Relationship of diet to the fatty acid composition of human adipose tissue structural and stored lipids\textsuperscript{1,2}

Catherine J Field, MSc, Aubie Angel, MD, and Michael T Clandinin, PhD

ABSTRACT  The habitual intake of 20 healthy free-living subjects was determined by two 7-day food records. Documented fatty acid intakes were utilized to examine the influence of fatty acid intake on fatty acid composition of stored and structural lipids in subcutaneous adipose tissue. Subjects with higher intakes of saturated fatty acids exhibited increased levels of total saturated fatty acids and decreased polyunsaturated fatty acids in adipose tissue triglycerides ($p < 0.01$). The dietary P/S ratio was significantly related to the saturated and polyunsaturated content of stored lipids. In the phospholipid fraction, relationships were found between dietary C18:2\textsuperscript{(6)} and the P/S ratio of phosphatidylcholine ($p < 0.05$). The essential fatty acid content of the two phospholipids studied was related to the dietary fats consumed. Relationships were identified between major fatty acids in the triglyceride and phospholipid fraction. Although diet was found to relate to fatty acid composition, the structural lipids in human adipose tissue appear more resistant to compositional change than stored triglycerides.  


KEY WORDS  Adipose tissue, diet fat composition, humans

Introduction

Fat is a major component of the North American diet. Changing the nature of fat consumed profoundly influences fatty acids available to the body. Thus, modifying the source of dietary fat may alter the composition of adipose tissue. Animal research has provided convincing evidence for enhanced deposition of linoleic acid in carcass fat with an increase in the proportion of this fatty acid in the diet (1). Dietary fat also influences levels of trans-fatty acid (2), saturated fatty acids (3), and cholesterol (4) in adipose tissue.

The composition of human adipose tissue does not appear to be of constant fatty acid composition. Despite quantitative differences, within an individual there appears to be little difference in fat composition sampled from subcutaneous or deep body sites (5). Small but significant differences occur in levels of saturated and monoenoic fatty acids between proximal limb and central trunk fat depots (5). Significant compositional differences have been noted between the content of C16 and C18 fatty acids in infant and adult adipose tissue (6, 7). Reasons for these differences might be attributed to de novo synthesis rates (6), although diet could also be an important factor. Infant vs adult differences in adipose tissue composition rapidly disappear when the infant consumes a diet of fatty acid composition similar to that of the adult (8). Sex differences have been reported in adipose tissue composition of saturated and monounsaturated fatty acids (9). Whether reported sex differences simply reflect different dietary intakes is unknown. The role of genetics in determining adipose tissue composition has not been established. Differences reported between racial groups (10) are complicated by dissimilar fat intakes. In this regard, individuals consuming diets that differ from their habitual in-

\textsuperscript{1}From the Department of Nutritional Sciences, and Department of Medicine, Faculty of Medicine, University of Toronto, 150 College Street, Toronto, Ontario.
\textsuperscript{2}Address reprint requests to: Dr MT Clandinin, Department of Foods and Nutrition and Department of Medicine, The University of Alberta, 6-125A Clinical Sciences Building, Edmonton, Alberta T6G 2G3.

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take develop adipose tissue fatty acid composition differing radically from that of their kinsmen (10). The magnitude of effects that are attributable to sample site, sex, and age are small compared with dietary fatty acids, which appear likely to be the dominant determinant of adipose tissue fatty acid composition (11).

Human studies aimed at establishing the relationship between diet and adipose tissue composition have focused on linoleic acid. Examining habitual diets of adults (10) or manipulating dietary fat on a short- (7) or long-term (12) basis indicates that adipose tissue linoleic acid content is related to the nature of fat consumed. Research on infants has also illustrated that the fatty acid profile of fat fed influences adipose tissue composition (13).

Based on some of the available literature, a mathematical relationship has been proposed relating the fatty acid composition of diet to the human adipose organ (14). Recently, when two groups of individuals, one with newly diagnosed coronary heart disease and one of apparently healthy individuals, were compared, it was concluded that a lower concentration of C18:2(6) and C20:3(6) in adipose tissue may contribute to the presence of coronary heart disease independently of other known risk factors (15). In this study (15), dietary C18:2(6) levels were positively correlated with adipose tissue C18:2(6) concentration.

The fatty acid composition of structural lipids of various cell membranes in experimental animals is also influenced by the composition of fat fed (16, 17). Changing the fatty acid composition alters membrane physical properties (18). These alterations occur rapidly and are reversed by changing the nature of fat fed (16). Changes in membrane lipid composition have been shown to influence various membrane associated functions such as liver plasma membrane glucagon stimulated adenylate cyclase activity (17) and mitochondrial ATPase (19). Studies in animals suggest that dietary fat may influence insulin receptor function by altering membrane fatty acid composition (20). Therefore, it is logical to hypothesize that diet fat intake alters membrane structure and function in human adipocytes. The present study of free living human subjects examined the hypothesis that the intake of fatty acids relates to the composition of stored and structural lipids in adipose tissue.

