INTRODUCTION

Linoleate (18:2n−6) is the main polyunsaturated fatty acid (PUFA) in the diet and in the body. Despite an extensive history of research into linoleate metabolism and its role in nutrition and health, little is known in humans about linoleate partitioning toward β-oxidation. In weight-stable adults, the proportion of linoleate in fatty acids in adipose tissue reflects habitual linoleate intake (1–3). Thus, β-oxidation is probably the main route of dietary linoleate utilization; however there is little quantitative evidence supporting this assumption.

During food restriction resulting in a weight loss of 10–25 kg in obese humans, the percentage of linoleate in adipose tissue remains unchanged compared with values before weight loss (4–6). This suggests that during energy deficit β-oxidation of linoleate occurs at a rate similar to that of other long-chain fatty acids. Nevertheless, linoleate depletion from the body can exceed that of other long-chain fatty acids in weight-cycling animals despite adequate linoleate intake (7). An energy deficit caused by fat malabsorption or disease that generates the need for parenteral nutrition leads to a risk of linoleate deficiency and possible depletion of long-chain n−6 PUFA, eg, arachidonate (20:4n−6; 8, 9). Thus, there are 2 mechanisms by which food restriction and weight loss incur a potential risk of linoleate deficiency: 1) reduced linoleate intake because mammals are unable to synthesize linoleate except from the small amounts of dietary hexadecadienoate (16:2n−6) present in some common edible green vegetables (10), and 2) increased linoleate β-oxidation because it may exceed linoleate intake during weight loss. Hence, it would be beneficial to assess the risk of linoleate deficiency with weight loss in humans and to determine the significance of increased linoleate β-oxidation as a risk for linoleate deficiency.

β-Oxidation of linoleate in obese men undergoing weight loss1–3

Stephen C Cunnane, Robert Ross, Jody L Bannister, and David JA Jenkins

ABSTRACT

Background: In animals, the whole-body content and accumulation of linoleate can be measured and compared with its intake to determine linoleate β-oxidation. This method can also provide quantitative information about the β-oxidation of linoleate in humans.

Objectives: The objectives of the study were to 1) use the whole-body fatty acid balance method to quantify whole-body concentrations of linoleate in humans, 2) estimate the distribution of linoleate between adipose and lean tissue, and 3) assess the effect of weight loss on linoleate stores and β-oxidation in obese humans.

Design: Nine healthy obese men underwent supervised weight loss for 112 d (16 wk). Magnetic resonance imaging data and fatty acid profiles from fat biopsies were both used to determine linoleate stores in adipose and lean tissue and in the whole body. Linoleate β-oxidation was calculated as intake – (accumulation + excretion).

Results: Mean weight loss was 13 kg and linoleate intake was 24 ± 6 mmol/d over the study period. Whole-body loss of linoleate was 37 ± 18 mmol/d, or 28% of the level before weight loss. Combining the intake and whole-body loss of linoleate resulted in linoleate β-oxidation exceeding intake by 2.5-fold during the weight-loss period.

Conclusions: All dietary linoleate is β-oxidized and at least an equivalent amount of linoleate is lost from the body during moderate weight loss in obese men. The method studied permits the assessment of long-term changes in linoleate homeostasis in obese humans and may be useful in determining the risk of linoleate deficiency in other conditions. Am J Clin Nutr 2001;73:709–14.

KEY WORDS Adipose tissue, linoleate, magnetic resonance imaging, obesity, weight loss, exercise, β-oxidation

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Fatty acid profiles of 4 plasma lipid classes were measured to determine whether linoleate changes in blood after weight loss were proportional to those in adipose tissue or to weight loss itself. If so, they could potentially serve as a substitute for the time consuming and expensive magnetic resonance imaging (MRI)–based measurements of changes in whole-body linoleate.

