Patterns of dietary fat intake have been associated with saturated fat, and an increase in the relative consumption of fat, a decrease in the ratio of polyunsaturated fat, a marked "trafficking" of dietary oleic, linolenic, and stearic acids in fasted or fed lean rats. Am J Physiol Endocrinol Metab 278: E1124–E1132, 2000.—Increasing evidence supports the notion that there are significant differences in the health effects of diets enriched in saturated, as opposed to monounsaturated or polyunsaturated fat. However, the current understanding of how these types of fat differ in their handling by relevant tissues is incomplete. To examine the effects of fat type and nutritional status on the metabolic fate of dietary fat, we administered 14C-labeled oleic, linolenic, or stearic acid with a small liquid meal to male Sprague-Dawley rats previously fasted for 15 h (fasted) or previously fed ad libitum (fed). 14CO2 production was measured for 8 h after tracer administration. The 14C content of gastrointestinal tract, serum, liver, skeletal muscle (soleus, lateral, and medial gastrocnemius), and adipose tissue (omentum, retroperitoneal, and epididymal) was measured at six time points (2, 4, 8, 24, and 48 h and 10 days) after tracer administration. Plasma levels of glucose, insulin, and triglyceride were also measured. Oxidation of stearic acid was significantly less than that of either linolenic or oleic acid in both the fed and fasted states. This reduction was in part explained by a greater retention of stearic acid within skeletal muscle and liver. Oxidation of oleate and stearate were significantly lower in the fed state than in the fasted state. In the fasted state, liver and skeletal muscle were quantitatively more important than adipose tissue in the uptake of dietary fat. In contrast, adipose tissue was quantitatively more important than skeletal muscle or liver in the fed state. The movement of carbons derived from dietary fat between tissues is a complex time-dependent process, which varies in response to the type of fat ingested and the metabolic state of the organism. Dietary fat; skeletal muscle; adipose tissue; fuel partitioning; triglyceride.

Over the course of human evolution, the nutrient content of the diet has changed dramatically (16). Compared with ancient times, the diet of modern societies is characterized by an increase in the consumption of fat, a decrease in the ratio of polyunsaturated fat to saturated fat, and an increase in the relative consumption of ω-6 fatty acids (15). These changes in the patterns of dietary fat intake have been associated with an increase in the prevalence of diabetes, coronary artery disease, and obesity (7, 35, 37). A growing body of literature suggests that the metabolic effects of diets high in saturated fat are quite different from those high in either monounsaturated fats or ω-3 polyunsaturated fats (10, 18). Diets high in saturated fat have been associated with reductions in insulin sensitivity and increases in serum low-density lipoproteins and body weight (14, 21–23, 28, 30, 38). One hypothesis that has been offered is that, as different fatty acid types are incorporated into cell membrane phospholipid, alterations in membrane fluidity, clotting, and vascular reactivity are produced (12, 24, 29, 33). An alternative hypothesis is that the differences in the health effects of these fats come from differences in the metabolic handling of these different fatty acids by relevant tissues.

We have been interested in how abnormalities in the metabolism of dietary fat might relate to the development of obesity. Our previous studies examined the movement of 14C-labeled oleic acid between the gastrointestinal (GI) tract, skeletal muscle, liver, and adipose tissue in genetically obese Zucker rats. These studies demonstrated a reduction in the oxidation and excessive storage of a dietary fat tracer in obese rats relative to lean. More specifically, the previous studies suggested a defect in the handling of dietary fat by skeletal muscle in both obese and reduced obese rats. To more completely define the time course of the handling of dietary fat, we have also performed dietary fat tracer studies in lean Sprague-Dawley rats (4). These studies suggested that the movement of carbons derived from dietary fat between tissues is a complex and dynamic process over time, perhaps better described by the term "trafficking" than by the more widely used term "partitioning." They showed that, in lean rats, skeletal muscle and liver are quantitatively more important than adipose tissue in the early clearance of dietary fat. The conclusions of these studies, however, are limited to the specific type of dietary fat used (oleate) and the metabolic state studied (only previously fasted rats were studied). Would the results of these studies be the same if another fatty acid tracer had been used or if the study had been conducted in fed rats? We wondered what the most representative fatty acid tracer would be to use in studies of the metabolism of dietary fat. A basic assumption in all tracer studies is that the tracer will behave metabolically in an identical manner to the tracee. Most metabolic tracer studies have used 14C- or
13C-labeled palmitic acid or, less commonly, oleic acid. This has been done on the basis of studies in which the fractional uptake of different fatty acids by skeletal muscle was found to be similar by arteriovenous balance (19). It is less clear that the handling of different fats by the GI tract, adipose tissue, and liver is similar, or that the intracellular storage or oxidation of the different fats is similar. In addition, many previous studies have been conducted in the fasted state. We speculated that some of the effects of diets varying in fatty acid composition were due to differences in the postigestive movement of these different fatty acids between tissues. In addition, we and others (2, 34) have demonstrated that lipoprotein lipase in adipose tissue and muscle is regulated in a manner that would predict that the tissue-specific clearance of dietary fat might be quite different in the fed state than in the previously fasted state.