Methods

Subjects

Subject selection and study procedures were approved by the University of Toronto Human Ethics Committee. Thirty-two healthy volunteers aged 21–35 yr completed a food frequency questionnaire and then recorded their food intake for 1 wk. Based on apparent consistency in daily fat intake, 11 female and 10 male subjects were selected for an adipose tissue needle biopsy.

Anthropometric measurements and fasting blood levels of cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and glucose were determined for each subject to demonstrate the absence of obesity and known disorders affecting lipid metabolism. One female subject was rejected because of abnormal blood cholesterol and LDL cholesterol levels. Height, weight, mid-upper-arm circumference, and skinfolds (triceps, biceps, subscapular, suprailiac) were measured by the same investigator to estimate body fat content (21).

Analysis of dietary intake

Each subject recorded his/her intake for a second 7-day period immediately preceding the needle biopsy. Four to five months elapsed between the initial and second food record, thus including some degree of seasonal variation in the documented food intake. Food frequency questionnaires and personal interviews aided in coding accurately an individual’s food record. Energy, protein, total fat, saturated, monounsaturated, and polyunsaturated fatty acids, carbohydrate, and cholesterol intakes were determined using a computer program written for this project. The nutrient data base was adopted from the University of Guelph Nutrient Data Deck (Guelph 1971) with updated fatty acid information obtained from United States Department of Agriculture Handbook No 8 (22) and Nutrient Value of Foods (23). Limitations in determining C18:2(6) intake may have resulted from the data system used, as the polyunsaturated content of a food included other polyunsaturated fatty acids such as linolenic acid. Nutrient intake for each subject was calculated as the mean daily intake for the 14 days.

Adipose biopsy

Samples of adipose tissue were aspirated under sterile conditions at the Clinical Investigation Unit of Toronto General Hospital. After cleaning, the skin and subcutaneous region was anesthetized with 1.5 cc xylocaine (2% wt/wt), and a small incision approximately 3 mm long was made on the left gluteus. A #14 gauge needle with a 50 ml plastic syringe containing 3 ml physiological saline was inserted 1–2 cm into the incision. A fold of skin above the incision was tented up to permit short, sharp thrusts of the needle into the fat, while pulling on the plunger, to suck the loosened fragments into the barrel. Samples were immediately transferred to acid-washed tubes containing 1.5 mg/ml EDTA in physiological saline at 0°C. The adipose aspiration biopsy contained fragments of adipose tissue, liberated adipocytes, and stromal vascular elements as well as some blood.

Lipid extraction and analyses

Aspirated adipose tissue samples weighing 10–50 mg were washed and centrifuged three times (2500 g for 2
min) with 12 ml saline to remove red blood cells. To insure that all red blood cells were removed, the final suspension (1 ml) was assayed for hemoglobin using a hemoglobin kit (Sigma No 525, St Louis, MO) and a standard curve diluted tenfold. Hemoglobin was not detected in the washed samples of adipose tissue.

Adipose tissue samples in approximately 1 ml saline were extracted in 20 ml chlorform:methanol (2:1 vol/vol) with 50 μg ethoxyquin added using a modified Folch procedure (24). The modification involved adding in succession 6.7 ml chlorform:methanol (1:1 vol/vol), 8 ml chlorform:methanol (2:1 vol/vol), and 3.3 ml chloroform to the sample. This extract was filtered through solvent-washed filter paper (Whatman #1) into an extraction flask. The filter paper was then washed with an additional 2 ml chlorform:methanol (2:1 vol/vol) and saved for protein determination.

Triglycerides, cholesterol, and phospholipids were separated by thin layer chromatography on 1000 μ silica gel G plates (10 x 20 cm) (Analtech Uniplate, Montreal, Que.) in petroleum ether:diethyl ether:formic acid (60:40:1.6 vol/vol) by double development for 1 h followed by 45 min. Lipids were visualized under UV light after spraying plates with 3% (wt/vol) 27-dichlorofluorescin in 0.01 N NaOH. Heptadecanoic acid was added to the triglyceride band as an internal standard and this band along with cholesterol and phospholipid bands were recovered. Lipids were eluted from the silica with three solvent rinses, centrifuged, and the supernatant filtered through funnels plugged with glass wool. Cholesterol was eluted with hexane:diethyl ether (85:15 vol/vol), triglycerides with chloroform, chloriform:methanol (1:1 vol/vol) followed again by chloroform, and phospholipids with chloroform:methanol:acetic acid:water (25:15:4:2 by vol), methanol, and methanol:acetic acid:water (95:15:5 vol/vol). Phosphatidylcholine was added as an internal standard to the cholesterol fraction for subsequent quantitative analysis. Solvent was removed from lipid samples at 40°C under nitrogen. Triglycerides were saponified and methylated using methanolic KOH and boron trifluoride methanol reagents (25).

