Essential fatty acid deficiency in patients with severe fat malabsorption\textsuperscript{1,2}

Palle B Jeppesen, Michael S Christensen, Carl-Erik Høy, and Per B Mortensen

ABSTRACT Essential fatty acid deficiency is commonly described in patients receiving parenteral nutrition, but the occurrence in patients with severe fat malabsorption not receiving parenteral nutrition is uncertain. One hundred twelve patients were grouped according to their degree of fat malabsorption: group 1, < 10% (n = 52); group 2, 10-25% (n = 21); group 3, 25-50% (n = 24); and group 4, > 50% (n = 15). Fecal fat was measured by the method of Van de Kamer the last 2 of 5 d of a 75-g fat diet. Serum fatty acids in the phospholipid fraction were measured by gas-liquid chromatography after separation by thin-layer chromatography and expressed as a percentage of total fatty acids. The concentration of linoleic acid in groups 1, 2, 3, and 4 was 21.7%, 19.4%, 16.4%, and 13.4% respectively (P < 0.001). The concentration of linolenic acid in groups 1, 2, 3, and 4 was 0.4%, 0.4%, 0.3% and 0.3%, respectively (P = 0.017). Evidence of essential fatty acid deficiency, defined as a serum concentration of linoleic acid less than the lower limit if the 95% CI in patients without fat malabsorption (group 1), was 5% (121), 38% (9/24), and 67% (10/15) in groups 2, 3, and 4, respectively. A considerable portion of patients with gastrointestinal diseases resulting in malabsorption of > 25-50% of dietary fat intake and not treated with parenteral nutrition have biochemical signs of essential fatty acid deficiency. The clinical effect of these changes are yet to be elucidated. Am J Clin Nutr 1997:65:837–43.

KEY WORDS Essential fatty acid deficiency, serum phospholipids, linoleic acid, eicosatrienoic acid, Holman index, fat malabsorption, steatorrhea, inflammatory bowel disease, humans

INTRODUCTION Essential fatty acid deficiency (EFAD) may occur after long-term parenteral nutrition (1–4), protein-energy malnutrition (5, 6), or severe fat malabsorption (7–12). Fat malabsorption results in a reduced pool of essential fatty acids and changes the serum fatty acid profile, primarily linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), reflecting biochemical evidence of EFAD before the appearance of clinical manifestations. Clinical signs of EFAD, however, may not appear for weeks or months (13), which may impede the clinical interpretation of the biochemical changes supposed to result from EFAD. Biochemical evidence of EFAD has been associated with different ratios and concentrations of fatty acids. The Holman index, ie, the ratio of eicosatrienoic acid (20:3n-9) to 20:4n-6 in plasma phospholipids is commonly used (14).

EFAD has been related to a large range of clinical manifestations: dermatitis (15), increased water permeability of the skin (16–18), increased susceptibility to infection (19), lowered resistance to irradiation injury, and impaired wound healing (20). Less prominent are reports of hemolitic disturbances including hemolytic anemia, thrombocytopenia and diminished platelet aggregation (21), fatty infiltration of the liver (22), elevated hepatic enzymes, impaired chylomicron synthesis, and aggravated fat malabsorption (23). Furthermore, studies have focused on the effects of 18:2n-6 in cholesterol metabolism and eicosanoid synthesis (24). In rats, a reasonable range of 18:2n-6 intake supports longevity (25), whereas EFAD has been related to dysfunctional eicosanoid synthesis and diminished immune system status (26).

In humans the four families of polyunsaturated fatty acids are distinct insofar as transformations between families do not occur, although enzymes for elongation and desaturation are shared (27). The essential fatty acids 18:2n-6 and linoleic acid (18:3n-3), which are entirely of dietary origin, are the principal members of the n-6 and n-3 families, in contrast with the nonessential fatty acids of the n-7 and n-9 families, which can be synthesized from saturated fatty acids of endogenous origin.

The present study evaluated the range of changes in essential and nonessential fatty acids in serum from 112 consecutive patients not receiving parenteral nutrition referred to a gastrointestinal unit for possible malabsorption. The study evaluated the relation between the degree of malabsorption and biochemical evidences of EFAD.