To date, the trafficking of different dietary fats in the fasted and fed states has not been systematically examined. In an effort to more completely examine the effects that nutritional status and degree of saturation have on the metabolic fate of dietary fat after ingestion, we have performed tracer studies in both fasted and fed rats, using three dietary fat tracers: stearic acid (18:0), oleic acid (18:1, ω-9), and linoleic acid (18:3, ω-3). These studies demonstrate significant effects of both fat type and nutritional state on the trafficking of dietary fat.

METHODS

Animals. Male Sprague-Dawley rats (n = 135) weighing 250–300 g were obtained from Sasco or Harlan (17). The rats were housed in the Surgical Research Facility at the Denver Health Medical Center (DHMC) in a temperature-controlled, 12:12-h light-dark cycle environment. Before studies were performed, rats were fed a semisynthetic diet containing 21% protein, 56% carbohydrate, and 23% fat, with a polyunsaturated-to-saturated ratio of 2.1 (Research Diets no. D12449L) for 7 days before surgery. Protocols for these experiments were approved by the Animal Care and Use Committees at the University of Colorado Health Sciences Center and DHMC.

Surgery. Gastric feeding tubes were placed in a manner similar to that described by Elizalde and Scalaﬁani (17). Rats were fasted overnight before surgery. After anesthesia was introduced (ketamine 80 mg/kg + xylazine 12 mg/kg), a midline abdominal incision was made and the stomach was withdrawn from the abdominal cavity. A 3-mm incision was made along the midportion of the greater curvature of the stomach, and a purse-string suture was placed along the margin of the incision. A Silastic tube (no. AST062095, Dow Corning, Kinnsburg Instruments, Pasadena, CA) was introduced into the lumen of the stomach through this incision. The tube had been previously prepared with a drop of silicone rubber near the tip. The purse-string suture was then tightened and tied to secure the feeding tube in the gastric lumen. A small piece of marlex mesh (Davol, Cranston, RI) was then glued to the tube and the outer surface of the stomach with methyl-methacrylate to further secure the tube in place. The remaining length of the tube was brought through the abdominal wall, tunneled subcutaneously to an exit site in the interscapular region, and trimmed to length, and a luer loc hub from an intravenous catheter was glued to the tube.

The hub was then capped. Both surgical sites were closed with interrupted silk sutures. Rats recovered from surgery for 24 h. After recovery, and on a daily basis thereafter until studies were performed, a liquid meal containing 3 kcal (16% protein, 64% carbohydrate, and 20% fat; Ensure, Ross Laboratories) was introduced through the feeding tubes to acclimate the rats to being fed in this manner.

