Isotope tracer measures of meal fatty acid metabolism: reproducibility and effects of the menstrual cycle

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Uranga, Ana Paola, James Levine, and Michael Jensen. Isotope tracer measures of meal fatty acid metabolism: reproducibility and effects of the menstrual cycle. Am J Physiol Endocrinol Metab 288: E547–E555, 2005. First published October 26, 2004; doi:10.1152/ajpendo.00340.2004.—Oxidation and adipose tissue uptake of dietary fat can be measured by adding fatty acid tracers to meals. These studies were conducted to measure between-study variability of these types of experiments and assess whether dietary fatty acids are handled differently in the follicular vs. luteal phase of the menstrual cycle. Healthy normal-weight men (n = 12) and women (n = 12) participated in these studies, which were block randomized to control for study order, isotope ([1H]triolein vs. [14C]triolein), and menstrual cycle. Energy expenditure (indirect calorimetry), meal fatty acid oxidation, and meal fatty acid uptake into upper body and lower body subcutaneous fat (biopsies) 24 h after the experimental meal were measured. A greater portion of meal fatty acids was stored in upper body subcutaneous adipose tissue (24 ± 2 vs. 16 ± 2%, P < 0.005) and lower body fat (12 ± 1% vs. 7 ± 1%, P < 0.005) in women than in men. Meal fatty acid oxidation ([1H2O generation) was greater in men than in women (52 ± 3 vs. 45 ± 2%, P = 0.04). Leg adipose tissue uptake of meal fatty acids was 15 ± 2% in the follicular phase of the menstrual cycle and 10 ± 1% in the luteal phase (P = NS). Variance in meal fatty acid uptake was somewhat (P = NS) greater in women than in men, although menstrual cycle factors did not contribute significantly. We conclude that leg uptake of dietary fat is slightly more variable in women than in men, but that there are no major effects of menstrual cycle on meal fatty acid disposal.

Understanding the Storage and Oxidation of Dietary Macronutrients has a number of implications for obesity, body fat distribution, and insulin resistance. Björntorp et al. (1) and Márin et al. (20) helped pioneer the use of adding tracers to meals to assess the uptake of dietary nutrients into different tissue beds in vivo in humans, including the uptake of dietary fat into intra-abdominal adipose tissue. Other investigators have used meal fatty acid tracers to assess the oxidation of dietary fat (17, 27). We have combined these approaches in attempts to account for the fate of meal fatty acids in their entirety (16, 23, 24). Our goal is to use this approach to uncover the relative contribution of regional variations in fatty acid uptake as a determinant of interindividual differences in body fat distribution.

In addition to describing differences in meal fatty acid disposal between obese and lean subjects or between men and women, these techniques can be used to determine whether hormones (18), activity (27), or perhaps dietary interventions alter meal fatty acid disposal. Use of the meal fatty acid tracer technique should permit investigators to assess whether there are means to alter meal fat disposal in a way that could shunt dietary fat away from visceral fat and into a depot less associated with adverse health consequences. Performing studies to assess the effect of an intervention on meal fatty acid uptake into regional fat is best done using a paired study design (studies pre-and posttreatment). By using a [14C]-labeled fatty acid tracer for one study and a [3H]-labeled fatty acid for the other study, it is possible to avoid having the residual adipose tracer (20) invalidate the adipose tissue fatty acid uptake calculations.

The design of such paired studies to test the effect of an intervention on adipose tissue uptake of meal fatty acids is enhanced if one has foreknowledge of the degree of interindividual variability, as well as whether there are other confounding factors. For example, in women, the menstrual cycle is considered to be a potential confounding variable for some metabolic parameters (2, 3, 10, 26, 29). Slight differences in the resting metabolic rate and the thermic effect of food have been reported in women in different phases of the menstrual cycle (10, 26), although this has not been verified in other studies (21). Because of the possibility that energy metabolism may be affected by the menstrual cycle, we studied meal fatty acid metabolism in women only in the follicular phase of their menstrual cycle (23); this precaution is not necessary for studies of resting free fatty acid (9) or glucose (29) metabolism. The present study was done largely to assess whether controlling for the phase of the menstrual cycle is necessary in future studies of meal fatty acid metabolism. We also assessed the interindividual variability in meal fatty acid metabolism in men by using the same study design as for women. Herein, we report the uptake and oxidation of meal fatty acids in both men and women and the variability in the uptake in these two groups.

Materials and Methods

Subjects

This protocol was approved by the Mayo Institutional Review Board. Written, informed consent was obtained from 24 healthy, nonobese volunteers (12 males and 12 premenopausal females). The subjects were taking no medications, including oral contraceptives. A complete blood count, chemistry group, and lipid profile were documented to be within normal limits before the study. All volunteers were weight stable for at least 2 mo before the study and refrained from vigorous exercise for 2 days before the study.

