TG, S_f 20–400 [^{13}C]TG, and plasma [^{13}C]NEFA in control and postobese subjects. The AUC for each parameter was calculated for each subject individually as ^{13}C (μM) × 8 h, and results are presented as averages ± SEM. * $P < 0.05$ postobese versus control subjects.

in **Table 2**. Insulin AUC correlated with fasting NEFA and [^{13}C]NEFA AUC. Insulin AUC also correlated with fasting plasma TG fractions (total TG), as well as with postprandial changes (plasma TG AUC, S_f >400 AUC and S_f 20–400 AUC) but not with glucose (data not shown). Similar correlations were obtained with ASP, where ASP AUC correlated as well with fasting values (plasma TG, S_f >400 TG, plasma NEFA, and HDL cholesterol) and with postprandial changes (TG AUC, S_f >400 TG AUC, and NEFA AUC). Fasting leptin correlated only with postprandial NEFA (NEFA AUC).

DISCUSSION

Opinion remains divided as to whether obesity is, to any important degree, a consequence of altered energy expenditure and fatty acid oxidation (4–9, 11, 54). To date, little attention has been paid to the possibility that obesity may be the consequence of enhanced fatty acid trapping. The data in this study suggest that, at least in certain instances, this may occur and may be one mechanism in the pathophysiology of the disorder. This view derives from two principal observations in the present study. First, there was faster clearance of dietary TG from the plasma of the postobese women as compared with the control group. Second, and more pronounced, much less [^{13}C]oleate entered the plasma NEFA pool in the postobese group than in the control group. Both findings are consistent with enhanced fatty acid trapping by peripheral tissues in the postobese women compared with the control women.

The subjects in our postobese group had all undergone gastric bypass surgery more than 2 years before the study. Their weight had subsequently diminished to within the normal range and they were all weight stable for at least 6 months at the time of this study. The control subjects were selected to ensure there were no differences in age, BMI, percent body fat, waist/hip ratio, plasma TG, and habitual physical activity. To the extent possible, the only difference between the groups was that the members of one had a history of morbid obesity and gastric surgery, whereas those of the other did not.

Before reviewing the potential significance of the results, the effects of the surgical procedure on fat absorption must be considered. In principle, gastric bypass should not, and in fact does not (55–58), produce significant malabsorption because most of the absorptive segments of the intestine are intact. On the other hand, early after surgery, patients may suffer from dumping syndrome (37, 59). This occurs particularly after high carbohydrate liquid intake and is much less marked with solid foods (60), and our diet was designed accordingly. None of our subjects had symptoms of dumping syndrome and none suffered any distress after the meal used in this study, which was specifically designed to avoid this problem

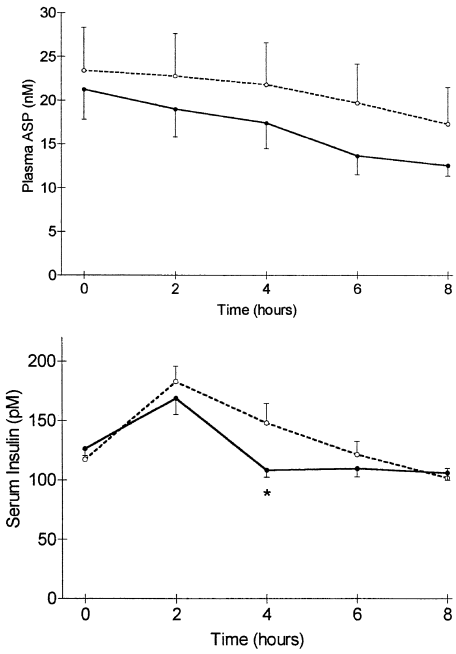

Fig. 7. Plasma acylation-stimulating protein (ASP) and insulin in control and postobese subjects. After the fat load had been administered, plasma ASP (top) and serum insulin (bottom) were measured at each time point for eight control subjects (open circles) and eight postobese women (closed circles). Results are expressed as the average ± SEM at each time point. * $P = 0.003$ for postobese versus control subjects at 4 h by two-way RM ANOVA.

