Differential oxidation of individual dietary fatty acids in humans

James P DeLany, Marlene M Windhauser, Catherine M Champagne, and George A Bray

ABSTRACT
Background: Dietary fatty acids that are more prone to oxidation than to storage may be less likely to lead to obesity.
Objective: The aim of this study was to determine the effect of chain length, degree of unsaturation, and stereoisomeric effects of unsaturation on the oxidation of individual fatty acids in normal-weight men.
Design: Fatty acid oxidation was examined in men consuming a weight-maintenance diet containing 40% of energy as fat. After consuming the diet for 1 wk, subjects were fed fatty acids labeled with $^{13}$C in the methyl or carboxyl position (10 mg/kg body wt). The fatty acids fed in random order were laurate, palmitate, stearate, oleate, elaidate (the trans isomer of oleate), linoleate, and linolenate blended in a hot liquid meal. Breath samples were collected for the next 9 h and the oxidation of each fatty acid was assessed by examining liberated $^{13}$CO$_2$ in breath.
Results: Cumulative oxidation over the 9-h test ranged from a high of 41% of the dose for laurate to a low of 13% of the dose for stearate. Of the 18-carbon fatty acids, linolenate was the most highly oxidized and linoleate appeared to be somewhat conserved. $^{13}$C recovery in breath from the methyl-labeled fatty acids was $\approx 30\%$ less than that from the carboxyl-labeled fatty acids. Oxidation of the long-chain fatty acids decreases with increasing carbon number. Am J Clin Nutr 2000;72:905–11.

KEY WORDS Lauric acid, myristic acid, palmitic acid, oleic acid, trans fatty acids, stearic acid, linoleic acid, linolenic acid, oxidation, dietary fatty acids, obesity

INTRODUCTION
The diversity in fatty acid structure resulting from differences in chain length, degree of unsaturation, and position and stereoisomeric configuration of the double bonds may affect the rate of fatty acid oxidation. Several early reports showed differences in fatty acid oxidation with use of $^{13}$C-labeled fatty acids in animals (1–3). Rats were shown to oxidize linoleate more than palmitate (1). In rat liver preparations, oxidation of the various fatty acids was as follows: linoleate $>$ butyrate $>$ linolenate $>$ acetate $>$ stearate (2). In mice the order of oxidation was oleate $>$ linoleate $>$ stearate (3). In contrast, in rats fed a fat-free meal or just the labeled fatty acids, the oxidation of linoleate and palmitate was similar (4). The general trend in these studies was that long-chain fatty acids were oxidized more slowly and unsaturated fatty acids were oxidized more rapidly than were saturated fatty acids. Measurement of fatty acid oxidation in rats with a more complete series of fatty acids showed that oxidation of the saturated fatty acids decreases with increasing carbon length (laurate $>$ myristate $>$ palmitate $>$ stearate) (5). For unsaturated fatty acids, 24-h oxidation was in the following order: linolenate $>$ oleate $>$ linoleate $>$ arachidonate. Through 7 h, the oxidation of oleate was greater than that of linoleate. Thus, the medium-chain fatty acids (8–14 carbons) were oxidized the most rapidly, with linolenate and oleate oxidation occurring nearly as rapidly.

Several studies in humans have been reported in which $^{13}$C-labeled substrates were fed to study fatty acid oxidation (6–8). The first such report was one in which various $^{13}$C-labeled substrates were examined for use in breath tests (7). In this study, octanoate was oxidized much faster than was palmitate. Another study from the same group used $^{13}$C-labeled lipids in children to diagnose fat malabsorption (8). Octanoate was oxidized much more rapidly than was oleate, which was oxidized much faster than was palmitate. A more recent study compared fatty acid oxidation in men consuming a test diet of normal foods to which $^{13}$C-labeled 18-carbon fatty acids differing in degree of unsaturation were added (6). Oxidation of oleate (18:1$\Delta_9$) was greater than that of linoleate (18:2$\Delta_9\Delta_12$), which was in turn greater than that of stearate (18:0). Therefore, considerable data in animals, and some data in humans, show that the short- and medium-chain fatty acids oleate and linoleate are oxidized rapidly, whereas the long-chain saturated fatty acids palmitate (16:0) and stearate are oxidized more slowly. Data regarding the effect of label position in humans are lacking. However, there is some evidence in humans and in rats that long-chain saturated fatty acids are only partially chain shortened (9, 10). In rats the location of label did not affect oxidation (11). The current study was undertaken to examine fatty acid oxidation in humans by using fatty acids labeled with $^{13}$C in the carboxyl or methyl position and to examine the role that chain length, degree of unsaturation, and stereoisomeric configuration of the double bonds play in determining the relative rates of oxidation of individual fatty acids.