METHODS

Weight-loss regimen

This study was done in obese men undergoing voluntary but supervised weight loss induced by a combination of food restriction and exercise. Subjects were healthy and had no disease conditions known to influence metabolism of PUFA. Each subject provided written consent and all procedures received ethical approval by both the University of Toronto and Queen’s University. The inclusion criteria were as follows: a body mass index (BMI; in kg/m²) >27, weight stability ±2 kg in previous 6 mo, no use of prescription drugs known to affect body weight, and (MRI)–based measurements of changes in whole-body linoleate.

Analysis of linoleate intake

Complete daily food intake records for the 112-d study period were obtained from each participant. Total intake of energy, protein, carbohydrate, fat, PUFA, and linoleate were analyzed for each subject at the University of Toronto by using a program based on US Department of Agriculture data (NUTRIPUT, version 2.02; US Department of Agriculture, Washington, DC).

Adipose tissue biopsies and plasma fatty acid analysis

Before and after weight loss, needle biopsies of subcutaneous fat were obtained from the anterior abdominal wall of each subject undergoing weight loss. Fat biopsies were obtained by suction into a 10-mL syringe through an 18-gauge needle without local anesthetic. The samples (5–20 mg) were stored from a different study with similar subjects. In that study, age linoleate excretion values were obtained from fecal samples not collected from the subjects undergoing weight loss so averaged from the cross-sectional areas of each scan as described previously (11, 13). These MRI measurements of lean and adipose tissue volume in living humans have been validated by us (13) and others (14) in comparison with the lean and fat composition of dissected human cadavers. The intraobserver CV for the in vivo MRI measurements was 2.1%. MRI data for skeletal muscle volumes differed from cadaver measurements by 1.3% (13).

Conversion of the MRI-based adipose tissue volumes and percentage fatty acid composition data to actual fatty acid mass (in mol) in total adipose tissue required that the adipose tissue volume be corrected for fatty acid density and water content in adipose tissue. The fat biopsies obtained for fatty acid analysis were too small for reliable measurements of fat density or water content. For this purpose, larger fat samples (100–400 g) from 5 different individuals not involved in the weight-loss study were obtained from the Cosmetic Surgery Institute (Toronto). Five 2–3-g weighed replicates of each individual sample were used for density measurements by volume displacement at 37°C. Four additional 2–3-g replicates from the same 5 individuals were used to determine the water content of adipose tissue by freeze-drying to constant weight over 7 d.

The percentage of linoleate in abdominal subcutaneous adipose tissue is reported to be the same as at other body sites, including visceral fat (15, 16); thus, the linoleate concentrations obtained in the single biopsy represented adipose tissue linoleate concentrations throughout the body. After the conversion factor to obtain fatty acid mass from adipose volume as measured by MRI was determined (see Results), the actual amount of linoleate in whole-body adipose tissue stores (in mol) was determined by multiplying the fatty acid mass in adipose tissue by the percentage of linoleate in the biopsies.

Linoleate excretion

To calculate linoleate balance and β-oxidation, a measure of linoleate excretion in feces was also needed. Fecal samples were not collected from the subjects undergoing weight loss so average linoleate excretion values were obtained from fecal samples stored from a different study with similar subjects. In that study, 3-d pooled fecal collections were obtained from 12 middle-aged adults who had all consumed the same metabolic diet over 4 wk.
The diet provided 20% of energy as fat and 25 mmol linoleate/d, which was similar to the diet of the subjects undergoing weight loss in the present study.

A 2-stage lipid extraction method was used (17). Two freeze-dried, homogenized 2-g replicates/subject were acidified and 20 mL heptane:diethyl ether:ethanol (1:1:1) containing heptadecanoic acid as an internal standard were added, vortexed for 5 min, and centrifuged at 2500 × g at 4°C for 10 min. The supernate was decanted and filtered. The centrifuged lipid extract was reextracted twice into 20 mL heptane:diethyl ether:ethanol:distilled water (1:1:1:1), centrifuged at 200 × g at 20°C for 10 min, decanted, and filtered. The lipid extracts were combined and rotary evaporated to dryness at 30°C. The dried fatty acid extracts were methylated and analyzed by capillary gas chromatography and corrected for relative response factors as described for plasma fatty acids. Fecal linoleate was quantified relative to the internal standard.