SUBJECTS AND METHODS

Patients

One hundred twenty-four consecutive patients admitted on an elective basis to the Department of Gastroenterology, Rigshospitalet, Copenhagen, for diagnosis and evaluation of

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malabsorption were included in the study. Patients receiving long-term parenteral nutrition or fluids, patients in an unstable condition as a result of acute disease or recent surgery (within the previous 3 mo), and patients with inflammatory bowel disease and signs of active inflammation were not considered as candidates. Twelve patients were excluded because of inability to eat the diet, incomplete stool collection, or withdrawal from the study at their own request.

One hundred twelve (46 males and 66 females) patients with a mean age of 44.5 y (range: 19–77 y) remained for investigation. Sixty-six patients had Crohn disease, of whom 52 had small-bowel resections between 15 and 265 cm in length (mean 104 cm). All but six Crohn patients had various degrees of colonic resection, of whom 32 had an ileostomy because of a total colectomy. All patients had quiescent Crohn disease according to the entrance criteria. All but 1 of 26 patients with ulcerative colitis were colectomized. Ten had a conventional ileostomy and 15 had a J pouch. Sixteen patients had intestinal resections because of mesenteric infarction (n = 6) or complications after surgery (n = 4), and four had intestinal bypasses for obesity (n = 3) or hypercholesterolemia (n = 1). One patient with polyposis and one with chronic constipation were colectomized and had ileostomies. One had radiation enteritis, two had celiac disease, and one had cholestatic liver disease of unknown origin. Remaining intestinal anatomy was deduced from surgical records. Normal small-bowel length was given as 350 cm. Remaining colon was given in quartiles, whereby 100% represented an intact colon and 0% represented a total colectomy. The intestinal anatomy of the population is given in Figure 1.

The study was approved by the Ethical Committee for Medical Research in Copenhagen and conducted according to the Helsinki II Declaration.

Methods

Patients were requested to fill in a record of their habitual consumption of food and beverages during a 3-d period 2–3 wk before admission. They were told to select a period representative of their common daily eating and drinking habits, to weigh out food components on a kitchen balance, and to measure energy-containing liquids to the nearest 100 mL. The average energy and fat consumption was calculated by a dietitian with the DANKOST computer program (28). Patients were usually admitted on a Monday and received a fixed diet prepared in the ward kitchen by the dietitians during the 5-d study period. The diet contained a known, constant amount of fat (~75 g/d) and a constant amount of energy to meet the usual needs of the patient as indicated from dietary records and an interview with the dietitian at admission. Non-energy-containing beverages were provided ad libitum and patients received their habitual medications. Remaining food was deducted by using the DANKOST computer program. Complete forty-eight-hour stool samples were collected the last 2 d of the 5-d study period and fat was determined in the homogenized feces by the method of Van de Kamer et al (29). Body weight and height and blood samples were obtained on the day of admission.

Fatty acid analysis

The blood samples were immediately centrifuged at 3000 rpm, 1000 × g, for 10 min at room temperature, and serum was stored at −20 °C until analyzed. The total lipid fraction from serum samples was extracted according to the method of Folch et al (30). To 200 μL serum, 1.5 mL methanol was added. Three milliliters chloroform was added and the mixture was shaken briefly; 0.2× the volume as saline (0.73% NaCl by wt) was then added, and the mixture was shaken vigorously. The mixture was left to separate into two phases, and the aqueous phase was discarded. The organic phase was filtered through a column of anhydrous Na2SO4, and the column was washed twice with 1 mL chloroform. The solvent was evaporated and the lipid fraction redissolved in 2 mL chloroform:methanol (1:95.5, by vol) containing 0.005% butylated hydroxytoluene (Sigma, St Louis).

One milliliter of the lipid extract was applied to thin-layer chromatography plates and developed by using heptan:isopropanol:acetic acid (95:5:1, by vol). The plates were sprayed with 2,7-diclorofluorescein and the lipid classes were visualized under an ultraviolet lamp. The phospholipid fractions (application zone) were scraped off for fatty acid analysis. The phospholipid fraction was saponified and methylated with BF3 (31). The fatty acid methyl esters were analyzed by using a Hewlett-Packard 5890, series II gas-liquid chromatograph equipped with a fused silica column (SP2380, 60 m, internal diameter 0.25 mm; Supelco Inc, Bellefonte, PA), helium as carrier gas, and a split ratio of 1:20. Initial temperature was 70 °C for 0.5 min, then temperature programming was as follows: 15 °C/min until 160 °C followed by 1.5 °C/min until 200 °C, which was maintained for 15 min, and finally a rate of 30 °C/min until 225 °C, which was maintained for 5 min. The fatty acid methyl esters were identified by using standard mixtures (NuChek Prep Inc, Elysian, MN).