Membrane phosphatidylcholine and phosphatidylethanolamine were separated on 200 μ silica gel coated microslices (Whatman MK6F) using chloroform:acetone:acetic acid:methanol:water (3.4:1:1:0.3 by vol) as the solvent system. Heptadecanoic acid was spotted on phosphatidylcholine and phosphatidylethanolamine bands and identified by chromatography with purified standards. Phospholipid bands were recovered and converted to methylsters using boron trifluoride methanol reagent (26).

Fatty acid methyl esters were separated and quantified by gas liquid chromatography using a Varian Vista 6000 gas chromatograph equipped with a flame ionization detector and a Vista 402 chromatography data system (Varian Instruments, Georgetown, Ont). Chromatography was performed using a Carbowax 20 M fused silica capillary column (25 m x 2.0 mm ID, Hewlett-Packard, Toronto). Helium was used as the carrier gas at a flow rate of 1.8 ml/min and an inlet pressure of 34 PSI. The inlet splitter was set at ~100:1 for analysis of triglycerides and ~30:1 for fatty acid analysis of phosphatidylcholine and phosphatidylethanolamine. Samples were injected at 250°C and the oven temperature was programmed from 185°C to 195°C at a rate of 5°C/min. Chromatography was completed within 40 min. Fatty acid methyl esters were identified with known standards.

**Protein determination**

Dried filter paper containing protein from biopsy samples was extracted by mincing the filter paper and agitating it for 1 h at 80°C in 3 ml 0.5 N NaOH (5). The slurry was transferred to centrifuge tubes, 1 ml NaOH added and centrifuged (2500 g for 30 min) to sediment the cellulose fraction. Protein content was determined in aliquots of supernatant (27).

**Cholesterol**

Cholesterol was extracted with internal standard and dissolved in chloroform (50 μl). Cholesterol was quantitated on Chromarods (type S, Technical Marketing Assoc, Mississauga, Ont) using a flame ionization detector (Iatroscan Model TH10, Technical Marketing Assoc, Mississauga, Ont). Analytical conditions were similar to those previously described (28). Rods were spotted with 1-3 μl of lipid extract and double developed for 40 min in petroleum ether (bp 37.7-65.9°C):diethyl ether:formic acid (97:4:1 vol/vol). Rods were scanned in the flame ionization detector to the end of the cholesterol band (~1.5 cm above the origin). To measure the phosphatidylcholine, added as an internal standard, rods were then developed for 40 min in chloroform:methanol:water (80:34:3 vol/vol) and scanned the full length of the chromarod. A Hewlett-Packard recording integrator (Hewlett-Packard Ltd, Mississauga, Ont) was used to calculate chromatograms. Linearity of detector response to cholesterol and phosphatidylcholine was determined for each rod using purified standards. Total cholesterol was determined relative to amount of internal standard added and expressed/mg protein in the total biopsy sample.

**Data analysis**

Simple correlation was used to identify relationships hypothesized to exist between dietary intake and adipose tissue fatty acid composition. Values for the entire group were used in the statistical analysis as each reported relationship remained significant when analyzed by sex. The ω3, ω6, major saturated and monounsaturated fatty acids, and total saturated, total polyunsaturated, and total monounsaturated fatty acids along with the polyunsaturated to saturated fatty acid (P/S) ratio of adipose triglycerides and phospholipids (phosphatidylcholine and phosphatidylethanolamine) were correlated with dietary fat, fatty acid, and carbohydrate intakes. Significant relationships were plotted using unlinear regression techniques identifying diet and adipose tissue as independent and dependent variables, respectively. Using dietary P/S ratio, two subgroups of subjects were also identified as having high vs low P/S intakes. Adipose tissue fatty acid composition was compared for these two subgroups by analysis of variance procedures. Significant relationships observed between diet and tissue composition were subsequently tested by multivariate analysis of variance procedures.

**Results**

**Characteristics of subjects**

Average age, height, weight, and estimated percent body fat indicate that percent body fat content of males and females corresponds
closely to ideal values (21) (Table 1). Average fasting blood cholesterol, LDL cholesterol, HDL cholesterol, and blood glucose levels (4.17, 0.54, 1.55, 2.31, 4.2 mmol/l, respectively), as with each individual value, fall within normal ranges (Table 2).

Average daily intake of selected nutrients is indicated (Table 3). Mean intakes for energy, protein, carbohydrates, polyunsaturated, saturated, monounsaturated, and total fatty acids calculated for the first and second 7-day food records did not differ significantly when compared by one-way analysis of variance procedures. Due to problems in assessing cholesterol intake with food composition data, a reliable estimation of cholesterol intake could not be obtained. The relationship between adipose tissue fatty acid composition and diet was examined by expressing adipose tissue fatty acid composition as a function of intake of saturated, monounsaturated, polyunsaturated, total fatty acids, and carbohydrate on a body weight basis, as a percent of total energy, and dietary fatty acid composition as a percent of total fat.

