Meal fatty acid uptake in human adipose tissue: technical and experimental design issues

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Romanski, S. A., Rita M. Nelson, and Michael D. Jensen. Meal fatty acid uptake in human adipose tissue: technical and experimental design issues. Am J Physiol Endocrinol Metab 279: E447–E454, 2000.—The adipose tissue uptake of dietary fat has been studied using fatty acid radio-tracers incorporated into a meal, followed by adipose tissue biopsies. A number of experimental design issues, including the use of isotopic tracers to measure meal fatty acid oxidation and plasma appearance of tracer, as well as the heterogeneity of adipose tissue fatty acid uptake, have been addressed. We examined these questions in a study of 24 volunteers (12 men and 12 women) who consumed a meal containing [3H]triolein and [14C]triolein. Slight differences in the purity of [3H]triolein vs. [14C]triolein were found, which could affect the apparent adipose tissue uptake of meal fatty acids. The adipose tissue triglyceride specific activity from bilateral biopsy sites agreed well, implying that a unilateral biopsy is satisfactory for measuring tracer uptake. Meal fatty acid oxidation measured using [3H]triolein and [14C]triolein was well correlated ($r = 0.79$, $P < 0.0001$). The peak tracer appearance in plasma chylomicrons occurred 1 h after the ingestion of a second, unlabeled meal. Our findings have implications for the experimental design of future meal fatty acid tracer/adipose tissue biopsy studies.

[14C]triolein; adipose tissue biopsy; body composition; chylomicrons

Directly measured the uptake of dietary fat by adipose tissue in humans in vivo by administering meals containing a radiolabeled fatty acid tracer and performing adipose tissue biopsies after meal absorption. This approach allows the uptake of dietary fat into adipose tissue TG to be traced.

With the use of this approach, a number of important observations have been made. For example, meal fatty acid uptake was not strongly correlated with adipose tissue LPL activity (1, 18). In addition, meal TG fatty acid uptake was greater in abdominal subcutaneous than femoral adipose tissue (20, 21) and greater in intra-abdominal than abdominal subcutaneous adipose tissue (18). Finally, hormonal treatments that change body fat distribution appear to alter adipose tissue meal fatty acid uptake (20). The major strength of this experimental approach is the sure knowledge that the tracer found in the adipose tissue reflects the uptake of meal fatty acids.

Some unanswered questions remain regarding this general study design. A substantial fraction of meal fatty acids is oxidized (16), yet these studies of adipose tissue uptake have not included a measure of meal fatty acid oxidation (1, 18–21). Some studies use [14C]oleic acid (21), whereas other studies use [3H]oleic acid (18) or both tracers in a sequential fashion (19). The assumption that these tracers provide equal quantitative estimates of meal fatty acid uptake has not been tested, nor has it been evaluated whether estimates of meal fatty acid oxidation using these different tracers provide comparable results. The meal fat challenges provided in some studies (1, 18–21) were enormous (80–120 g). These massive fat loads could result in prolonged chylomicronemia and altered regional meal fatty acid storage. Finally, investigators have attempted to predict total meal fatty acid uptake by multiplying the regional uptake of meal fatty acids by total body fat (21). To confidently make this extrapolation, the regional heterogeneity of adipose tissue meal fatty acid uptake must be known.

The following studies were therefore undertaken to examine several technical aspects of the meal fatty acid tracer/adipose tissue biopsy study design. We assessed the comparability of [3H]- and [14C]-labeled TG

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fatty acid tracers to assess adipose tissue meal fatty acid uptake and meal fatty acid oxidation (3H2O and 14CO2 production). Bilateral adipose tissue biopsies were performed to determine the heterogeneity of meal fatty acid uptake. Finally, the peak and duration of meal fatty acid oxidation and tracer chyomicronemia were examined to optimize the design of future studies.