Protocol

The study was designed to assess meal fatty acid metabolism in the follicular and luteal phases of the menstrual cycle in women as well as men by using the same study design as for women. Herein, we report the uptake and oxidation of meal fatty acids in both men and women and the variability in the uptake in these two groups.

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as to assess the reproducibility of the measures. To measure adipose tissue meal fatty acid uptake on two occasions, it was necessary to use labeled [14C]triolein for one study and [3H]triolein for the other study. Because tracers can differ with regard to purity (24), the studies were designed to randomize for meal tracer administration. One-half of the subjects received the [14C]triolein for the first study and [3H]triolein for the second study, and one-half received the opposite schedule. For the women participating, a similar allocation was used, and, in addition, the studies were block randomized such that one-half of the women had their luteal phase study as their first study and one-half had the follicular phase study as the first study. In this way, there was an even distribution of meal tracers across first and second studies and follicular and luteal phase studies. To assess meal fatty acid oxidation using comparable methodology on both study days, we included a carbon-labeled isotope, [1-13C]triolein (798 ± 62 mg for men and 590 ± 68 mg for women), on the [14C]triolein meal day. Breath was collected for 14C enrichment on both study days; the samples from the [1-14C]triolein study day served as the background enrichment for each individual for their [1-14C]triolein/[3H]triolein study day.

Subjects consumed all of their meals in the Mayo Clinic General Clinical Research Center (GCRC) for 1 wk before the study to ensure consistent macronutrient intake (50% carbohydrate, 35% fat, and 15% protein). They were instructed not to eat anything except what was provided for them throughout the study, and food intake was adjusted to maintain a stable weight if necessary. Each volunteer’s energy requirement was estimated using the Harris-Benedict formula (8) and usual daily activity. The macronutrient intake the week before the tracer studies for the men and women, respectively, was as follows: energy intake, 2,929 ± 100 and 2,080 ± 48 kcal/day; protein intake, 114 ± 4 and 81 ± 2 g/day; carbohydrate intake, 376 ± 13 and 267 ± 13 g/day (193 ± 8 and 136 ± 5 g/day simple carbohydrate); and fat intake, 117 ± 4 and 83 ± 2 g/day (44 ± 7 and 30 ± 8 g/day saturated fat).

The volunteers underwent all body composition measurements before the study. Ten of the women were studied in the follicular and luteal phases of their menstrual cycle, whereas two women underwent both studies in the follicular phase of the menstrual cycle. The studies were 2 wk apart for both men and women, except for the two women in whom we conducted two follicular phase studies. All female volunteers had a negative pregnancy test before participating in the study.

The volunteers were admitted to the Mayo Clinic GCRC the evening before the study. The morning of the study, after an overnight fast, a catheter was placed in a forearm vein and used to collect blood samples. Before the test meal was consumed, baseline breath and urine samples were collected for measurement of background 14CO2 (7) and 3H2O specific activity (SA).

Before arising from bed, participants had their basal metabolic rate measured at 0700 (24-h clock), after a 12-h overnight fast. At 0800, the volunteers consumed a meal providing one-third of their resting energy expenditure as determined by indirect calorimetry (586 ± 20 kcal for men and 436 ± 22 kcal for women). The meal consisted of a liquid formula (Ensure Plus; Ross Laboratories, Abbott Park, IL) containing 57% monounsaturated fat, 57% polyunsaturated fat), and 15% protein to provide 22 kcal for men and 15 kcal for women.) The meal consisted of a liquid formula (Ensure Plus; Ross Laboratories, Abbott Park, IL) containing 57% monounsaturated fat, 57% polyunsaturated fat), and 15% protein to provide 22 kcal for men and 15 kcal for women.) The meal consisted of a liquid formula (Ensure Plus; Ross Laboratories, Abbott Park, IL) containing 57% monounsaturated fat, 57% polyunsaturated fat), and 15% protein to provide 22 kcal for men and 15 kcal for women.) The meal consisted of a liquid formula (Ensure Plus; Ross Laboratories, Abbott Park, IL) containing 57% monounsaturated fat, 57% polyunsaturated fat), and 15% protein to provide 22 kcal for men and 15 kcal for women.)

Adipose tissue and meal lipids were extracted using standard (5) procedures, and the TG SA was measured as previously described (24). The lipid was extracted from the tissues and accurately weighed and counted on the scintillation counter to <2% counting error. The adipose tissue TG SA (3H and 14C, in dpm/mg lipid) was calculated for each site.