Mean fatty acid composition of adipose tissue triglycerides, phosphatidylcholine, and phosphatidylethanolamine (Table 4) reflected fatty acid composition of the diet to varying degrees. Trace amounts of fatty acids with chain lengths < 16 carbons were not measured in phospholipid classes. In addition to fatty acids illustrated, C12:0, C18:4(4), C20:4(3), and C22:3(6) in the triglyceride and phospholipid fractions were also measured and included in the total. Ninety-one percent of fatty acids present in human adipose tissue triglycerides consisted of C16:0, C16:1, C18:0, C18:1, and C18:2(6). Major fatty acids found in phosphatidylcholine and phosphatidylethanolamine were C16:0, C16:1, C18:0, C18:1, C18:2(6), C18:3(3), C20:4(6), and C20:5(3).

### Relationships between diet and triglyceride fatty acid composition

Linear regression procedures indicated relationships between intake of carbohydrates, polyunsaturated, saturated, monounsaturated and total fatty acids, and the fatty acid composition of human adipose triglycerides. On a body weight basis, an increase in mean total fat and saturated fatty acid intake was associated with a highly significant increase in adipose tissue levels of total saturated C16:0 and C18:0 fatty acids and a decrease in C20:4(6) (p < 0.01; Figs 1 and 2, respectively). High intakes of both total and saturated fatty acids were also significantly associated with decreases in C18:3(3) (p < 0.01) and the P/S ratio of triglyceride fatty acids (p < 0.05).

Dietary intake of monounsaturated fatty acids and carbohydrates correlated with fatty acid composition of adipose tissue triglycerides. On a body weight basis, higher monounsaturated fatty acid intake was associated with increased levels of C16:0, C18:0, and total saturated fatty acids (p < 0.01), and decreased levels of C18:3(3), C20:4(6), and P/S ratio (p < 0.05) in the triglyceride fraction. When expressed as a percent of fat, increases in mono-

### Table 1

<table>
<thead>
<tr>
<th>Subject descriptive data</th>
<th>All (n = 20)</th>
<th>Males (n = 10)</th>
<th>Females (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.4 ± 3.0*</td>
<td>26.2 ± 2.9</td>
<td>26.7 ± 3.1</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>168.8 ± 8.9</td>
<td>174.9 ± 8.3</td>
<td>162.6 ± 3.9</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>62.0 ± 9.0</td>
<td>68.3 ± 8.2</td>
<td>55.6 ± 3.6</td>
</tr>
<tr>
<td>% Body fat</td>
<td>17.3 ± 5.8</td>
<td>12.3 ± 2.6</td>
<td>22.2 ± 3.0</td>
</tr>
</tbody>
</table>

* Mean ± SD.

### Table 2

<table>
<thead>
<tr>
<th>Average fasting blood lipid and glucose levels</th>
<th>All</th>
<th>Males (M)</th>
<th>Females (F)</th>
<th>Normal range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>4.17 ± 0.5†</td>
<td>3.92 ± 0.3</td>
<td>4.42 ± 0.6</td>
<td>&lt;7.00</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.54 ± 0.2</td>
<td>0.58 ± 0.3</td>
<td>0.51 ± 0.2</td>
<td>&lt;1.80</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>1.55 ± 0.4</td>
<td>1.34 ± 0.3</td>
<td>1.77 ± 0.4</td>
<td>(M) 0.80–1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(F) 0.90–2.10</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>2.31 ± 0.6</td>
<td>2.22 ± 0.3</td>
<td>2.39 ± 0.7</td>
<td>2.05–4.65</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.2 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>4.1 ± 0.4</td>
<td>4.0–6.0</td>
</tr>
</tbody>
</table>

* Based on Toronto General Hospital reference standards.
† Mean ± SD.
TABLE 3
Mean daily intake of selected nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Group</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2674 ± 902‡</td>
<td>3357 ± 733</td>
<td>1991 ± 341</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>95.0 ± 33</td>
<td>121.0 ± 27</td>
<td>69.0 ± 11</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>325.7 ± 124</td>
<td>398.1 ± 136</td>
<td>253.3 ± 49</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>102.0 ± 34</td>
<td>126.5 ± 24</td>
<td>77.5 ± 23</td>
</tr>
<tr>
<td>Saturated</td>
<td>38.2 ± 14</td>
<td>47.1 ± 10</td>
<td>29.2 ± 10</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>35.2 ± 13</td>
<td>44.6 ± 9</td>
<td>25.8 ± 7</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>17.5 ± 7</td>
<td>21.0 ± 7</td>
<td>14.0 ± 4</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.49 ± 0.19</td>
<td>0.47 ± 0.23</td>
<td>0.51 ± 0.16</td>
</tr>
</tbody>
</table>