MATERIALS AND METHODS

Subjects. Written, informed consent was obtained from 24 nonobese, healthy volunteers (12 males and 12 premenopausal females). The subjects were taking no medications, including oral contraceptives. All volunteers were weight stable for at least 2 mo before the study and refrained from vigorous exercise for 2 days before the study. The women were studied in the follicular phase of their menstrual cycle. Subjects consumed all of their meals in the 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 through 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 (10) and usual daily activity. The macronutrient intake the week before the tracer study for the entire group was as follows: energy intake 2,533 ± 478 (SD) kcal/day; protein intake 100 ± 18 g/day; carbohydrate intake 325 ± 62 g/day (53 ± 7% simple carbohydrate); fat intake 100 ± 20 g/day (37 ± 7% saturated fat).

Materials. L-[14C]triolein (Du Pont NEN Research Products) and [3H]triolein (American Radiolabeled Chemical and Du Pont NEN Research Products) were used in these studies.

Assays and methods. Chylomicron particles were separated by ultracentrifugation of 2.0 ml of fresh plasma (23), and the TG concentrations (11) were measured on a small portion of the sample. The remainder of the sample was subjected to a Dole extraction (3) to measure chylomicron TG specific activity (SA) and tracer concentration in plasma. Initially, chylomicron and nonchylomicron samples were tested to assess whether 3H or 14C appeared in plasma phospholipids. Because none was found in the initial five studies, the remaining samples were assayed only for TG content and radioactivity.

Adipose tissue and meal lipids were extracted using standard procedures (7), and the TG SA was measured as described by Marin et al. (21). The lipid was extracted from the tissues, accurately weighed, and counted on the scintillation counter to <2% counting error. The adipose tissue TG SA (3H and 14C; dpm/mg lipid) was calculated for each site. The meal aliquots were subjected to serial dilution to enable comparison of the ratio of 3H to 14C at the same radioactivity level in adipose tissue and meal. This was found to be necessary because slight but significant differences in the apparent 3H-to-14C ratio were noted between meal samples with different amounts of radioactivity (Fig. 1). Each batch of triolein tracers was assayed for radiochemical purity by measuring the radioactivity in the TG and non-TG fractions by HPLC (12). To assess the proportion of radioactivity present in oleate, the TG fraction was subjected to alkaline hydrolysis, followed by conversion to a phenacyl derivative and injection on a second HPLC (22). The non-TG fraction (nonesterified fatty acids) was also derivatized and assayed for purity by HPLC (22). Urine water was assayed for 3H2O concentration using dual-channel (3H and 14C) liquid scintillation counting because a small amount of 14C was noted that affected the 3H dpm if we relied on single channel counting.

Total body and regional fat and fat-free mass was measured using dual-energy X-ray absorptiometry (DEXA; DPX-IQ, Lunar Radiation, Madison, WI; see Ref. 15). Total body water was measured with 2H2O (26). Oxygen consumption and carbon dioxide production were measured by indirect calorimetry using a DeltaTrac Metabolic Cart (Yorba Linda, CA). Volunteers were acclimatized to the hood for the first 10 min of each 30-min measurement. The basal metabolic rate measurements were made at 0700, after a 12-h overnight fast, before the volunteer had arisen from bed.

Protocol. The volunteers underwent all body composition measurements before the adipose tissue biopsy study. A complete blood count, chemistry group, and lipid profile were documented to be within normal limits before the study. 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 consuming the test meal, baseline breath and urine samples were collected for measurement of background 14CO2 (9) and 3H2O (13) SA.

At 0800, the volunteers consumed a meal providing 40% of their resting energy expenditure as determined by indirect calorimetry (509 ± 50 kcal). The meal consisted of a liquid formula (Ensure Plus; Ross Laboratories) containing 57% carbohydrate, 27% fat (16% saturated fat, 27% monounsaturated fat, 57% polyunsaturated fat), and 15% protein to which 20 μCi of L-[13C]triolein and 40 μCi of [3H]triolein had been added as previously described (12). The mean ± SD TG content of the tracer meals was 21.3 ± 4.0 g. The volunteers were also provided with normal (solid food) meals at 1300 and 1800, consistent with their previously determined pattern. These meals provided a total 1,902 ± 391 kcal with the same distribution of protein, carbohydrate (complex and simple), and fat (saturated and unsaturated) as the diets provided during the week before the study. To determine the exact amount of L-[14C]triolein and [3H]triolein consumed,
quadruplicate 50-μl samples of the meal were counted using
dual-channel liquid scintillation counting. The meal was
weighed to the nearest 0.1 g. Aliquots of the meal were also
saved for measurement of meal lipid (14C) and (3H) SA (see
above).