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2 Supported by the US Department of Agriculture (grant 94-34323-0308).
3 Address reprint requests to JP DeLany, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124. E-mail: delanyjp@mhs.pbr.edu.
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SUBJECTS AND METHODS

Subjects

The participants in this study were 4 normal-weight, healthy men (67.2 ± 2.1 kg, 16.8 ± 9.7% body fat, 29.5 ± 7.5 y). Subjects gave informed consent by signing forms approved by the Louisiana State University Institutional Review Board.

Protocol

For 7 d before they were administered the test fatty acid, subjects were fed a standard diet of defined composition to meet their energy requirements. After 5 d of this standard diet, breath samples were collected after consumption of the liquid meal without any added fatty acids and after consumption of the lunch meal to determine the background 13C contribution of the test diet. Then, a fatty acid oxidation test was performed every 2–4 d, while the subjects continued to consume the standard diet. The labeled fatty acids were administered to each subject in random order.

Diets

The nutrient composition of the diet was similar to that of the typical American diet. The menu was formulated by using Moore’s Extended Nutrient database (MENu; Pennington Biomedical Research Center, Baton Rouge, LA) to contain 15% of energy as protein, 45% as carbohydrate, and 40% as fat (42% saturated, 36% monounsaturated, and 22% polyunsaturated fatty acids), with a ratio of polyunsaturated to saturated fatty acids of 0.51. Linoleic acid constituted 91% of the polyunsaturated fatty acids, with linolenic acid (8%) and arachidonic acid (1%) accounting for the remainder. A 4-d rotating menu was used, with one menu always fed the day before the test days and another menu always fed on the test days.

Each labeled fatty acid was blended in a heated (85°C) liquid meal (Ensure; Ross Laboratories, Columbus, OH) that was allowed to cool slightly and given to the subjects as breakfast (0800). When many similar procedures were used in a study of deuterated fatty acids, the absorption of even stearate in triacylglycerol form (both the high melting point tristearin and the lower melting point mixed-acid triacylglycerol) was ≈95% (12). The same lunch meal was consumed on each fatty acid test day. Dinner was served at the completion of the oxidation test.

The labeled fatty acids were synthesized by commercial isotope companies (Cambridge Isotopes, Andover, MA; Isotec Inc, Miamisburg, OH; Medical Isotopes, Inc, Pelham, NH; and MSD Isotopes, Dorval, Canada) with the 13C label in either the carboxyl or methyl end of each fatty acid (Table 1). The chemical purity and isotopic enrichment of each substrate were checked by gas chromatography–mass spectrometry. All labeled fatty acids used in these experiments were ≥98% chemically pure and isotopically enriched. The dose of the long-chain saturated 13C-labeled fatty acids was initially 15 mg/kg, but was subsequently reduced to 10 mg/kg body wt, which was the dose used for each 13C-labeled fatty acid.

Pilot study

A pilot study was conducted to compare 2 methods of delivering the labeled fatty acids. On 2 separate days, 1 subject received either a capsule containing [13]C-palmitate with the breakfast meal or [13]C-palmitate blended in a hot (85°C) liquid meal. The capsule protocol was used previously in humans (6), but we were concerned about whether the long-chain saturated fatty acids with high melting points [eg, palmitic acid (62°C) and stearic acid (69°C), both with melting points well above body temperature] would be absorbed effectively even if a correction was made for differential absorption. Thus, to enhance absorption we also administered fatty acids in a hot blended mixture, similar to the method used by Emken et al (12) in their studies of deuterium-labeled fatty acids, in which they observed ≈95% absorption of long-chain saturated fatty acids.

Breath tests

On the day of a fatty acid oxidation test, the labeled fatty acid was ingested in a hot blended meal. After subjects finished the meal, they lay down under the hood of a metabolic cart (2900Z; SensorMedics, Yorba Linda, CA) for measurements of oxygen consumption and carbon dioxide output. The flow meter was calibrated daily by using a 3.0-L syringe and the analyzers were calibrated daily by using 2 standard span gases and room air. The test was carried out for 9 h. Every 30 min a breath sample was collected for measurement of 13C enrichment by using a 60-mL syringe with a latex tubing mouthpiece. Background diet 13C enrichment was subtracted from the fatty acid generated 13C enrichment. The fraction of the ingested dose of 13C in breath carbon dioxide was calculated by using 1) the background-diet-adjusted amount of 13CO2 in breath, 2) carbon dioxide production measured by indirect calorimetry, and 3) the amount and enrichment of the dose administered (13).