* Values are indicated for the entire group, males and females.
† Mean daily cholesterol intake 454.1 ± 229.6 mg.
‡ Mean ± SD.

unsaturated fatty acid intake correlated with decreases in C20:4(6) and C22:4(6) triglyceride fatty acids (p < 0.01). Carbohydrate intake, as a percent of energy, was negatively related to triglyceride levels of C16:0, C18:0, and total saturated fatty acids, and positively associated with levels of total polyunsaturated fatty acids, total ω6 fatty acids (p < 0.05), C20:3(6), and

TABLE 4
Average percent composition of major fatty acids in adipose tissue stored and structural lipids

<table>
<thead>
<tr>
<th>Fatty acid*</th>
<th>Triglycerides</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>2.98 ± 0.4†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>16:0</td>
<td>2.32 ± 0.7*</td>
<td>4.85 ± 2.5*</td>
<td>3.1 ± 3.7</td>
</tr>
<tr>
<td>20:5(3)</td>
<td>0.20 ± 0.02</td>
<td>4.68 ± 3.2*</td>
<td>3.54 ± 5.5*</td>
</tr>
<tr>
<td>22:6(6)</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:0</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:1</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:2</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:3</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:4</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:5</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
<tr>
<td>24:6</td>
<td>0.11 ± 0.05*</td>
<td>2.78 ± 2.7*</td>
<td>2.02 ± 0.04*</td>
</tr>
</tbody>
</table>

* In addition to the data presented C8:0, C10:0, C12:0, C13:0, C17:0, C18:4 (4), C20:4 (3), C22:3 (6), C23:0, and minor fatty acids of C14, C15, C16, C18, C22 carbons in chain length were measured.
† Mean ± SD (%wt/wt).
‡ Composition relates significantly to diet (p < 0.05).
§ Mean fatty acid composition; (males, females).
# Includes all isomers.
† SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, MUFA = monounsaturated fatty acids.
FIG 1. Adipose tissue levels of triglyceride fatty acids expressed as a function of mean dietary fat intake calculated on a body weight basis. Relationship between fat intake and A, total saturated fatty acids ($r = 0.56$); B, C16:0 ($r = 0.57$); C, C18:0 ($r = 0.54$); D, C20:4(6) ($r = -0.60$).
FIG 2. Adipose tissue levels of triglyceride fatty acids expressed as a function of mean dietary saturated fatty acid intake and A, total saturated fatty acids ($r = 0.57$); B, C16:0 ($r = 0.56$); C, C18:0 ($r = 0.58$); D, C20:4(6) ($r = -0.59$).
P/S ratio (p < 0.01) of the triglyceride fraction. No significant relationships were found between carbohydrate intake and the monounsaturated fatty acid content of adipose triglycerides.

Polyunsaturated fat intake was related to the composition of adipose triglycerides. Increased intake of C18:2(6)/unit body weight was associated with a decrease in content of C18:1 (p < 0.03; Fig 3) and total monounsaturated fatty acids in the triglyceride fraction (p < 0.05). A positive relationship was noted between polyunsaturated fatty acid intake and level of C22:4(6) (p < 0.05). Increase in dietary P/S ratio was accompanied by increased levels of total w6 fatty acids (p < 0.05) of adipose tissue triglycerides. Levels of total polyunsaturated and C18:2(6) fatty acids also increased significantly in the triglyceride fraction with increases in dietary P/S ratio (p < 0.05).

Using analysis of variance procedures, the difference in adipose tissue triglycerides fatty acid composition was examined in two groups of five subjects with mean dietary P/S ratios of 0.35 ± 0.02 and 0.59 ± 0.08. Significant differences were noted between adipose tissue total w6 (p < 0.05), C18:2(6) (p < 0.05), and total saturated (p < 0.02) fatty acids and the P/S ratio (p < 0.03) of adipose tissue triglycerides (Table 5). These differences in the fatty acid composition of adipose triglycerides reflected the high vs low dietary P/S ratio.