After consuming the test meal, blood and breath samples
(for 14CO2 SA) were obtained hourly for 8 h, every 2 h for an
additional 4 h, and then every 4 h until the next morning.
The blood samples were analyzed for plasma chylomicron TG
and nonchylomicron TG (3H) and (14C) SA. Indirect calorim-
etry was performed hourly for 8 h at the 10th hour and the
next morning (24 h after the test meal). Urine was collected
for 24 h after the test meal to calculate 3H2O losses and to
assess the concentration of 3H2O in body water after 24 h.
The volunteers remained seated or lying in bed during the
first 8 h after consuming the test meal except as needed to
void. After 8 h, the volunteers were allowed to walk around
the room or the GCRC. Because the intravenous saline infu-
sion was continued to maintain venous access, the physical
activity of the volunteers was necessarily limited.

Twenty-four hours after the test meal consumption, adi-
pose tissue biopsies were obtained using sterile technique
under local anesthesia. Biopsies were taken from the left
and right abdominal subcutaneous, gluteal, and femoral regions.
After the adipose tissue biopsies, the intravenous catheter
was removed, and the volunteers ate breakfast and were
dismissed.

Calculations. Meal fatty acid oxidation for the 24 h after
the test meal was calculated using both the (3H) and (14C)
tracers to determine 3H2O and 14CO2 production, respec-
tively. The production of 3H2O was calculated by multiplying
the concentration of 3H2O in body water (using a urine
sample obtained 24 h after the test meal) by total body water
as measured by the 3H2O space and adding the 3H2O lost in
the urine over the 24 h. This value (total 3H2O dpm produced)
was divided by the total [3H]triolein consumed to calculate
the fraction of meal fatty acids oxidized in the first 24 h after
the meal.

The 14CO2 production was determined by multiplying the
14CO2 SA by the CO2 production rate, as measured by indi-
rect calorimetry, at each time point. The nocturnal CO2
production rate time points were not measured in this study,
although the 14CO2 SA was measured as described above. To
estimate nocturnal CO2 production rates, we used data from
a previous study (24) to develop a nonlinear model to predict
CO2 production throughout the night. This model used the
1800 and 0800 O2 consumption and CO2 production rates for
each individual. The data from this previous study (24) were
used because the size and composition of the evening meal
was similar to the evening meal used in the present study.

The model that was developed predicted ~75% of the vari-
ance in CO2 production rates of an independent sample.
Because the 14CO2 production rate is the product of the
14CO2 SA and CO2 excretion and because the majority of the
changes in 14CO2 SA occurred during the daytime (Fig. 2),
the errors associated with the use of modeled nocturnal CO2
production values should have had little impact on the 24-h
(area under the curve) 14CO2 production rates. To calculate
meal fatty acid oxidation using [14C]triolein, the area under
the 14CO2 curve was divided by the amount of [14C]triolein
consumed.

Meal fatty acid uptake into adipose tissue was calculated
as follows. The adipose tissue TG SA (dpm/mg) was divided by
the meal TG SA (dpm/mg) to predict the meal TG fatty acid
uptake (mg meal TG/g adipose tissue TG). We used the
average uptake values for the two different triolein tracers
for this calculation, except for the two volunteers who had
previously received a 3H fatty tracer (see Subject Charac-
teristics), in whom only the 14C values were considered. Visceral
fat mass was predicted using the computed tomography (CT)
measures of intra-abdominal and subcutaneous adipose tissue
combined with DEXA-measured abdominal fat as previ-
ously 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. The site-specific concentration of meal TG
per gram in adipose tissue TG was multiplied by the TG mass
to estimate total meal TG uptake in the different adipose
tissue depots as proposed by Marin et al. (21).