Tracer feeding experiments. Rats were allowed to recover from surgery for 7 days before tracer studies were performed. During this time, rats were weighed each day. Any rats that lost >10% of their presurgery body weight during the postoperative period were removed from the study. 14C feeding studies were conducted as previously described (3, 4). Before tracer was administered, rats were either fasted for 15 h (fasted) or allowed to eat ad libitum (fed). At 0830 on the morning of the study, rats were given a tracer amount of linoleic, oleic, or stearic acid labeled at the 1 position with 14C (8.3 × 106 dpm total dose, specific activity = 52–55 μCi/mmol; Amersham) in olive oil followed by a “chase” of cold olive oil to ensure complete delivery of the tracer through the feeding tube. This was immediately followed by an Ensure liquid meal, as described above. The total nutrients delivered in this test meal then included 4 kcal, with 48% fat, 35% carbohydrate, and 17% protein. The fat content of the meal was 163 mg, with 65% monounsaturated, 23% polyunsaturated, and 12% saturated fat. Test meals had the same composition in all groups with the exception of the tracer quantity of the labeled fatty acid being tested. After administration of the tracer, rats were placed in an airtight respiratory chamber. Room air was passed through barium hydroxide lime (Baralyme, Allied Healthcare Products, St. Louis, MO) to remove CO2 and then passed through the chamber at a flow rate of 3.0 l/min. The effluent CO2 from the chamber was collected over 20-min intervals in 3.0-ml aliquots of 2:1 mixture of methanol and methylbenzenothionium (hyamine) hydroxide. The 14C content of these samples was then measured with a Beckman LS6500 scintillation counter. Background 14C activity, determined by counting a sample containing only scintillation fluid and hyamine hydroxide, was subtracted from experimental values. Exhaled CO2 was collected in this manner for 8 h after tracer administration or until the time of tissue collection for the 2- and 4-h time points. Rats representing time points of >8 h were returned to their cages and given ad libitum access to food before tissue collection at the later time points.

Determinations of 14C content in tissues. At 2, 4, 8, 24, and 48 h and 10 days after the administration of tracers, tissues were collected for determination of 14C content. Studies were performed in a random order with regard to nutritional state (fed vs. fasted), tracer (linolenate, oleate, or stearate), and time point to minimize any effect of season or systematic laboratory/procedural drift on the data. Rats were deeply anesthetized with pentobarbital, and skeletal muscle samples including lateral gastrocnemius (mixed fiber type), medial gastrocnemius (predominantly glycolytic), and soleus (predominantly oxidative) were removed and cleaned of any visible fat and connective tissue. Samples were frozen in liquid nitrogen and then stored at −80°C until analyzed. A sample of blood was obtained from the vena cava, and rats were then euthanized with an intracardiac injection of pentobarbital. Blood was centrifuged, and the serum was stored at −20°C until analyses of insulin, triglyceride, and glucose.
were performed. The entire liver was removed and weighed. Samples of liver were then frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\). The GI tract was removed, stripped of all omental fat, and weighed. Epididymal and retroperitoneal fat pads were also removed and weighed.

The GI tract liver and muscle samples were homogenized in ice-cold 0.9% saline. Duplicate samples (0.5 ml) of tissue homogenate were then digested with 1.0 ml of tissue solubilizer (Solvable; NEN) at 50°C overnight. Samples were bleached with H2O2, and 14C content was determined by scintillation counting. The 14C content of serum samples was also determined in this manner.

The 14C content of adipose tissue samples was measured after extraction of lipid with chloroform-methanol (2:1, vol/vol). Phases were separated with the addition of H2SO4 and centrifugation. The lower phase was removed and allowed to dry overnight under nitrogen gas, and 14C content was measured by scintillation counting. The lipid content of the remaining carcass was determined by lipid extraction after homogenization.

After other assays. Insulin levels were measured with a commercially available radioimmunoassay (Linco catalog no. R1-133). Serum triglyceride was assayed by measuring glycerol released after acid hydrolysis of the sample (Sigma kit no. 337-b). Serum glucose was measured with a Yellow Springs Instruments model 1500 glucose analyzer.

Calculations and statistics. The 14C content of each tissue was calculated from the 14C activity per gram of tissue multiplied by the total weight of the tissue. Whole body skeletal muscle 14C content was calculated by multiplying the average 14C activity per gram in lateral and medial gastrocnemius by the body mass times the percent skeletal muscle [38% of body weight as estimated from the tissue data of Caster et al. (11)]. Similarly, whole body adipose tissue 14C content was calculated by multiplying the average 14C activity per milliliter of serum by 0.0385 (%body mass accounted for by erythrocyte 14C activity per gram in lateral and medial gastrocnemius by the body mass times the percent skeletal muscle [38% of body weight as estimated from the tissue data of Caster et al. (11)]. Whole body adipose tissue 14C content was calculated by multiplying the average 14C activity per milliliter of serum by 0.0385 (%body mass accounted for by serum) times body mass (11). All data are presented in graphic form as the mean ± SE of 4–6 rats per time point per tracer per metabolic state. The data were inspected for apparent group differences, and one- (fasted vs. fed, for hormone and substrate data) or two-way (fasted/fed and/or stearate/oleate) ANOVA was performed between groups (tissue, time point) where appropriate (Sigma Stat, SPSS, Chicago, IL). Trends within a group across time were tested for with a pairwise multiple comparison procedure.