### Whole-body linoleate balance

In animal studies, linoleate intake, excretion, and whole-body accumulation can all be measured directly (7). In the present study, linoleate intake and the loss of linoleate stores from adipose tissue were measured directly in each person undergoing weight loss. Lean tissue linoleate was estimated from lean tissue volume as measured by MRI (13) and literature values indicating 4% fat weight and 20% linoleate in total fatty acids of lean tissue (18–21). Thus, total lean tissue linoleate was measured directly in each person undergoing weight loss, and the decrease in lean tissue content of linoleate was attributed to weight loss.

### RESULTS

#### Body weight

Body weight loss was 13.0 ± 4.9 kg; 27% of the loss was of initial adipose tissue and 3% was of initial lean tissue. The mode of exercise did not significantly affect the results so data for both aerobic and resistance exercise were combined.

#### Adipose and lean tissue linoleate

The percentage of linoleate decreased significantly in subcutaneous fat after weight loss (Table 1). Besides linoleate, the only other n−6 PUFAs that were consistently present in adipose tissue were dihomo-γ-linolenate (20:3n−6) and arachidonic acid. They totaled <1% of adipose tissue fatty acids and their proportions did not change significantly with weight loss in either group. n−3 PUFAs were present in adipose tissue biopsies but their content in the body was not quantified in this study.

The density of subcutaneous fat was 0.82 ± 0.01 kg/L (n = 5). The actual proportion of fatty acids in adipose tissue was 88.3 ± 3.5% (n = 5). Multiplying the fat density by the percentage of fatty acids in adipose tissue provided the factor 0.724, which was used to correct adipose tissue volume for the total mass of adipose fatty acids. Correction of adipose tissue volumes for fatty acid mass and multiplication by the percentage of linoleate in the biopsies gave the total mass of linoleate in adipose tissue. There was 14.7 mol linoleate in adipose tissue before weight loss and 10.5 mol after weight loss (Table 2).

#### Linoleate intake, excretion, and β-oxidation

Linoleate intake was 24 ± 6 mmol/d, or 3.2% of energy intake (Table 2). Linoleate intake was significantly positively correlated with total energy intake (r = 0.65, P < 0.01) and with total fat intake (r = 0.88, P < 0.0001). Linoleate excretion was 0.8 mmol/d, or 4.0 ± 2.4% of daily linoleate intake. Linoleate excretion was not affected by differences in BMI. Average daily linoleate β-oxidation during the weight-loss period was calculated by subtracting the loss of whole-body linoleate from total linoleate intake after first deducting linoleate excretion. Over the 112-d study period, 6.77 ± 2.63 mol (60 mmol linoleate/d) was β-oxidized. Thus, during the weight-loss period

### Data analysis

All data are expressed as means ± SDs. Unpaired Student’s t tests were used to determine whether BMI affected fecal linoleate excretion and to determine possible effects of type of exercise on adipose tissue fatty acid composition. Paired t tests were used to determine the significance of effects of weight loss on linoleate β-oxidation, the percentage of linoleate in adipose tissue, and adipose tissue density at different temperatures.

GRAPH PAD IN PLOT version 4.0 (Graph Pad Software, San Diego) was used for regression analyses.