Statistics. All data are presented as means ± SD unless
otherwise stated. The fractional oxidation of meal fatty acids
in the 24 h after test meal consumption was compared be-
tween 3H and 14C tracers using a paired t-test. Linear re-
gression analysis was used to determine whether the two
means of assessing meal fatty acid oxidation were concor-
dant; CO2 fixation constants were not employed for this
comparison. Repeated-measures ANOVA was performed to
determine whether the relative amounts of 3H and 14C trac-
ers in the meal, chylomicrons, and adipose tissue were the
same. Comparison of adipose tissue SA between the right
and left sides was performed with paired t-tests, and Bland-
Altman plots were used to assess the degree of agreement
between values obtained from a single-sided biopsy and the
mean SA from bilateral adipose tissue biopsy results. A
Bonferroni correction was used when multiple statistical
tests were performed on data that were not part of the a
priori hypothesis testing. ANOVA was used to assess the
effect of tracer lot on the ratio of 3H to 14C in chylomicron
particles and adipose tissue (see below).

RESULTS

Subject characteristics. The men and women were
30 ± 5 and 28 ± 8 yr old, respectively. The body mass
index values of the men and women were 23.3 ± 2.0
and 21.0 ± 1.8 kg/m2. Total body water, as measured
by the 2H2O space, was 72 ± 2% of fat-free mass as
measured by DEXA in both men and women. Baseline
glucose and TG concentrations were normal in all sub-
jects. After completion of the studies, we discovered
that one male and one female volunteer had partici-
pated in studies that included the administration of
(\[^{3}\text{H}\]palmitate within the previous 2 yr. The residual \[^{3}\text{H}\] in adipose tissue of these two volunteers (the half-time of fatty acids in adipose tissue is \(~12\) mo (21)) rendered the adipose \[^{3}\text{H}\] data from their studies unusable, and thus meal uptake values are calculated solely using the adipose \[^{14}\text{C}\] lipid SA data. There was no \[^{3}\text{H}\] in the baseline (prestudy) urine samples from these volunteers; therefore, meal fatty acid oxidation was calculated for these two individuals using both approaches.

**Effects of variations in isotopic purity.** Two separate lots of \[^{3}\text{H}\]triolein and two separate lots of \[^{14}\text{C}\]triolein were used for this study. The manufacturer’s purity was listed as 98% for all tracers. Our initial HPLC test of isotopic purity did not confirm this degree of purity, but the results were not sufficiently disparate to cause immediate concern. After reviewing the results of the first two studies (Table 1), however, we reexamined the tracer purity using more complete HPLC procedures. For the \[^{3}\text{H}\]triolein, oleate was 82% of the radiolabeled tracer purity using more complete HPLC procedures. Of all daytime values. The meal ratio is that obtained upon counting an aliquot with similar amounts of radioactivity to the adipose tissue. The chylomicron value is the mean of \[^{3}\text{H}\]triolein and \[^{14}\text{C}\]triolein administered (1, \[^{3}\text{H}\] and \[^{14}\text{C}\] from the first lots; 2, \[^{3}\text{H}\] and \[^{14}\text{C}\] from the second lots; and 3, \[^{3}\text{H}\] from the first lot and \[^{14}\text{C}\] from the second lot).

### Table 1. Adipose tissue and meal \[^{3}\text{H}\]-to-\[^{14}\text{C}\] ratios

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<th>[^{3}\text{H}] to [^{14}\text{C}] Ratios</th>
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The ratio of \[^{3}\text{H}/^{14}\text{C}\] in the test meal, the chylomicron fraction of fresh plasma, and adipose tissue biopsied 24 h after meal consumption. The lipid extraction procedures were the same for the meal, chylomicrons, and adipose tissue. The chylomicron value is the mean of all daytime values. The meal ratio is that obtained upon counting an aliquot with similar amounts of radioactivity to the adipose tissue sample from that volunteer. Prefixes 1, 2, and 3 refer to the different combinations of lots of \[^{3}\text{H}\]- and \[^{14}\text{C}\]triolein administered (1, \[^{3}\text{H}\] and \[^{14}\text{C}\] from the first lots; 2, \[^{3}\text{H}\] and \[^{14}\text{C}\] from the second lots; and 3, \[^{3}\text{H}\] from the first lot and \[^{14}\text{C}\] from the second lot).
meal ratio, relative uptake in different tissue beds was unaffected by the choice of tracer.