### RESULTS

Rat weights and hormone and substrate concentrations. Mean rat weights after the placement of gastric feeding tubes declined by 7 g. This decline was not statistically significant. Weights returned to baseline and were stable by postoperative day 5. Serum levels of insulin, glucose, and triglyceride seen in fasted or fed rats are given in Table 1. Because the caloric content and macronutrient composition of the chronic diet and test meals were similar, these values were not different in the different fatty acid tracer groups. Therefore, data from the three tracer groups (stearate, oleate, and linoleate) either fasted or fed) have been pooled. Serum insulin levels were significantly higher in fed rats for 2 h after the tracer administration (P < 0.003). Interestingly, at 48 h there was a trend for insulin levels to be higher in fasted rats (P = 0.056). This is likely due to rebound hyperphagia after the initial period of fasting. The rise in insulin levels seen in fasted rats over time was significant (P < 0.05, 48 h vs. 2, 4, or 8 h). Serum glucose levels were measured over the time course of the experiment. There were no significant differences between groups at any time point. As might be expected, the mean level of triglyceride was significantly higher in fed rats compared with fasted rats at early time points (P = 0.039 between groups at 2 h, P < 0.001 at 4 h). The increase in serum triglyceride levels seen in fasted rats was also significant (P < 0.05 at 4 h vs. 8 h and at 4 vs. 8 h). The increase in serum triglyceride levels seen in fasted rats was also significant (P < 0.05 at 4 h vs. 8 h and at 4 vs. 8 h).

Tracer oxidation. The production of 14C-labeled CO2 is shown in Fig. 1. In all groups, tracer oxidation appears to peak between 4 and 8 h. The striking finding is the reduced production of 14CO2 in the animals that received labeled stearate (P < 0.05). Oxidation of dietary oleate or stearate over the 8 h after administration was found to be significantly greater in fasted rats (P < 0.05), although this effect was not seen in the linoleate group. Fed effects were most pronounced over the first 2 h after meal administration.

Tracer content in serum and tissues. Differences in tracer oxidation could be due to decreased GI absorp-

### Table 1. Serum insulin, glucose, and triglyceride levels after administration of dietary fat tracers with a small meal

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Insulin, ng/ml</th>
<th>Glucose, mg/dl</th>
<th>Triglyceride, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fasted</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>3.36 ± 0.61*</td>
<td>0.84 ± 0.28</td>
<td>175.1 ± 12.9</td>
</tr>
<tr>
<td>4 h</td>
<td>2.83 ± 0.33*</td>
<td>0.99 ± 0.25</td>
<td>190.5 ± 14.6</td>
</tr>
<tr>
<td>8 h</td>
<td>2.11 ± 0.26*</td>
<td>0.64 ± 0.12</td>
<td>158.3 ± 14.2</td>
</tr>
<tr>
<td>24 h</td>
<td>3.08 ± 0.42</td>
<td>2.23 ± 0.43</td>
<td>177.2 ± 16.9</td>
</tr>
<tr>
<td>48 h</td>
<td>2.28 ± 0.24</td>
<td>3.33 ± 0.43</td>
<td>146.7 ± 12.9</td>
</tr>
<tr>
<td>10 day</td>
<td>2.67 ± 0.65</td>
<td>1.62 ± 0.36</td>
<td>179.3 ± 12.9</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8–12 rats/group. Time 0 represents the time of tracer administration. Rats fasted overnight are compared with rats allowed to eat ad libitum overnight. Because there were no significant differences, data from rats administered different fat types (dietic, linoleic, and stearic acids) were pooled. Significant differences were observed in insulin levels between fasted and fed rats at the 2-, 4-, and 8-h time points (P < 0.003). No significant differences in serum glucose levels were found at any time point. Triglyceride levels were significantly higher in fed rats at the 2- and 4-h time points (P < 0.04). Triglyceride levels declined significantly in fed rats between 2 and 8 h and rose significantly in fasted rats between 8 and 48 h.
The 14C content of serum is depicted in Fig. 3. The values represent 14C content within the total serum pool at each time point. 14C activity within the serum pool could be associated with dietary fat being absorbed, nonesterified fatty acids released into the circulation after lipoprotein lipase-mediated hydrolysis, or 14C in bicarbonate or dissolved CO2. The only significant difference found was between tracer groups at the 2-h time point (P = 0.02), with the difference being largely accounted for by the difference between linoleic acid and stearic acid.