(37). Most importantly, the postobese women had normal fecal fat content and thus differences in fat absorption cannot account for our findings.

The first important observation is that over the course of the study there was no evidence of any significant difference in energy expenditure between the two groups. Therefore, fatty acid utilization was not impaired in the postobese group. In addition, because there was no difference in fasting plasma TG between the two groups, any differences in plasma clearance and tissue trapping of dietary fatty acids cannot be attributed to this variable, and we believe that is a strength in this study.

There were some differences in postprandial clearance of TG between the two groups, but they were not large. Although total postprandial TG did not differ significantly between the two groups, postprandial S_f >400 TRL return to fasting levels occurred slightly earlier in the postobese group compared with the control group. This was more profound for the stable isotope analyses: postprandial S_f >400 [^{13}C]TG fraction returned to fasting levels 4 h earlier in the postobese group, although there was no significant difference in the overall [^{13}C]TG between the groups.

Clearance of plasma TG is a two-step process. Our data suggest that the first step, hydrolysis of chylomicrons by lipoprotein lipase, may be increased in postobese subjects, and indeed it has been demonstrated that lipoprotein lipase activity increases after weight loss (61–63). The second step involves the fate of the fatty acids that are released from the chylomicron particle, which can either be released into plasma or enter the peripheral cell (adipocyte or myocyte). The two groups differ sharply with regard to the fate of the released dietary fatty acids. A much smaller proportion enters the plasma NEFA pool in the postobese group compared with the control group. Thus the [^{13}C]oleate AUC was three times smaller in the postobese group than in the control subjects. Furthermore, the [^{13}C]oleate AUC/[^{13}C]TG AUC ratio was three times larger in the control subjects compared with the postobese group.

Our data dovetail with those reported by Binnert et al. (64, 65) in their study of postprandial fatty acid metabolism in obese women compared with normal weight women.

TABLE 2. Correlation of ASP and insulin AUC to postprandial changes

	ASP AUC		Insulin AUC		Leptin	
	r	*P*	*r*	*P*	*r*	*P*
Fat mass					0.556	0.025
Fat-free mass	0.504	0.047				
HDL cholesterol	0.500	0.049				
TG						
Fasting	0.578	0.02	0.443	0.08		
AUC	0.575	0.02	0.689	0.003		
S_f >400 TG						
Fasting	0.492	0.05				
AUC	0.526	0.036	0.720	0.002		
^{13}C AUC			0.570	0.021		
S_f 20–400 TG						
Fasting			0.542	0.03		
AUC						
NEFA						
Fasting	0.572	0.021	0.510	0.04		
AUC	0.591	0.016			0.520	0.039
^{13}C AUC			0.568	0.022		

Pearson correlation coefficient (*r*) and significance level (*P*) are shown for correlation between ASP area under the curve (AUC), insulin AUC, and fasting leptin for n = 16 (eight control and eight postobese subjects).

Using similar methodology, they also observed diminished entry of [^{13}C]oleate into the plasma NEFA pool. On the other hand, they observed no differences in the metabolism of medium-chain TG.

Plasma levels of insulin, ASP, and leptin were also measured in this study. For both insulin and ASP there was a trend toward lower fasting and postprandial levels in the postobese subjects compared with the control subjects. Given the lower plasma glucose levels and evidence of enhanced fatty acid trapping in peripheral tissues, it is possible that these lower levels might reflect enhanced tissue sensitivity to these two agonists. However, as is always the case in such analyses, these are inferences, not direct observations. It is of interest that the plasma leptin levels were also lower in the postobese subjects than in the control subjects. Whether this predisposes them to regain weight remains to be determined.

In summary, these data point to enhanced fatty acid trapping by peripheral tissues in postobese subjects and raise the possibility that such an abnormality might be important in the pathogenesis of obesity. Further studies are obviously necessary to test such a hypothesis.

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