Mass spectroscopy

The abundance of 13CO2 was measured by using a dual-inlet isotope ratio mass spectrometer (Finnigan Delta S, Bremen, Germany). Breath samples from the 60-mL syringe were placed in a 20-mL evacuated tube (no additive, nonsterile) and placed in an autosampler attached to an automated breath carbon dioxide trapping device (Finnigan). The device cryogenically purifies and transfers the carbon dioxide to the mass spectrometer for isotopic analysis. Several samples of a 5% CO2 standard that had been calibrated against known 13CO2 standards were analyzed with each run to be sure that the trapping device was extracting carbon dioxide from the samples properly and that the mass spectrometer was giving accurate and precise enrichment measures. The measured enrichment of this standard gas was stable, giving a mean enrichment of −54.64 ± 0.14‰ over a 2-y period. If the internal precision of the mass spectrometer was >0.15‰, or if the transfer of carbon dioxide from the sample was low, the samples were reanalyzed. The CV of repeat measures of baseline 13C enrichment in breath samples was 0.7%. The mean (±SD) of 4 duplicate samples with low enrichment was 3.02 ± 0.10‰, whereas that for 4 duplicate enriched samples was 34.19 ± 0.44‰.

### TABLE 1

Position of the 13C label in the fatty acids tested

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Carboxyl label</th>
<th>Methyl label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurate (12:0)</td>
<td>C-1</td>
<td>C-12</td>
</tr>
<tr>
<td>Palmitate (16:0)</td>
<td>C-1</td>
<td>C-16</td>
</tr>
<tr>
<td>Stearate (18:0)</td>
<td>C-1</td>
<td>C-18</td>
</tr>
<tr>
<td>Oleate (cis 18:1n−9)</td>
<td>C-1</td>
<td>C-18</td>
</tr>
<tr>
<td>Elaidate (trans 18:1n−9)</td>
<td>C-1</td>
<td>C-18</td>
</tr>
<tr>
<td>Linoleate (18:2n−6)</td>
<td>C-1</td>
<td>C-18</td>
</tr>
<tr>
<td>Linolenate (18:3n−3)</td>
<td>C-1</td>
<td>C-18</td>
</tr>
</tbody>
</table>

1Not done.
Data analysis

Cumulative fatty acid oxidation was analyzed by using a one-way analysis of variance with the general linear models procedure. Waller-Duncan K-ratio t tests were used to determine differences between means. A t test was used to determine whether there was a carryover effect of the previously ingested labeled fatty acid. Simple regression analysis was carried out to determine the relation between oxidation and chain length and degree of unsaturation. All statistical tests were conducted by using SAS 6.12 for WINDOWS (SAS Institute, Cary, NC) and significance was set at P < 0.05. Data are presented as means ± SEMs.

RESULTS

Pilot study

There was a striking difference in oxidation of the labeled palmitate between the 2 methods of administration (Figure 1). When the fatty acid was blended in a hot liquid meal, the recovery of 13C in breath samples was 10 times higher than when the fatty acid was given in a capsule with a meal. The dose of the long-chain saturated fatty acids we used initially (15 mg/kg) was based on giving the fatty acid in a capsule. Thus, because of the high absorption observed when the fatty acid was given with the hot blended liquid meal, we reduced the dose to 10 mg/kg.

Reproducibility and carryover

Reproducibility of the oxidation of fatty acids was examined for 2 fatty acids. Data on the oxidation of laurate (12:0), which is highly oxidized, and of palmitate, which is less highly oxidized, are shown in Figure 2. The repeat studies showed cumulative recoveries of 42.5% and 46.3% of the label from laurate. For palmitate the oxidation was 12.4% and 15.3% in the 2 trials, implying that oxidation was similar with repeat measurement.

To determine whether there was any carryover effect on baseline 13CO2 enrichment from the previous administration of a labeled fatty acid, we examined the initial baseline enrichment on the background test diet day and on the morning of the first day a labeled fatty acid was administered (before any 13C-labeled fatty acids were given). The initial baseline 13CO2 for the 4 subjects was −21.90 ± 0.41‰, whereas the average for the morning of all test days was −21.30 ± 0.83‰ (P < 0.04). This difference of 0.60‰ in baseline breath 13CO2 would have had little, if any, effect on the oxidation measures because the baseline enrichment was subtracted from each subsequent time point. In addition, an enrichment of this magnitude is low compared with the enrichment observed at each time point, except for the first few time points (Figure 3).