14C content of the liver is depicted in Fig. 4. The most striking finding is the retention of tracer within the liver in the animals receiving labeled stearic acid at 8 and 24 h. Also of note is the retention of labeled linoleic acid in the livers of fasted rats relative to fed rats. A number of significant differences between groups were identified. At the 8-h time point, there is a significant effect of tracer type (P < 0.001), with stearate being significantly higher than either oleate or linolenate. At the 24-h time point, there was also a significant effect of fat type (P < 0.001), with labeled...
stearate content in the liver being significantly greater than either oleate or linolenate. In addition, a significant difference was seen between the fasted and fed linolenic acid groups at this time point ($P < 0.05$). At the 48-h time point a significant effect of fat type was seen, with stearic acid again being greater than oleic acid ($P < 0.01$).

$^{14}$C content of skeletal muscle is depicted in Fig. 5. Although less dramatic than what was seen in the liver, again the main finding is increased retention of the stearate tracer. This difference was statistically significant at the 24-h time point ($P < 0.001$), with the stearic acid group being greater than either the oleic or linolenic acid groups.

The $^{14}$C content of adipose tissue is depicted in Fig. 6. As was seen in our previous studies, tracer content in adipose tissue continues to rise after it has already peaked in liver and skeletal muscle. In fact, it doesn't peak until 48 h after ingestion in most groups, and it doesn't peak until 10 days in the stearic acid groups. The other obvious finding in this tissue was the marked feeding/fasting effects in the unsaturated fat groups. Differences between fed and fasted groups were significant ($P < 0.002$) at all time points, and differences between fat types (linolenic, oleic, and stearic) were significant at the 8-h, 24-h ($P < 0.001$), and 10-day ($P = 0.007$) time points. The differences at the 8- and 24-h time points are the result of the greater adipose tissue tracer content in the oleate- and linolenate-fed groups. At the 10-day time point, the differences are due to the greater adipose tissue tracer content in the fasted stearate groups.

DISCUSSION

In this study, we have attempted to examine the effects of degree of saturation and nutritional state on the postprandial movement of dietary fat tracers between tissues. Both appear to have major effects on fat trafficking. Specifically, the postprandial oxidation of the saturated fat stearic acid was found to be considerably less than that of either oleic or linolenic acids. One way of understanding this reduction in the oxidation of stearic acid after ingestion is to follow the content of tracer in several tissues over time. These relationships...
for linolenic acid and stearic acid administered to fasted rats are depicted in Fig. 7, which suggests that the reduction in the oxidation of stearic relative to linolenic acid is not due to a delay in GI absorption but rather to a retention of this fatty acid within the liver and skeletal muscle pools. The movement of stearic acid to the ultimate site of oxidation appears to be restricted; its trafficking "shifted to the right" in relationship to time. Most of the recent work examining the effects of dietary fat type on insulin sensitivity has focused on the degree to which the ingestion of these fats influences plasma membrane fluidity (33). It is also possible that differences in fluidity between fatty acids with differing degrees of saturation affect their movement from the GI tract to the plasma compartment, into liver and skeletal muscle, and ultimately into mitochondria where they are oxidized. Saturated fat may be a less suitable substrate for lipoprotein lipase, may bind less effectively to fatty acid-binding proteins, or may be less able to move freely within cell membranes (9, 26). These alterations in fatty acid movement may underlie some of the effects seen with diets enriched in saturated fat.