Meal fatty acid oxidation measured with $[^{3}H]$- vs. $[^{14}C]$triolein. The majority (70%) of $^{14}CO_2$ production occurred during the first 10 h after consumption of the labeled meal. The breath $^{14}CO_2$ SA peaked (1,907 ± 747 dpm/mmol) at 6–7 h (Fig. 2) and returned to relatively low levels by the following morning (277 ± 76 dpm/mmol). Meal fatty acid oxidation averaged 30 ± 7% using $^{3}H_2O$ production values and 21 ± 5% using $^{14}CO_2$ production values during the 24 h after the test meal ($P < 0.0001$, $^{3}H_2O$ vs. $^{14}CO_2$). There was a positive correlation between the two approaches of calculating integrated meal fatty acid oxidation ($r = 0.79$, $P < 0.0001$; Fig. 4). In Fig. 4, open symbols depict the values from subjects who received the first (less pure) lot of $[^{3}H]$triolein. Note that there was no effect of the tracer lot combination on the meal fatty acid oxidation comparison.

Duration of meal tracer chylomicronemia. The TG concentrations in the chylomicron fraction of plasma and the $^{14}C$ concentration in the chylomicron and nonchylomicron fractions of plasma are depicted in Fig. 5. Plasma chylomicron and nonchylomicron $^{14}C$ (and $^{3}H$) TG were detected in plasma within 1 h of consuming the labeled meal. The major peak in chylomicron $^{14}C$ appeared 1 h after the consumption of the second meal that was provided 5 h after the test meal. The $^{14}C$-labeled TG in chylomicrons decreased throughout the remainder of the day, reaching near baseline levels at 24 h after the test meal.

The $^{14}C$ content of nonchylomicron TG is presented in Fig. 5C. The sharp peak in tracer concentration after the second meal is absent; however, the pattern of tracer presence in plasma is similar to that seen for the chylomicron $^{14}C$ TG. Twenty-four hours after the test meal, more $^{14}C$ was present in the nonchylomicron TG (97 ± 10 dpm/ml) than in the chylomicron TG (12 ± 2 dpm/ml).

Disposition of meal TG fatty acids. As noted above, 30 ± 7% of meal fatty acids were oxidized (using the $^{3}H_2O$ water method) during the 24 h after consumption of the labeled meal. We estimated that 31 ± 12% of meal fatty acids were stored in subcutaneous adipose tissue (19 ± 8% in upper body subcutaneous and 12 ± 6% in lower body subcutaneous fat); 38 ± 15% of meal
TG fatty acids were therefore unaccounted for after 24 h (a small portion remained in the circulation). If only the [14C]triolein data are used to assess subcutaneous meal fatty acid uptake for the volunteers who received the less pure lot of [3H]triolein, the percentage of meal fatty acids stored in subcutaneous adipose tissue (upper body and lower body) and percentage unaccounted for were 30.13% (20.8 and 12.6%) and 37.613%, respectively.

**DISCUSSION**

These studies were conducted to address a number of unresolved technical issues with respect to the meal fatty acid tracer/adipose tissue biopsy study design. It has been taken for granted that [3H] and [14C] fatty acid tracers could be used interchangeably to quantitatively measure meal fatty acid uptake by adipose tissue (19). We found, however, that slight differences in the purity of [3H]- vs. [14C]triolein are not uncommon and that these differences affect the apparent adipose tissue uptake of meal fatty acids. Although the absolute errors in predicting the portion of meal fatty acids taken up by adipose tissue might be small, the error would be consistent. Thus it may not be possible to make paired comparisons before and after an intervention using two different tracers unless the purity of the two is known to be identical. Differences in apparent adipose tissue fatty acid uptake could be due to tracer differences rather than intervention effects. We also examined the reliability of extrapolating from a single biopsy site to a larger adipose tissue depot fatty acid uptake. There was excellent agreement between the adipose TG SA from the right and left side biopsy sites. The slight asymmetry we observed was much less than the regional differences in meal fatty acid uptake.