O2 consumption and CO2 expiration were measured by indirect calorimetry using a DeltaTrac Metabolic Cart (Sensor Medics, Yorba Linda, CA). Plasma glucose concentrations were measured with a Beckman glucose analyzer (Beckman Instruments, Fulton, CA). Plasma insulin concentrations were measured using a chemiluminescence method with the Access Ultrasensitive Immunoenzymatic assay systems (Beckman, Chaska, MN). The plasma TG concentrations were measured (11), and a 1.0-mL plasma sample was subjected to a Dole extraction (4) to measure TG radioactivity

Body fat and fat-free mass (FFM) were measured using dual-energy X-ray absorptiometry (DEXA; DPX-IQ, Lunar Radiation, Madison, WI) (15). Intra-abdominal adipose tissue area was measured using a single-slice computed tomography (CT) at the L2–3 level (14). Total body water was measured with 2H2O (25). Urine water was assayed for 3H2O concentration using liquid scintillation counting. The triolein tracer was assayed for radiochemical purity by measuring the radioactivity in the TG and the non-TG fractions by HPLC (13). The 24-h urine sample was also assayed for nitrogen content to allow for calculation of substrate oxidation.

Assays and Methods

Adipose tissue and meal lipids were extracted using standard (5) procedures, and the TG SA was measured as previously described (24). The lipid was extracted from the tissues and accurately weighed and counted on the scintillation counter to <2% counting error. The adipose tissue TG SA (3H and 14C, in dpm/mg lipid) was calculated for each site.

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Calculations

Visceral fat mass was predicted using the CT measurements of intra-abdominal and subcutaneous adipose tissue combined with DEXA-measured abdominal fat, as previously described (14). Upper body subcutaneous fat was taken as upper body fat (DEXA) minus visceral fat. Leg fat was measured using the region-of-interest program with the DEXA instrument.

Substrate oxidation at each time point in was calculated using indirect calorimetry and urinary nitrogen excretion rates (6). Integrated substrate oxidation over the first 10 h of the study was

Materials

[1-14C]triolein and [9,10-3H]triolein were purchased from NEN Life Science Products (PerkinElmer, Boston, MA). [1-13C]triolein and 3H2O (Isotech, Miamisburg, OH) were also used in these studies.

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calculated with the area under the curve of fatty acid and carbohydrate oxidation for each individual. Meal fatty acid oxidation for the 24 h after the test meal was calculated with either the $^2$H$_2$O or $^{14}$CO$_2$ production to determine the percentage of meal fatty acids oxidized using the $^2$H or $^{14}$C tracers, respectively. The production of $^2$H$_2$O was calculated by multiplying the concentration of $^2$H$_2$O in body water (using a urine sample obtained 24 h after the test meal) by total body water, as measured by the $^2$H$_2$O space, and adding the $^2$H$_2$O lost in the urine over the 24 h. This value (total $^2$H$_2$O dpm produced) was divided by the total [H]triolein consumed to calculate the fraction of meal fatty acids oxidized in the first 24 h after the meal. The $^{14}$CO$_2$ production was determined by multiplying the $^{14}$CO$_2$ SA by the $^{14}$CO$_2$ production rate, as measured by indirect calorimetry, at each time point. The nocturnal $^{14}$CO$_2$ production rate time points were not measured in this study, although the $^{14}$CO$_2$ SA was measured. Nocturnal $^{14}$CO$_2$ production rates were estimated as previously described (24). To calculate meal fatty acid oxidation using [$^{14}$C]triolein, the area under the $^{14}$CO$_2$ curve was divided by the amount of [$^{14}$C]triolein consumed. A similar approach was used to calculate [1-$^{13}$C]triolein oxidation, except that each $^{13}$CO$_2$ value on the [1-$^{13}$C]triolein day was corrected for the background level from the control study day.

Meal fatty acid uptake into adipose tissue was calculated as follows. The adipose tissue TG SA (dpm/g) was multiplied by the site-specific (lower body and upper body subcutaneous) adipose TG mass to estimate the amount of tracer (which reflects the amount of meal TG) stored in that body fat region. The regional adipose tracer uptake was divided by the total meal tracer content to determine the fraction of the tracer, and thus the meal fat, that was stored in the different adipose tissue depots (19). The gluteal values are given only for comparison with femoral values and were not used to calculate lower body meal fatty acid uptake. This is because the two sites are quite similar and because the body composition techniques we use do not allow us to quantify the gluteal depot in isolation.