Carboxyl- and methyl-labeled fatty acids

The recovery of 13CO2 from the methyl or carboxyl position of each fatty acid is shown in Figure 3. The pattern of 13CO2 recovery and the timing of peak enrichment of each fatty acid were similar whether the fatty acid was labeled in the carboxyl or methyl position. However, the recovery of 13C in breath after feeding the methyl-labeled fatty acid was 74 ± 16% of that seen when feeding the carboxyl-labeled fatty acid. The major exception to this was oleate, for which recovery of the methyl label was 92 ± 18% of that for the carboxyl label, significantly higher than for all other fatty acids except linolenate (methyl label recovery: 82 ± 0.2%). Excluding oleate, the recovery of the methyl label was 69 ± 11% of that for the carboxyl label.

Oxidation of individual fatty acids

The oxidation of laurate was the highest of all fatty acids tested (Figure 4). The next most highly oxidized fatty acid was linolenate (18:3n−3), followed by elaidate, linoleate, and...
oleate, which showed similar rates of oxidation. The oxidation of elaidate (trans 18:1n−9) appeared to be slightly higher than that of oleate (cis 18:1n−9) and the peak oxidation appeared to be delayed by ≈30 min (Figure 5). The 2 long-chain saturated fatty acids were the least oxidized, with only 13% of stearate oxidized over the 9-h test.

The ranking, from highest to lowest, of the oxidation of the fatty acids over 9 h was fairly consistent whether we considered the carboxyl- or methyl-labeled fatty acids or according to the overall average regardless of the label position (Table 2). For the carboxyl-labeled fatty acids, the order of oxidation from lowest to highest was as follows: laurate > linolenate > elaidate > linoleate > oleate > palmitate > stearate. When the average of the carboxyl and methyl data were used, the order was laurate > linolenate > elaidate > oleate > linoleate > palmitate > stearate.

**DISCUSSION**

The results of our study of fatty acid oxidation in humans are similar to those observed in radioisotopic tracer studies of rats (5). In our studies, as well as in those of rats, oxidation of saturated fatty acids decreased with increasing carbon length (laurate > palmitate > stearate). The relation between oxidation and fatty acid carbon length was highly significant (r² = 0.94; Figure 6). In the study in rats, the 24-h oxidation of unsaturated fatty acids was in the following order: linolenate > oleate > linoleate (5). These results are similar to ours, in which the oxidation of the 18-carbon fatty acids was significantly correlated with the number of double bonds (Figure 6). There was a nearly perfect linear relation (r² = 0.9997) between oxidation and the number of double bonds for stearate, oleate, and linoleate. The trans fatty acid elaidate was more highly oxidized and in Figure 6 appears above this regression line. The essential fatty acid linoleate appeared to be less oxidized and appears below the observed regression line. These findings are also similar to those of a 6-h study in which uniformly 14C-labeled fatty acids were infused into the external iliac vein in pigs (14). Although the absolute numbers in this study were different, the pattern was similar to that observed in our human feeding study, with palmitate being more highly oxidized than stearate (19.1% compared with 6.6%) and oleate more highly oxidized than linoleate (30.1% compared with 13.1%).
TABLE 2
Cumulative 13CO2 recovery in breath over 9 h1

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Carboxyl label</th>
<th>Methyl label</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>15.8 ± 2.8c,d [4]</td>
<td>12.0 ± 0.9c [3]</td>
<td>14.2 ± 2.9c [7]</td>
</tr>
<tr>
<td>cis 18:1n−9</td>
<td>17.9 ± 3.8c [4]</td>
<td>16.2 ± 3.6c,d [4]</td>
<td>17.0 ± 3.6c,d [8]</td>
</tr>
<tr>
<td>trans 18:1n−9</td>
<td>20.5 ± 3.0c [3]</td>
<td>—c [0]</td>
<td>20.5 ± 3.0c [3]</td>
</tr>
</tbody>
</table>

1±SD; n in brackets. Means within columns with different superscript letters are significantly different, P < 0.05.
2Not done.
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unsaturated fatty acids, the n−3 fatty acid linolenate was the most highly oxidized and linoleate was the least oxidized. The recovery of label from methyl-labeled fatty acids was ≈30% lower than that from the carboxyl-labeled fatty acids, except for oleate, for which the difference was only 8%. Finally, differences in the rates of oxidation of individual fatty acids may partially explain differences in weight gain observed in animals fed different types of dietary fat and may also play a role in human obesity.

REFERENCES