Production of [14CO2] or [13CO2] from isotopically labeled meal fatty acid tracers has been used to estimate meal fatty acid oxidation (16); however, generation of [3H2O] from [3H] meal fatty acid tracers appears not to have been examined. Despite the differences in tracer purity, there was a good correlation between meal fatty acid oxidation predicted using [14CO2] production and [3H2O] production. As expected, the meal fatty acid oxidation predicted using [3H2O] generation was substantially greater than that predicted using [14CO2] production. The kinetics of tracer appearing in the circulation and breath ([14CO2]) were examined. The peak time of meal fatty acid oxidation, as measured by breath [14CO2] excretion, coincided with a peak [14C] chylomicronemia. Virtually all of the fatty acid tracer was cleared from the chylomicron portion of plasma within 24 h, although a small amount of residual tracer remained in the nonchylomicron TG fraction of plasma. Taken together, these findings suggest that the experimental design of meal fatty acid tracer/adipose tissue biopsy studies may need to be modified.

The extent to which apparently minor variations in isotopic tracer purity affected the predicted meal fatty acid uptake in adipose tissue was surprising. We anticipated that the relative presence of [3H] and [14C] in chylomicrons and adipose tissue should reflect their presence in the meal, especially considering all samples were handled in an identical fashion and were counted at the same level of radioactivity. We initially considered that isotope discrimination might explain the differences in the ratio of [3H] to [14C] between the meal and adipose tissue but reexamined the purity of the different tracers before coming to this conclusion. We found a significant association between isotopic impurity in our tracer lots and variations in relative uptake by adipose tissue (Table 1). This finding strongly suggests that the fatty acid composition of the tracer is a more likely explanation for the lack of ideal agreement between the ratio of [3H] to [14C] in the meal and adipose tissue. For example, medium-chain fatty acids are less readily reesterified than long-chain fatty acids (28) and thus are more likely to be oxidized. If the impurities in one of our tracers were medium-chain fatty acids, this could have affected the ratio of [3H] to [14C] between the meal and adipose tissue. Note that for the second combination of tracers (Table 1) the ratio of [3H] to [14C] in chylomicrons and adipose tissue was nearly identical and was significantly greater than that observed in the meal. This would be consistent with the presence of an isotopically labeled fatty acid in the meal that was not incorporated into chylomicrons. Differences in tracer purity between [3H]- and [14C]-
triolein were associated with 7–10% differences in the apparent adipose tissue uptake of meal fatty acids. For example, if subcutaneous meal fatty acid uptake was estimated at 30% with one tracer it might be 27% with another tracer. Thus, when comparing absolute rates of meal fatty acid uptake between $^{14}$C- and $^3$H-labeled tracers, it is important to ensure that the isotopic purity of the two compounds is virtually identical.

As expected, meal fatty acid oxidation predicted using the generation of $^3$H$_2$O was substantially greater than that predicted using the production of $^{14}$CO$_2$. This is likely because of significant fixation of carbons generated from $\beta$-oxidation of fatty acids (27). There may also be incomplete appearance of hydrogen (generated from the oxidation of $^3$H fatty acids) via exchange with the body hydrogen pool, although this is thought to occur less extensively (26). The relatively good agreement between meal fatty acid oxidation rates measured using the two approaches (Fig. 4) suggests that the production of $^3$H$_2$O after the administration of $^3$H fatty acid tracers may be a reliable and straightforward means to measure integrated meal fatty acid oxidation. Although the same is likely true for the use of $^{14}$C or $^{15}$C fatty acid tracers, it is necessary to perform additional, condition-specific experiments to document the degree of carbon fixation when these compounds are used. In addition, variations in the natural abundance of $^{13}$C in foods require that additional control experiments be carried out when this tracer is used for meal studies.