Statistics

All data are presented as means ± SE unless otherwise stated. Comparisons of plasma insulin, glucose, and TG results over time during the different study days was done using a repeated-measures ANOVA with factors for time of sample and study day (day 1 vs. day 2, $^2$H vs. $^{14}$C, follicular vs. luteal), and between groups (men vs. women). A similar approach was used to compare meal fatty acid uptake and oxidation between groups and study days, except that the time factor was not used because these values were integrated 24-h measurements. In addition, a repeated-measures factor for between-site (upper body subcutaneous fat vs. lower body fat) differences in meal fatty acid uptake was included. If significant differences were found using the repeated-measures ANOVA, comparisons of results between follicular and luteal phases or study 1 and study 2 for women and between study 1 and study 2 for men were done using a paired $t$-test. Comparisons between men and women were done using a nonpaired $t$-test. A Bonferroni correction was used when multiple statistical tests were performed on data that were not part of the a priori hypothesis testing. Part of the statistical analysis included tests for normality and constant variance; only the data for leg uptake of meal fatty acids in women did not pass the test for normal distribution and the constant variance (see RESULTS). To determine whether the variance in leg uptake data for women was significantly different than the variance in other sites and in men, a modified Levine test was used. In brief, the absolute difference between the median value and each individual’s value for a site and study day was calculated. A rank sum test was then applied to assess whether there were significant differences between the two sets of observations.

RESULTS

Subject Characteristics

Men and women participating in this study were well matched for age (Table 1) but otherwise displayed the typical sex differences. Men were taller and heavier than women, with less body fat and more FFM. Men had more visceral fat than women, whereas women had more leg fat. Women had somewhat more upper body nonscursal (subcutaneous) fat. Total body water was 76 ± 1% of FFM.

The plasma cholesterol concentrations were greater in men than in women (174 ± 6 vs. 153 ± 6 mg/dl, $P = 0.02$), and HDL cholesterol concentrations were less in men than in women (48 ± 3 vs. 65 ± 4 mg/dl, $P = 0.002$). The mean fasting plasma triglyceride concentrations were greater in men than in women (909 ± 130 vs. 602 ± 64 μmol/l, $P = 0.049$) and not different ($P = 0.81$) in women between the follicular phase and luteal phase studies.

Plasma progesterone concentrations in the follicular and luteal phases of the menstrual cycle were 2.4 ± 0.4 and 16.1 ± 3.5 ng/ml ($n = 10$), respectively. The plasma progesterone concentrations for the two women studied only in the follicular phase of their cycles averaged 2.8 ng/ml (average between-study difference = 0.45 ng/ml).

Tracer-Determined Meal Fatty Acid Metabolism

Overall analysis of the percentage of the meal taken up in upper body subcutaneous and lower body subcutaneous fat between men and women was performed (Fig. 1). There was no effect of the study (study 1 and study 2) or of the isotope ($^2$H vs. $^{14}$C) when the pooled analysis was performed. Thus subsequent analyses were able to focus on mean values of study 1 and study 2 between sexes and comparisons between follicular and luteal phases of the menstrual cycle with the knowledge that isotope effects and study order were not statistical confounders.

Regional subcutaneous meal fatty acid uptake. Meal fatty acid uptake into adipose tissue is presented in two ways. The tracer-determined meal fatty acid metabolism

| Table 1. Subject characteristics |
|---|---|---|
| **Men** | **Women** | **$P$ Value** |
| Age, yr | 29±2 | 32±2 | NS |
| Height, m | 1.81±0.03 | 1.64±0.02 | <0.0001 |
| Weight, kg | 80.3±2.2 | 59.3±1.6 | <0.0001 |
| BMI, kg/m$^2$ | 24.6±0.6 | 22.2±0.4 | <0.05 |
| %Body fat | 18.0±1.5 | 30.1±1.2 | <0.0001 |
| Fat, kg | 13.36±1.12 | 16.78±0.91 | <0.05 |
| FFM, kg | 65.9±2.5 | 41.3±1.1 | <0.0001 |
| Visceral fat, cm$^2$ | 61±11 | 26±3 | <0.001 |
| Abdominal SQ, cm$^2$ | 89±11 | 96±9 | NS |
| Upper body nonscursal fat, kg | 6.90±0.52 | 8.84±0.51 | <0.05 |
| Visceral fat, kg | 1.74±0.27 | 1.15±0.12 | <0.05 |
| Leg fat, kg | 4.72±0.41 | 6.79±0.37 | <0.001 |

Values are means ± SE. Body fat and leg fat were measured by dual-energy X-ray absorptiometry; computed tomography (CT) abdominal subcutaneous (SQ) and visceral fat areas were measured using a single-slice CT at the L2–3 interspace. BMI, body mass index; FFM, fat free mass; NS, not significant.
assessed by the percentage of the meal fatty acid tracer) stored in the different depots.