### TABLE 1

<table>
<thead>
<tr>
<th>Fatty acid composition in subcutaneous adipose tissue total lipids and plasma cholesteryl esters before and after weight loss in obese men</th>
<th>% by wt of total fatty acids</th>
<th>Before</th>
<th>After</th>
<th>% by wt of total fatty acids</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate</td>
<td>27.0 ± 1.9</td>
<td>26.2 ± 1.8</td>
<td>14.0 ± 1.6</td>
<td>14.1 ± 1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stearate</td>
<td>4.5 ± 1.0</td>
<td>5.0 ± 1.3</td>
<td>1.4 ± 0.2</td>
<td>1.1 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleate</td>
<td>54.0 ± 2.1</td>
<td>54.3 ± 2.6</td>
<td>23.0 ± 1.7</td>
<td>25.1 ± 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleate</td>
<td>12.7 ± 1.6</td>
<td>12.0 ± 1.3</td>
<td>52.5 ± 3.3</td>
<td>50.4 ± 5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachidonate</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.6</td>
<td>8.3 ± 1.1</td>
<td>8.2 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Linolenate</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.6</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoate</td>
<td>&lt;0.1</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 ± SD; n = 9.

2 Significantly different from before weight loss, P < 0.05 (Student’s t test).
DISCUSSION

We showed here that the whole-body linoleate content and its distribution between lean and adipose tissue can be determined in living humans. We estimated that 6.5% of whole-body linoleate concentrations in these obese men was in lean tissue (Table 2). The proportion of dietary linoleate utilized by lean tissue could not be estimated by using the present method. With respect to weight loss, the main observation was that obese men undergoing voluntary weight loss induced by a combination of food restriction and exercise β-oxidized 2.5-fold more linoleate than they consumed. Our present data show that when exercise accompanies moderate food restriction, adipose tissue linoleate is depleted slightly but significantly more rapidly than are other long-chain fatty acids measurable in adipose tissue. Exercise is well known to increase fat oxidation (25) and linoleate is readily β-oxidized (24, 26–28), especially during high intensity exercise of short duration (29).

Even after correction for a possible 30% underreporting of fat intake (30), linoleate β-oxidation was still more than twice that of linoleate intake. Thus, our data show that during moderate weight loss, dietary linoleate is completely β-oxidized as a fuel and whole-body linoleate losses are at least equivalent to what was consumed and β-oxidized from the diet. Because only 28% of whole-body linoleate stores were lost during the study period and subjects continued to consume linoleate, they were presumably not at imminent risk of linoleate deficiency. Nevertheless, the present method provides an approach to assessing this risk either with longer-term weight loss or in clinical situations characterized by weight cycling or in which wasting is a risk, eg, in patients with cystic fibrosis (9), with malabsorption (8), or who need total parenteral nutrition (31).

Despite β-oxidizing all of the linoleate consumed for 16 wk and a 28% decrease in whole-body linoleate content, plasma fatty acid profiles did not reflect this significant change in linoleate homeostasis. In the 4 main plasma lipid classes, serum linoleate was not significantly affected by weight loss nor was the classic fatty acid marker of dietary linoleate deficiency, eicosatrienoate (20:3n–9), increased significantly after weight loss. This may have been due in part to the adequate linoleate intake during the study period. It may also have been that plasma fatty acid profiles are not sensitive to significant changes in linoleate metabolism when linoleate intakes are adequate. The significant positive correlation between changes in plasma cholesterol linoleate and weight loss or adipose tissue loss suggests that this fraction of plasma linoleate could potentially substitute for the MRI-based adipose tissue measurements in estimating linoleate β-oxidation during weight loss involving food restriction and exercise.