The timing of the peak appearance of radiolabeled meal fatty acid tracer in the plasma chylomicron fraction deserves mention for two reasons. Note that the peak occurred 6 h after the ingestion of the labeled meal, 1 h after the ingestion of a second meal that did not contain a radiolabeled fatty acid. This phenomenon has been previously reported with meals of differing fatty acid content (5) and may reflect delayed fat absorption, delayed epithelial chylomicron secretion, or delayed transport of chylomicrons from the lymphatic system into the circulation. In the context of adipose tissue biopsy studies, it should be recalled that adipose tissue LPL activity increases significantly ~6 h after an insulin challenge (25). Thus induction of LPL would appear to coincide with the peak appearance of meal-derived fatty acids into the circulation. Attempts to relate adipose tissue LPL activity to in vivo measures of meal fatty acid uptake (1) may require two biopsies, one at the time of peak radiolabeled chylomicronemia (for measurement of LPL activity) and a second 24 h later (when the vast majority of the tracer has been cleared).

Meal fatty acid oxidation rates using $^{14}$C-triolein in this study are not substantially different from those reported by Jones et al. (16). The continued presence of $^{14}$CO$_2$ in breath 24 h after consumption of the labeled meal suggests ongoing oxidation of the meal fatty acids and/or continued release of $^{14}$C that mixed in body carbon pools.

We calculated the percentage of meal TG fatty acids stored in subcutaneous adipose tissue 24 h after the labeled meal and found it to be similar to that reported by Marin and colleagues (20, 21) despite the dramatic differences in the fat content of the meals provided. It is unlikely that 24-h meal fatty acid oxidation in the studies by Marin and colleagues was as great as the ~30% we observed, given the limited ability of humans to increase fat oxidation in response to greater dietary fat intake (6). After accounting for subcutaneous meal fatty acid uptake and meal fatty acid oxidation, a significant portion of meal fat disposal remained unaccounted for. Marin and co-workers (18, 20) reported substantial uptake of meal TG fatty acids in visceral adipose tissue, suggesting that some or all of the "missing" dietary fatty acids in our study were taken up by intra-abdominal fat, a site inaccessible to biopsy except in patients undergoing surgery (18, 20). Given the similarity in the percent meal fatty acid uptake in subcutaneous adipose tissue between our study and that of Marin and colleagues (20, 21) and the possible dissimilarity in meal fatty acid oxidation, future studies will be needed to test for differences in intra-abdominal fat storage under conditions of varying dietary fat intake. Other possible storage sites for the missing fatty acids include intramuscular TGs, intrahepatic TGs, or a greater concentration of meal fatty acids in subcutaneous sites that were not biopsied in these studies. We have collected adipose tissue from the triceps ($n = 4$) and lower leg (calf) regions ($n = 1$) in research volunteers undergoing meal fatty acid tracer studies, however, and the lipid SA in these sites was less, rather than greater, than the abdominal subcutaneous lipid SA (unpublished data).

In summary, these studies have addressed several technical issues that relate to studies employing the meal fatty acid tracer/adipose tissue biopsy technique. We found that slight differences in the purity of the tracers used for these studies can result in small but consistent differences in the apparent uptake of meal fatty acids by adipose tissue. Future studies will need to consider issues of isotope purity in the study design. We found that a unilateral adipose tissue biopsy is highly likely to provide representative data with respect to meal fatty acid uptake. Integrated values for meal fatty acid oxidation using the generation of $^3$H$_2$O agrees well with those predicted using $^{14}$CO$_2$ production rates; however, the measures cannot be used interchangeably. The peak appearance rates of meal-derived tracer in plasma occur after the ingestion of a second, unlabeled meal, and this suggests that "extra" biopsies may be needed (for LPL measurement) if one hopes to relate adipose tissue LPL activity to in vivo measured meal fatty acid uptake. Oxidation accounted for a significant fraction of dietary fatty acid disposal during the first 24 h after meal ingestion. Documenting the contribution of this component of dietary fatty acid metabolism should be considered an important aspect of future studies using this technique.

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REFERENCES