The meal fatty acid uptake in abdominal, femoral, and gluteal fat in men averaged 0.66 ± 0.11, 0.40 ± 0.06, and 0.45 ± 0.08 mg meal fat/g adipose tissue lipid. The uptake in abdominal fat was greater (P = 0.02) than in femoral fat, but not significantly greater (P = 0.14) than in gluteal fat. The uptake of meal fatty acids in femoral and gluteal fat was not significantly different, nor were there differences between study 1 and study 2 or the uptake measured with [14C]- vs. [3H]triolein. For women, the meal fatty acid uptake in abdominal, femoral, and gluteal fat averaged 0.41 ± 0.04, 0.28 ± 0.03, and 0.28 ± 0.03 mg meal fat/g adipose tissue lipid, respectively. The uptake in abdominal fat was greater than in femoral fat (P = 0.01) and gluteal fat (P = 0.002). The uptake of meal fatty acids in femoral and gluteal fat was not significantly different. Abdominal subcutaneous meal fatty acid uptake averaged 0.38 ± 0.05 and 0.40 ± 0.06 mg meal fat/g adipose tissue lipid in the follicular and luteal phases of the menstrual cycle, respectively (n = 10, P = 0.77). Femoral adipose tissue uptake of meal fatty acids in the follicular and luteal phases was 0.31 ± 0.06 and 0.23 ± 0.02 (P = 0.25), and gluteal uptake was 0.26 ± 0.03 and 0.26 ± 0.04, respectively (P = 0.97).

Expressing the fate of meal fatty acids as the percentage of the total consumed, we found that 24 ± 2% of meal fatty acid was taken up into upper body subcutaneous fat in women and 16 ± 2% in men (P < 0.005, women vs. men). There was no difference in the uptake between the first study and the second study in men or women and no difference between the follicular and luteal phases of the menstrual cycle in women. There was no difference between study 1 and study 2 for lower body subcutaneous meal fatty acid uptake in men, with an average of 7 ± 1% of meal fat taken up in lower body fat. For women, the average uptake (both studies) of meal fatty acids in lower body fat was 12 ± 1% (P < 0.005 vs. men), and there was no difference between study 1 and study 2. In the follicular phase of the menstrual cycle, 15 ± 2% of meal fatty acid was stored in the lower body subcutaneous fat, whereas in the luteal phase, 10 ± 1% was stored in lower body subcutaneous fat. The constancy of the variance and the skewness of femoral meal fatty acid uptake data were such that we analyzed the menstrual cycle effects with a rank sum test. Still, the difference between the follicular and luteal phase leg uptake data was not statistically significant (P = 0.08). This statistical/analytic issue was not encountered with other aspects of the meal tracer data. The variance in the leg data for women was not significantly (P = 0.10–0.20) greater than the variance in the other uptake data, however, according to the modified Levine test.

**Meal fatty acid oxidation.** Meal fatty acid oxidation over 24 h in men was 21 ± 3% with [14C]triolein and 22 ± 1% with [13C]triolein (without a carbon fixation correction factor). For women, these values were 21 ± 2 and 25 ± 2%, respectively [P = not significant (NS) for both 14C vs. 13C and men vs. women]. For all subjects, [14C]triolein oxidation was estimated to be 21 ± 2% over 24 h, and [13C]triolein oxidation was 24 ± 1% over 24 h (P = 0.12). The lack of between-study differences in 14C vs. 13C meal fatty acid oxidation implies that any observed differences in nonoxidative meal fatty acid disposal are not downstream effects related to major differences in oxidative metabolism of meal fat. As calculated using the 1H2O generation from the [3H]triolein study, 24-h meal fatty acid oxidation in men was 52 ± 3% and in women was 45 ± 2% (P = 0.04 men vs. women). If we used the acetate correction factor of 50% (28), the [14C]triolein and [13C]triolein oxidations were 42 ± 3 and 47 ± 2%, respectively, over 24 h for all 24 subjects (P = NS vs. the 1H2O-estimated 24-h meal fatty acid oxidation of 47 ± 2%).

**Unaccounted-for meal fatty acids.** With data from the [3H]-triolein study day, the percentage of meal fatty acids that could not be accounted for was greater in men than in women (29 ± 2 vs. 21 ± 4%, respectively), but the difference was not statistically significant (P = 0.10).