**Relationship between diet and phospholipid fatty acid composition**

Polyunsaturated fatty acid intake correlated with the fatty acid composition of membrane phospholipids. Increase in mean dietary C18:2(6) was associated with an increase in P/S ratio of fatty acids in phosphatidylcholine (p < 0.05; Fig 4). For phosphatidylethanolamine, increase in C18:2(6) intake was associated with a decrease in level of C22:6(3) (p < 0.01) and also in the levels of C20:3(6), C20:4(6), total

**TABLE 5**

Difference in triglyceride fatty acid composition between two groups of subjects consuming diets of high versus low P/S ratios

<table>
<thead>
<tr>
<th>Triglyceride fatty acid composition</th>
<th>Level of significance</th>
<th>p&lt;</th>
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<tbody>
<tr>
<td>Dietary P/S ratio</td>
<td>0.35 ± 0.2</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>Total w6</td>
<td>11.81 ± 1.1</td>
<td>13.38 ± 2.0</td>
</tr>
<tr>
<td>C18:2(6)</td>
<td>11.47 ± 1.1</td>
<td>12.83 ± 1.8</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.44 ± 0.08</td>
<td>0.62 ± 0.10</td>
</tr>
<tr>
<td>Total SFA</td>
<td>27.38 ± 2.5</td>
<td>23.09 ± 1.9</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 5).
† SFA = saturated fatty acids.
\( \alpha3 \) fatty acids, and total monounsaturated fatty acids \((p < 0.05)\). Increase in the dietary P/S ratio was accompanied by significantly lower levels of total monounsaturated fatty acids \((p < 0.05)\) and higher levels of C22:4(6) \((p < 0.03)\) in phosphatidylethanolamine. In phosphatidylcholine, increase in dietary P/S ratio was associated with an increase in C18:1 \((p < 0.05)\). Increased intake of saturated fatty acids, as a percent of total fat, correlated negatively with the level of C22:4(6) in phosphatidylethanolamine \((p < 0.01)\). As monounsaturated fatty acid intake increased as a percent of total fat, levels of C16:0 \((p < 0.03)\) and C20:4(6) \((p < 0.04)\) in phosphatidylcholine decreased (Fig 5). Increased monounsaturated

FIG 4. P/S ratio of adipose tissue phosphatidylcholine fatty acids expressed as a function of mean dietary C18:2(6) intake \((r = 0.48)\).

FIG 5. Fatty acid composition of adipose tissue phosphatidylcholine, expressed as a function of mean dietary monounsaturated fatty acid intake calculated as a percent of total fat. Relationship between monounsaturated fat intake and (▲) C16:0 \((r = -0.49)\); and (△) C20:4(6) \((r = -0.48)\).
fatty acid intake correlated negatively with the level of C20:5(3) (p < 0.01) and C18:0 (p < 0.05) in phosphatidylethanolamine. An increase in monounsaturated fatty acid intake was associated with a decrease in C16:1 (p < 0.05) and an increase in C18:3(3) (p < 0.01), C20:3(6), and total ω3 fatty acids (p < 0.05) in the phosphatidylcholine fraction of adipose tissue. On a body weight basis carbohydrate intake was associated with decreased levels of total ω3 fatty acids (p < 0.01) and increased levels of C22:4(6) (p < 0.01) in phosphatidylethanolamine. Increased carbohydrate intake was associated with significantly higher levels of C22:6(3), C22:4(6) (p < 0.01), total PUFA content, and P/S ratio (p < 0.05) of phosphatidylcholine. Thus it is apparent that transitions in intake of each of the major dietary fat constituents and carbohydrates are associated with distinct changes in fatty acid composition of both the essential and nonessential fatty acids present in membrane phospholipids.

Relationships within lipid classes

A linear relationship was found between the levels of total ω6 and ω3 fatty acids in phospholipids (Fig 6). Increased total ω3 fatty acids in phosphatidylethanolamine were positively associated with levels of total ω6 fatty acids (p < 0.05; Fig 6A). For phosphatidylcholine, higher levels of total ω3 fatty acids were associated with decreased levels of total ω6 fatty acids (p < 0.05; Fig 6B). A relationship between ω3 and ω6 fatty acids was not found in the triglyceride fraction. A negative linear relationship was noted between levels of total polyunsaturated and total monounsaturated fatty acids in adipose triglycerides (p < 0.01).

Relationship between lipid classes

A positive linear relationship was found between the C18:1 level in adipose tissue triglycerides and C18:1 level in phosphatidylethanolamine for male subjects (p < 0.03), and the level of C18:2(6) in these two lipid classes in the adipose tissue of female subjects (p < 0.02; Fig 7). For female subjects, a positive linear relationship was also noted between the levels of C18:3(3) in adipose triglycerides and phosphatidylethanolamine (p < 0.02). In addition, levels of total monounsaturated fatty acids in triglyceride and phosphatidylethanolamine

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**FIG 6.** Relationship between total ω3 fatty acids and total ω6 fatty acids within adipose tissue. A, phosphatidylethanolamine (r = 0.45); B, phosphatidylcholine (r = −0.46).
Discussion

Relationship between diet and triglyceride fatty acid composition

Fatty acid composition of human adipose tissue found in this study agrees with earlier reports (11). Due to slow turnover rates, the triglycerides stored in adipose tissue are believed to be a qualitative measure of fat intake over the previous year (6). In the present study, both total and saturated fatty acid intakes were significantly related to the saturated and polyunsaturated fatty acid composition of adipose tissue triglycerides (Figs 1 and 2). The similar relationships between total, saturated, and monounsaturated fatty acid intakes may be a result of dietary correlations found between saturated fatty acid intake and intake of monounsaturated and total fatty acids (r = 0.87 and r = 0.93, respectively). The negative relationship observed between dietary saturated fatty acids and C20:4(6), C18:3(3), and P/S ratio in adipose tissue triglycerides could have resulted from a diminished C18:2(6) intake, since saturated fatty acid intake/ unit body weight was inversely related to the polyunsaturated fatty acid content of dietary fat.