In a previous study that systematically examined the effect of chain length and degree of saturation on the oxidation of dietary fat, Leyton et al. (25) found some of these same features. This study suggested that fatty acid oxidation increased with higher degrees of unsaturation but was inversely related to chain length. In addition, there was increased retention of saturated fat in liver 24 h after tracer administration compared with either mono- or polyunsaturated fats. However, this study had a number of important limitations. First, the rats used were quite young (21 days) and weighed 60–80 g. Second, measurements did not take into account the different specific activities of the different tracers used. This is to say that the $^{14}$C per nanomole of carbon varied between tracer groups as a result of differing chain lengths. Third, the effect of nutritional state was not studied. Fourth, in these studies relevant tissues, including skeletal muscle, GI tract, and adipose tissue...
pose tissue were not sampled; as a result, it was not possible to speculate on the mechanisms underlying the reduced oxidation of saturated fat. Finally, serum, carcass, and liver distribution of tracer was examined only at a single time point. Another study, by Bottino et al. (6), also found lower oxidation of a saturated fat tracer compared with mono- or polyunsaturated fat tracers. By sampling multiple tissues over a more extensive time course, the current study extends the observations of Leyton et al. and Bottino et al.

A second finding of the current study was that the oxidation of dietary fat was in general lower in the fed compared with the fasted state. This reduction in tracer oxidation seen in fed rats was associated with an increase in the fraction of the dietary fat tracer stored in adipose tissue. This is depicted in Fig. 8, which compares the trafficking of oleic acid in the fed and fasted states. These data are in accord with predictions made by Tan et al. (36) many years ago on the basis of estimates of total body muscle and adipose tissue lipoprotein lipase activities in the fasted or fed states. Specifically, in fasted rats, skeletal muscle and liver play a quantitatively more important role in the clearance of dietary fat than in that of adipose tissue during the 8 h after ingestion, independent of the type of fat. However, in the fed state, adipose tissue plays an important role right from the start. It was surprising to see the tracer content in adipose tissue rise between 24 and 48 h after administration. This is in line with data obtained by Marin et al. (27) in humans, which showed that a dietary fat tracer gradually accumulates in adipose tissue for 1 mo after ingestion. This finding highlights the importance of adipose tissue as a "storage site of last resort" for dietary fat. In contrast, it appears that skeletal muscle and liver play important roles in the storage of dietary fat after periods of negative caloric balance.

In this study, fat tracers were administered in the diet. This design is a departure from the traditional approach used in classic studies of chylomicron triglyceride (Tg) metabolism, in which the tracer is delivered into the vascular compartment (1, 8, 20), but it has been used in studies of both animals and humans by a number of investigators (5, 31). The traditional experimental design that creates a steady state allows quantitative measures to be calculated of the rate of appearance or disappearance of the metabolite of interest to...
and from the vascular compartment. A central issue in this experimental design, then, is the specific activity (SA) of the tracer in the vascular compartment. In the present study, the plasma SA is not constant over the duration of the study. In particular, in the fed state the plasma Tg concentration is increased relative to the fasted state. This difference would produce a decreased SA of $^{14}$C/Tg within the plasma compartment in the fed state, and a reduction in the rate of $^{14}$CO$_2$ evolution would be an expected consequence. An alternative conceptual framework that we have employed in the current study is to “trace the meal.” Given this as the starting point, the relevant SA is the SA of tracer in the meal. A reduction in the production of $^{14}$CO$_2$ as a result of dilution of the tracer upon entering the plasma compartment in fed rats does not mean that the assessment of the oxidation is artificially low; it simply suggests an etiology for that reduction. The present study attempts simply to follow the movement of dietary fat tracers through the body. No attempt is made to quantitatively assess hepatic Tg production or to comment on peripheral Tg clearance. Because the tracer is placed directly into the relevant pool (the meal), the behavior of the tracer should model the behavior of dietary fat quite well. An SA for tracer in the dietary fat pool could have been calculated; however, because the different tracers behave differently, this method seems inappropriate. Alternatively, an SA could be calculated as labeled saturated fat per total saturated fat in the meal, labeled polyunsaturated fat per unlabeled polyunsaturated fat, and labeled monounsaturated fat per unlabeled monounsaturated fat in each experimental condition. Because all meals contained the same dose of tracer, yet the content of monounsaturated fat in the meal was high and the saturated fat was quite low, this type of calculation would make the reduced oxidation seen with stearic acid even more prominent. The data are presented as disintegrations per minute, as this is the most conservative way to present the data. A problem introduced by this design is that the metabolites are not in steady state, and as a result the quantitative analyses that can be made to the experimental design, then, is the specific activity (SA) of the tracer in the vascular compartment. In the present study, the plasma SA is not constant over the duration of the study. In particular, in the fed state the plasma Tg concentration is increased relative to the fasted state. This difference would produce a decreased SA of $^{14}$C/Tg within the plasma compartment in the fed state, and a reduction in the rate of $^{14}$CO$_2$ evolution would be an expected consequence. An alternative conceptual framework that we have employed in the current study is to “trace the meal.” Given this as the starting point, the relevant SA is the SA of tracer in the meal. A reduction in the production of $^{14}$CO$_2$ as a result of dilution of the tracer upon entering the plasma compartment in fed rats does not mean that the assessment of the oxidation is artificially low; it simply suggests an etiology for that reduction. The present study attempts simply to follow the movement of dietary fat tracers through the body. No attempt is made to quantitatively assess hepatic Tg production or to comment on peripheral Tg clearance. Because the tracer is placed directly into the relevant pool (the meal), the behavior of the tracer should model the behavior of dietary fat quite well. An SA for tracer in the dietary fat pool could have been calculated; however, because the different tracers behave differently, this method seems inappropriate. Alternatively, an SA could be calculated as labeled saturated fat per total saturated fat in the meal, labeled polyunsaturated fat per unlabeled polyunsaturated fat, and labeled monounsaturated fat per unlabeled monounsaturated fat in each experimental condition. Because all meals contained the same dose of tracer, yet the content of monounsaturated fat in the meal was high and the saturated fat was quite low, this type of calculation would make the reduced oxidation seen with stearic acid even more prominent. The data are presented as disintegrations per minute, as this is the most conservative way to present the data. A problem introduced by this design is that the metabolites are not in steady state, and as a result the quantitative analyses that can be performed on the data are limited. However, we believe that this limitation is counterbalanced by the physiological nature of the experiment and the comprehensive tissue tracer content information obtained at multiple time points.