**Reproducibility of meal fatty acid uptake into adipose tissue.** We assessed the reproducibility of meal fatty acid uptake data between the first and second study independently of the isotope ([3H]triolein vs. [14C]triolein), because the initial analysis indicated no isotope effect. To assess the intraindividual variation, we calculated the absolute difference between the fatty acid uptake during the first study and the mean of both studies. The absolute difference between the percentage of meal fatty acids stored in upper body subcutaneous fat in the first study and the mean of both studies was 4 ± 4% (mean ± SD, range 0–13%) for women and 4 ± 3% (mean ± SD, range 0–10%) for men. The extreme example is a woman in whom 15% of meal fatty acids was stored in upper body subcutaneous fat in the first study and 41% in the second study (average uptake of 28%); there was a 13% difference in meal fatty acid uptake between either of her two studies and the mean of both studies. To put this in perspective, the interindividual variation can be appreciated by recalling that 24 ± 9% (mean ± SD, range 9–42%) and 16 ± 9% (mean ± SD, range 3–42%) of meal fatty acid uptake was in upper body subcutaneous fat in women and men, respectively. The intraindividual variation in lower body subcutaneous meal fatty acid uptake, assessed using the same approach, was 4 ± 4% (mean ± SD,
range 0–13%) for women and 4 ± 3% (mean ± SD, range 0–5%) for men. The interindividual variation in meal fatty acid uptake is reflected by the 12 ± 6% of meal fatty acids stored in lower body subcutaneous fat (mean ± SD, range 7–35%) for women and 7 ± 5% (mean ± SD, range 2–20%) for men. There was a correlation between the uptake of meal fatty acids in leg adipose tissue in study 1 and study 2 ($r = 0.58, P = 0.048$) for men; however, the correlation between the uptake of meal fatty acids in abdominal adipose tissue between study 1 and study 2 was not statistically significantly ($r = 0.41, P = 0.18$). There was not a significant correlation between either upper body ($r = 0.27, P = 0.39$) or lower body ($r = 0.01, P = 0.98$) meal fatty acid uptake values between the two studies in women.

The individual values for the percentages of meal fatty acids taken up into abdominal and leg fat for study 1 and study 2 for men are depicted in Fig. 2; the same approach is used to depict these data for the follicular and luteal phase studies in women in Fig. 3.

The interpretation of the data we gathered on regional meal fatty acid uptake, meal fatty acid oxidation, and intraindividual variability is potentially impacted by the substrate, hormonal, and energy expenditure milieu in which the measurements are made. Thus below are presented these study outcomes.

**Plasma Insulin and Glucose Responses**

There were no significant differences between the glucose and insulin concentrations on study day 1 vs. study day 2, the [$^{3}$H]- vs. [$^{14}$C]triolein study days, or the follicular vs. luteal study days. Figure 4, top, shows the average plasma insulin responses, and Fig. 4, bottom, depicts the average plasma glucose responses in men and women for both study days.

**Plasma Triglyceride Responses**

There were no significant differences between the plasma triglyceride concentrations on study day 1 vs. study day 2 or the [$^{3}$H]- vs. [$^{14}$C]triolein study days for either men or women. For women, plasma triglyceride concentrations were not different between the follicular and luteal study days (Fig. 5, top). Men had consistently greater plasma triglyceride concentrations than women ($P < 0.05$); the average 24-h plasma triglyceride concentrations in men and women are shown in Fig. 5, bottom. In addition, the increase in plasma triglyceride concentrations above fasting was greater in men than in women (peak increase $1,037 ± 285$ vs. $262 ± 45$ μmol/l, respectively, $P = 0.02$).

The concentration of the meal tracer in plasma triglycerides was examined as an indicator of the entry/removal of chylomicron triglycerides from the circulation. Figure 6 shows the plasma concentration (dpm/ml) of [$^{14}$C]triglyceride and [$^{3}$H]triglyceride from the two study days in men and women. The amount of tracer incorporated in the meals was not different between men and women ($57 ± 5$ vs. $56 ± 8$ μCi, respectively, of [$^{3}$H]triolein and $21 ± 4$ vs. $21 ± 6$ μCi, respectively, of [$^{14}$C]triolein). The pattern of tracer presence in plasma was similar to the one that we previously observed: a
gradual increase in [3H]- or [14C]triglyceride occurred in the first 4 h after the ingestion of the meal with the tracer, followed by a peak at 6 h (1 h after the ingestion of lunch). The plasma [14C]triglyceride and [3H]triglyceride concentrations were consistently greater in men than in women on both study days, consistent with a lesser clearance of meal-derived triglycerides in men.

Energy Metabolism

The basal metabolic rates for men and women in study 1 and study 2 and in women in the follicular vs. luteal phase of the menstrual cycle are provided in Table 2. As expected, basal metabolic rates were higher in men than in women, but there was no difference between the different study days in men and women. The 24-h respiratory exchange ratios (RER; averages of study 1 and study 2) for men and women are depicted (see Fig. 7, bottom). As assessed by repeated-measures ANOVA with factors for time (min) and sex, men had a lower ($P < 0.05$) RER than women at several time points throughout the day (180, 300, 360, and 420 min). The RER throughout the day was not different in women in the follicular and luteal phases of the menstrual cycle (Fig. 7, top). The estimated energy expenditure from fatty acid oxidation over the 10 h during which frequent indirect calorimetry measures were done is also provided in Table 2.