<p>| TABLE 2 |
| Whole-body linoleate and linoleate balance in obese men during a 16-wk period of supervised weight loss1 |</p>
<table>
<thead>
<tr>
<th>Whole-body linoleate (mol)</th>
<th>Adipose tissue</th>
<th>Lean tissue</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before weight loss</td>
<td>12.92 ± 2.94</td>
<td>1.76 ± 0.11</td>
<td>14.68 ± 2.71</td>
</tr>
<tr>
<td>After weight loss</td>
<td>8.81 ± 2.43</td>
<td>1.69 ± 0.07</td>
<td>10.50 ± 2.25</td>
</tr>
<tr>
<td>Change</td>
<td>−4.11 ± 1.792</td>
<td>−0.07 ± 0.05</td>
<td>−4.18 ± 2.033</td>
</tr>
<tr>
<td>Linoleate balance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake (mmol/d)</td>
<td>24 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excretion (mmol/d)</td>
<td>0.8 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% of linoleate intake)</td>
<td>0.40 ± 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-body accumulation</td>
<td>−37 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disappearance1</td>
<td>(mmol/d)</td>
<td>60 ± 23</td>
<td></td>
</tr>
<tr>
<td>(% of linoleate intake)</td>
<td>250 ± 96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1r ± SD; n = 9.

2P < 0.05 (Student’s t test).

3Sum of the change in adipose and lean tissue linoleate.

4Equivalent to β-oxidation: intake − (accumulation + excretion).

Plasma fatty acid profiles

Before weight loss, the percentage of linoleate in adipose tissue was correlated with the percentage of linoleate in plasma free fatty acids distribution between lean and adipose tissue can be determined in

| TABLE 3 |
| Change in adipose tissue linoleate and linoleate β-oxidation in relation to weight loss in obese men1 |
| Change in adipose tissue linoleate as a function of the reduction in adipose tissue (9.0 ± 4.5 kg1) during weight loss | r | P |
| (ΔLinoleate, −4.11 ± 1.79 mol) | 0.97 | <0.0001 |
| (ΔLinoleate, −0.7 ± 0.8%) | 0.74 | <0.02 |
| Change in linoleate β-oxidation (6.77 ± 2.63 mol) as a function of the change in body weight (kg) or the change in adipose linoleate (mol) | r | P |
| (ΔBody weight, −12.9 ± 4.9 kg) | 0.82 | <0.02 |
| (ΔAdipose tissue linoleate, −4.11 ± 1.79 mol) | 0.83 | <0.005 |

1r ± SD.
β-OXIDATION OF LINOLEATE DURING WEIGHT LOSS

The possible effect of underreporting of food intake on whole-body linoleate has been discussed but other potential sources of error deserve comment. Fecal linoleate was measured from samples that were not from the subjects undergoing weight loss but the commonly observed 4% excretion of fat (and linoleate) was observed (32, 33). Although not measured in the subjects undergoing weight loss, values in the expected range for fat density and percentage of water in adipose tissue (34) were obtained from other obese subjects undergoing elective surgery. Skeletal muscle linoleate decreases slightly during exercise without weight loss (18) but we estimate that this change would not have significantly affected changes in whole-body linoleate in the present study because >98% of the whole-body reduction in linoleate with weight loss was from adipose tissue.

Calculating linoleate balance while excluding long-chain n-6 PUFAs and eicosanoids was not a significant source of error. Less than 3% of linoleate intake accumulates in the adult human body even when it is consumed at ≤38% of total fat intake (35). About 1% of dietary linoleate is converted to arachidonate in adult humans under normal dietary conditions (36) and an energy deficit inhibits the desaturation and chain elongation enzymes that are required to convert linoleate to longer-chain n-6 PUFAs (23). Thus, negligible linoleate should disappear through this route during weight loss. Under no circumstances reported does eicosanoid excretion by humans exceed 1 mg/d (37), which represents <0.0002% of the linoleate intake in this study. Thus, our estimate of linoleate β-oxidation during weight loss appears to be robust and consistent with the relevant literature (18, 27, 32, 38).

One important limitation to this method is that MRI equipment and the necessary software to obtain whole-body lean and adipose tissue volumes are not widely available for research purposes. Lean tissue biopsies are also more difficult to obtain than are adipose biopsies. Lean tissue will contain a higher proportion of adipose tissue volumes are not widely available for research purposes. Lean tissue biopsies are also more difficult to obtain than are adipose biopsies. Lean tissue biopsies are also more difficult to obtain than are adipose biopsies.

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