A second limitation is the nutritional context in which the studies were performed. The results of this study describe the behavior of these fatty acids in the setting of a small meal containing a relatively high fat content, in particular a relatively high content of monounsaturated fat, given to rats chronically consuming a diet containing 23% fat. This baseline diet was chosen because it more closely mimics the diet consumed by humans than the 10% fat content of standard rat chow. The composition of the baseline diet, the caloric content of the test meal, the fat content, and the type of fat in the test meal likely play important roles in determining the overall pattern of trafficking of dietary fat. The specific effect of varying these parameters on trafficking, however, would need to be determined experimentally. Third, the exact biochemical nature of the compounds labeled with $^{14}$C is not known. It is possible that both the chain length and degree of saturation may change after ingestion (13). However, these possibilities do not alter the conclusion that ingested stearic acid is metabolized differently than ingested linoleic acid. Finally, $^{14}$CO$_2$ recovered may not quantitatively reflect total rates of dietary fat oxidation because of dilution in the bicarbonate pool and fixation of label within isotopic exchange reactions (32). These differences may even be systematically different between groups, in particular between the fed and fasted states. However, because of the number of tissues tested, the length of the time course examined, the consistent picture seen when the data are taken as a whole, and the lack of accepted approaches to correcting tracer oxidation estimates in the fed state, it seems reasonable to conclude that the present studies do suggest important differences in the metabolic handling of different fatty acids that should be considered in future studies.

In summary, the present study sought to examine the effects of nutritional state and the type of fatty acid ingested on the trafficking of dietary fat. Each of these appears to have important effects. Most studies of fat metabolism have utilized palmitate or oleate tracers administered to fasted animals or humans. It has been assumed that the metabolic behavior of these tracers is similar to that of other fats and that experimental results obtained in the fasting state can be extrapolated to the fed state. The present study suggests that these assumptions have limitations. In particular, the effect of fat type may vary depending on the tissue and the time point examined. Although stearate was exsively stored in liver and skeletal muscle at intermediate time points, the content in adipose tissue was actually reduced at these same time points relative to the unsaturated fats studied. The effect of the degree of saturation on the oxidation of a fatty acid ingested in the diet appeared to be dramatic. These differences should be considered when a fat tracer is selected for metabolic studies. Perhaps most importantly, these findings may have relevance to understanding the effects of saturated fat on insulin sensitivity and the health effects of diets high in saturated fat.

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