DISCUSSION

We assessed whether the metabolic fate of meal fatty acids is different in the follicular vs. luteal phase of the menstrual cycle and at the same time measured the reproducibility of these types of experiments. Twenty-four healthy, nonobese volunteers participated in two studies of meal fatty acid metabolism 2 wk apart. The studies were block randomized to avoid possible confounding effects of study order or meal tracer ([3H]- or [14C]triolein) effects and were scheduled to coincide with the follicular and luteal phases of the menstrual cycle for 10 of the 12 women. There were no significant differences in energy expenditure or total or meal fatty acid oxidation between the follicular and luteal phases of the menstrual cycle in lean, healthy women. The variability in meal fatty acid uptake into leg fat in women was somewhat greater than in abdominal fat in women and abdominal and leg fat in men, although the difference was not statistically significant ($P = 0.10–0.20$). To the extent that the issue of variability in this parameter influences the design of future studies, we should consider that it could be a sex-related difference in meal fatty acid disposal that is independent of or related to the menstrual cycle.

Surprisingly little research has been done to examine whether regional adipose tissue lipid uptake is affected by menstrual cycle in women. Rebuffé-Schrie et al. (22) measured both in vitro lipolysis and lipoprotein lipase (LPL) activity in femoral and abdominal adipose tissue samples taken from women in the follicular and luteal phases of their menstrual cycle.
cycle. She found no differences in either of these fat cell parameters as a function of the menstrual cycle, whereas important differences were seen when fat cells were taken from women during pregnancy or lactation (22). LPL activity is thought to be a major determinant of triglyceride uptake by adipose tissue, although it has not been possible to detect strong correlations between LPL activity and triglyceride uptake into adipose tissue in vivo (19, 23). Nevertheless, the data presented in the report of Rebuffé-Scrive et al. do not hint at differences in LPL activity or differences in the variability of LPL activity during the follicular phase of the menstrual cycle. Unfortunately we did not measure LPL activity in this study and thus cannot ascertain whether the variations in uptake of meal fat by adipose tissue were associated with differences in LPL activity.

We assessed whether substrate oxidation by indirect calorimetry and isotopic techniques differed between the two study days (study 1 vs. study 2, follicular vs. luteal) and whether the insulin and triglyceride responses were comparable. If we had discovered differences in one or more of these factors, it would have introduced confounding variables regarding the meal fatty acid oxidation and uptake data. Because none of these factors differed significantly between study days, either in average values or in variance, it is easier to draw conclusions regarding the metabolic fate of dietary fat. The somewhat greater variability of leg adipose tissue meal fatty acid uptake in women could be a reflection of a confounding effect of the menstrual cycle phase; no such variability was noted in men. This variability was not seen in other factors that could influence the results: energy expenditure or triglyceride or insulin responses. Intraindividual differences in resting energy expenditure averaged only 2 and 7% in men and women, respectively; the thermic effect of food after breakfast differed by only an average of 3–4 kcal/5 h between the two studies; the intraindividual differences in average plasma triglyceride concentrations were only 6 and 8% in men and women; and the intraindividual differences in average plasma insulin concentration was only 11 and 8% in men and women, respectively. The outlying value of leg meal fatty acid uptake values in women in the follicular phase of the menstrual cycle is unlikely to be due to analytic/sampling error. We have previously assessed the intrastudy analytic/sampling error (24) for femoral meal fatty acid uptake and found it to be less than one-half of the between-study variation we observed in women in the present experiments.

With the use of carbon-labeled triolein tracers, there was no detectable difference in meal fatty acid oxidation between studies. This implies that meal fatty acid oxidation did not differ between the two study days. When we corrected the $^{13}$C or $^{14}$C meal fatty acid oxidation data for carbon fixation using the acetate correction factor (28) of 50%, the carbon-labeled meal fatty acid oxidation was comparable to the $^3$H meal fatty acid oxidation.

In this study, men had a somewhat greater proportional fat oxidation throughout the day than did women, as assessed by the lower RQ at several time points. They also had slightly greater meal fatty acid oxidation, as measured by the generation of tritiated water from the [3H]triolein-labeled meal. Although this could be due to true sex differences in substrate oxidation under the conditions of these experiments, a more likely cause is a slightly negative energy intake relative to energy expenditure in men compared with women; energy deficits tend to be accounted for by fat oxidation (12). Unfortunately, because we did not have true 24-h energy expenditure measurements (such as are possible with whole room calorimeters), we cannot be certain of the exact energy balance in our volunteers.