As linoleic acid is an essential fatty acid, its presence in adipose tissue can be assumed to have originated from the diet. Predicted relationships between dietary polyunsaturated fatty acids and the polyunsaturated fatty acid composition of triglycerides were not observed. This relationship may have been ob-
sured by limitations in measuring C18:2(6) intake from the computer data base used. Although C18:2(6) is the major polyunsaturated fatty acid in the diet, other unsaturated fatty acids, such as linolenic acid, were combined in determining total polyunsaturated fatty acid intake. Linolenic fatty acid intake may affect independently adipose tissue composition. In addition, the relationship between dietary and tissue concentrations of C18:2(6) is not necessarily linear, since in mice it was found that at low intake, the diet level of C18:2(6) influenced the ratio of the amount of C18:2(6) oxidized to that deposited (1). Whether this relationship is also true for man or at the higher C18:2(6) intakes measured in this study is unknown.

Early animal work has indicated that the relationship between dietary fatty acid intake and adipose tissue composition may be related to the dietary pattern, rather than the quantity of the fatty acid in the diet (2). Significant differences were noted in adipose C18:2(6) concentration of dialysis patients consuming similar amounts of C18:2(6) but different levels of saturated fatty acids (29). This may indicate that the relative proportion of polyunsaturated to saturated fatty acids may be of greater significance in determining polyunsaturated fatty acid content of human adipose tissue triglycerides than total C18:2(6) intake.

In the present study the dietary P/S ratio correlated with the polyunsaturated fatty acid (C18:2(6), total ω6, total PUFA) content and P/S ratio of the adipose triglyceride fraction. The relationship between dietary P/S ratio and adipose fatty acid composition was further demonstrated by comparing two groups of subjects representing a high (0.59 ± 0.08) vs low (0.35 ± 0.02) dietary P/S ratio. Significant differences were found between the groups in the relative proportions of saturated and polyunsaturated fatty acids (Table 5). Significant changes in adipose polyunsaturated fatty acid composition have been induced by changing the P/S ratio of diets fed from <0.3 to >1.2 (12). Examining the diets of the two groups of individuals with high and low concentrations of adipose tissue C18:2(6) demonstrated significant differences in the dietary P/S ratio, despite similar absolute intakes of C18:2(6) (30). In this regard, McMurchie et al (31) produced differences in the C18:2(6) and P/S ratio in phospholipids of cheek lipids by changing the dietary P/S from approximately 0.4 to 1.0. Examination of the two subgroups in the present study indicated no significant differences in the polyunsaturated fatty acid composition of phospholipids. Thus, a larger variation in dietary P/S than observed in the present study may be necessary to demonstrate differences in phospholipid fatty acid composition.

The observation that dietary C18:2(6) is inversely related to adipose tissue concentrations of C18:1 in both animals (2, 32) and man (7, 12) has led to the hypothesis that a regulatory mechanism exists to maintain the composition of depot fat at an optimal unsaturated to saturated ratio. It has been speculated that compensatory modifications occur in either or both the rates of biosynthesis and oxidation of specific fatty acids so as to minimize compositional changes in fatty acids (33). Controversy exists with regard to the existence of this mechanism (34), as changes in one dietary component inevitably involve other dietary alterations. A possible explanation for the inverse relationship found between dietary C18:2(6) and triglyceride C18:1 content is the negative correlation between C18:2(6) intake and the saturated fatty acid content of dietary fat (r = −0.90). It has been observed in mice that increased intake of saturated fatty acids induces desaturase activity in the liver, subsequently increasing the concentration of oleic acid in both the liver and adipose tissue (3).

A mathematical relationship has been proposed by Beynen et al (14) to predict adipose tissue C18:2(6) composition for large groups using mean dietary C18:2(6) intake. Entering the mean polyunsaturated fatty acid intake (as a component of dietary fat) of the entire group into the proposed formula, the predicted value of adipose tissue C18:2(6) fell within 2% of the mean obtained for subjects examined. A problem in accepting this model is that it is based on the assumption that all fatty acids have similar turnover kinetics. The fatty acids available for incorporation into adipose tissue depend on diet, amount and type of endogenously synthesized fatty acids, rate of fatty acid oxidation, and controls over incorporation into the lipid class. It is likely that both the amount and relative proportion of each fatty acid in the diet can modify these factors.