Consistent with previous studies (23), women stored a greater proportion of meal fatty acids in subcutaneous fat than men (Fig. 1). There was also a slightly lesser portion of meal fatty acids that could not be accounted for in women than in

| Table 2. Energy expenditure and substrate oxidation |
|---------------------------------|---------|---------|---------|---------|---------|---------|
|                                | Men     |        | Women   |        | Women   |        |
|                                | Study 1 | Study 2 | Study 1 | Study 2 | Follicular | Luteal |
| BMR, kcal/day                  | 1,751±63| 1,748±78| 1,283±51| 1,285±63| 1,278±47 | 1,289±66|
| TEF, kcal/5 h                  | 46±4    | 51±4    | 33±4    | 38±3    | 35±4    | 34±3    |
| TEF, %, breakfast              | 8±1     | 9±1     | 7±1     | 9±1     | 8±1     | 8±1     |
| Carbohydrate oxidation, kcal/10 h| 210±44 | 237±23  | 227±16  | 267±31  | 234±22  | 260±28  |
| Fat oxidation, kcal/10 h       | 272±39  | 298±25  | 158±18  | 136±17  | 150±16  | 144±19  |

Values are means ± SE. Men’s meal size was 590 ± 23 kcal, and women’s meal size was 435 ± 23 kcal. Values for the follicular and luteal phase studies are from 10 women who underwent a study during each phase, whereas the study 1 and study 2 values are those from all 12 women. Thermic effect of food (TEF) is expressed as a percentage of the energy in the meal expended as an increase in metabolic rate. BMR, basal metabolic rate. There were no significant differences between study 1 and study 2 values for men and women or follicular and luteal values for women.

![Fig. 7. Respiratory exchange ratio (RER) from indirect calorimetry throughout study days. Top: RER for the 10 women who had studies in both the follicular and luteal phases of their menstrual cycle. Bottom: average RER for both studies for men and women. Values are means ± SE.](http://ajpendo.physiology.org/ by 10.20.33.6 on July 7, 2017)
men. We hypothesized (23) that the meal fatty acids we could not account for after 24 h had been stored in visceral adipose tissue and subsequently reported that, by accounting for the meal fatty acid uptake into visceral fat, it is possible to account for 100% of meal fatty acid disposal (16). The data from the present study are consistent with a greater uptake of meal fatty acids into visceral fat in nonobese men compared with women.

In this report, we provide reproducibility data for the study of meal fatty acid uptake in adipose tissue by use of isotopic tracers. To present this data, we begin by assuming that, for an individual, the mean adipose tissue uptake of dietary fat for two studies is a better reflection of that person’s true average than the value from either study alone. The difference between the value from one study and the mean of two studies provides an estimate of how much the result from any given study will vary from a given individual’s average response. Using this approach, we previously reported the side-to-side differences for abdominal and leg meal fatty acid uptake for a single experiment (~4% for abdominal and 10% for femoral). In the present study, we found that between-experiment differences for abdominal meal fatty acid uptake averaged 16% for women and 26% for men (4 ± 24% and 4 ± 16%, respectively). The between-experiment differences were similar for leg uptake of meal fatty acids. The range of uptake values that we observed is also reported. These data will help investigators to perform power calculations to better design studies, especially for paired experiments to test the effects of an intervention on meal fatty acid uptake into adipose tissue. For example, detecting a 5% change in meal fatty acid uptake into either lower body or upper body adipose tissue beds by using a paired study design would require seven subjects (80% power, α of 0.05, 2-sided t-test). In contrast, detecting a 5% difference between groups (nonpaired t-test) in meal fatty acid uptake into upper body fat and lower body fat with the same power would require 52 and 24 subjects per group, respectively.

In summary, we found that more meal fatty acids were taken up in leg adipose tissue of women during the follicular phase of the menstrual cycle, but that this difference from uptake during the luteal phase was not statistically significant. There was somewhat greater variance and skewing of the leg adipose tissue meal fatty acid uptake data in women relative to upper body adipose tissue and meal fatty acid oxidation; this also contrasted with the consistency of the data variance in men. If there are differences in leg uptake of meal fatty acids between different phases of the menstrual cycle in women, it will take large studies to detect them. Examining and/or controlling for potential variables (LPL activity, adipose tissue blood flow, and so forth) in future studies may help address whether there are biological explanations for the intraindividual variability we observed. For the present, the phase of the menstrual cycle does not appear to greatly affect the planning for studies of meal fatty acid metabolism in women.

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