Since each fatty acid has its own unique
physiological-biochemical properties, altering a fatty acid within a lipid will alter the properties of the lipid to some extent. Adipose tissue fatty acid composition has been examined in relation to disorders such as coronary artery disease (35, 36), hypertension (37), and glucose tolerance (38). Cause and effect conclusions are not clear since most of these diseases are multifactorial, making controlled experiments difficult. Animal studies, however, indicate that the fatty acid composition of adipose tissue may be of physiological importance, as adipose tissue rich in saturated fat demonstrated reduced uptake and utilization of glucose (39). Further, in animals fed high saturated fatty acid diets, adipose tissue was not as sensitive to norepinephrine in vitro as that obtained from animals fed diets high in polyunsaturated fat (40).

Relationship between diet and phospholipid fatty acid composition

Total phospholipids extracted from the adipose biopsy in this study represent the mixture of cell membranes in adipose tissue, since it may be assumed that there is negligible phospholipid storage in human adipose tissue. Although there are other phospholipids, such as phosphatidylserine, phosphatidylinositol, and sphingomyelin, phosphatidylcholine and phosphatidylethanolamine are the major phospholipids in adipocyte membranes.

Fatty acid composition of major phospholipids in the adipose organ were related to the mean dietary intake of specific fatty acids. The relationship between diet and composition was found to be more consistent in the triglyceride fraction than in the phospholipids. This may be connected with the relative ability of the body to modify the fatty acid composition of different lipid classes. Phospholipids in various tissues have been found to reflect dietary differences in fatty acid intake to a much smaller extent than triglycerides (3, 29). This observation suggests endogenous control on phospholipid composition. In addition, phospholipids extracted from different tissue demonstrate differences in composition (32). In accordance with reports (16, 17) from other laboratories, this study demonstrated a higher percent of unsaturated fatty acids in phosphatidylcholine when compared with phosphatidylethanolamine (Table 4). Since C18:2(6)
cannot be synthesized one would suspect that a mechanism is necessary to conserve phospholipid stores of C18:2(6). Changing from a diet with a high P/S to low P/S ratio over a 6-wk period did not decrease phospholipid C18:2(6) levels in subjects examined (31). Although resistant to change, phosphatidylcholine and phosphatidylethanolamine in the adipocyte (39) and a variety of other membranes (16, 17) have been successfully altered by changing dietary fat.

By changing C18:2(6) intake of guinea pigs, Pavey et al (32) altered the polyunsaturated and saturated fatty acid composition of liver, muscle, and plasma phosphatidylcholine and phosphatidylethanolamine. In the animal model, diet-induced changes in the levels of ω3 and ω6 fatty acids in membrane phospholipids have been related to alterations in membrane function (17, 19). In the present study, the intake of C18:2(6)/unit body weight significantly related to the P/S ratio of fatty acids in phosphatidylcholine (Fig 4). In both phospholipid classes fatty acid intake was significantly related to the ω3 and ω6 fatty acids present, suggesting that the levels of dietary essential fatty acids in these membrane lipids were influenced by diet fat.

The two phospholipid fractions studied did not relate to diet in the same manner. For example, dietary polyunsaturated fatty acid composition was positively related to the C18:1 composition in phosphatidylcholine but negatively related to the C18:1 composition in phosphatidylethanolamine. Within the phospholipids, increases in total ω3 fatty acid content were associated with decreases in total ω6 fatty acids in phosphatidylcholine and increases in total ω6 fatty acids in phosphatidylethanolamine (Fig 6). This suggests that dietary ω6 and ω3 fatty acids were incorporated and/or turned over differently within the two phospholipids studied.

Relationship between lipid classes

The relationship found between the C18:2(6), C18:1 (Fig 7), and C18:3(3) content of adipose tissue triglycerides and phosphatidylethanolamine implied that these two lipids: a) derive fatty acids from a common precursor pool; or, b) that one lipid class provides fatty acids for synthesis of the other. Because stored triglycerides represent the largest body pool of
potentially available fatty acids, adipose triglycerides could provide a ready supply of fatty acids for rapidly turning over phospholipid pools. Since diglycerides are intermediate in the synthesis of triglycerides and phospholipids (33), subsequent studies could examine this perspective by examining triglyceride and phospholipid fatty acid composition in conjunction with diglyceride pools.

**Summary and implications**

The present study demonstrated that in healthy free-living individuals the nature of one's habitual diet with respect to both the amount and type of fatty acids is related to the fatty acid composition of structural and stored lipids in adipose tissue. The physiological importance of these compositional alterations is apparent in several animal models (17, 19, 40) but remains to be determined in human adipose tissue. Because of the central role of the adipose organ in metabolism, the whole-body implications of modest changes in function could be important.

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