Statistical analysis

Differences between groups were assessed by a nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks with the SIGMASTAT statistical program package (Jandel Corp, Erkrath, Germany). For all pair-wise-multiple comparisons, Dunn’s method was used as the post hoc test. A P value < 0.05 was considered to represent a significant difference between groups.

\[\text{FIGURE 1. Length of remaining small and large intestine in the four groups of patients with different degrees of fat malabsorption. Arrow indicate patients with a Holman index > 0.2.}\]
RESULTS

Fifty-two of the 112 patients evaluated had normal stool output of fat defined as < 10% (7–9 g/d) of dietary fat (group 1; Table 1). The 60 patients with steatorrhea were divided according to severity of malabsorption into three groups, who excreted 10–25% (21 patients, group 2), 25–50% (24 patients, group 3), and 50–100% (15 patients, group 4) of the dietary intake of fat (median 72–76 g/d, Table 1). Energy intake at home, calculated from the dietary records, and energy intake during the study period did not differ among the four groups. Habitual median fat intake was generally 10–17 g/d higher than the ∼75 g eaten daily in the study period (Table 1). No difference was seen in the four groups for hemoglobin or serum albumin concentrations or inflammatory activity measured by sedimentation. No difference was seen in body mass index and no correlation between body mass index and serum albumin could be detected (r = 0.09, P > 0.2).

The intestinal anatomy of the patients is illustrated in Figure 1. Intestinal resection was more frequent and extensive with increasing fat malabsorption, resulting in decreasing median lengths of remaining small intestine (350, 275, 205, and 200 cm) for groups 1–4, respectively (P < 0.001). Correlation between length of remaining small intestine and fat malabsorption was poor (r = 0.53) although significant (P < 0.001). In contrast, the extent of colonic resection did not differ significantly among the four groups, the median remaining colonic length being 0%, 0%, 50%, and 0% for groups 1–4, respectively (P = 0.34). No difference was detected between the relation of remaining small intestine and fat absorption in patients with Crohn disease and patients who had small intestinal resections from other causes (Crohn disease: linear correlation, x ± SD, intercept length = 0 cm, 36 ± 11%, slope α1 = 0.14 ± 0.04, r = 0.43; and other causes: linear correlation, intercept length = 0 cm, 42 ± 6%, slope α2 = 0.14 ± 0.02, r = 0.70, Pint = 0.64, Pα1=α2 = 0.93).

The concentrations of the main saturated and unsaturated long-chain fatty acids in the phospholipid fraction of serum, which has been suggested to be the optimal fraction for detection of changes in essential fatty acids because of its high concentration of polyunsaturated fatty acids (32), are given in Table 2. The concentration of 18:2n–6 decreased as a function of increasing fat malabsorption from 21.7% in patients without malabsorption (group 1) to 19.4%, 16.4%, and 13.4% in patients who malabsorbed 10–25%, 25–50%, and 50–100% of their fat intake, respectively (groups 2–4, P < 0.001; Table 2). Changes in other n–6 fatty acids were much less prominent. There were no significant changes in 20:4n–6, the denominator in the Holman index. Other fatty acids of the n–6 family actually increased in concentration (dihomo-γ-linoleic and docosapentaenoic acids), but total concentrations nevertheless decreased as a consequence of the dominance of 18:2n–6, which accounted for 52–65% of n–6 fatty acids (Table 2). Concentrations of 18:3n–3 were only ∼2% of the concentrations of 18:2n–6 and 5–7% of total n–3 fatty acids, which made the small but significant reduction in concentration from 0.4% to 0.3% (P = 0.017) in groups 1–4 insufficient to influence total concentrations of the n–3 fatty acids (Table 2).

Signs of EFAD may, as discussed later, include increased concentrations of n–9 and n–7 fatty acids, e.g. 20:3n–9, which increased threefold (0.2%, 0.3%, 0.5%, and 0.6% for groups 1–4, respectively; P < 0.001) and was the principal reason for the increase in the Holman index (0.03, 0.03, 0.05, and 0.06 for groups 1–4, respectively; P = 0.001; Table 2). However, total concentrations of n–9 fatty acids did not change because of unaffected concentrations of oleic acid (18:1n–9) constituting > 90% of the n–9 fatty acids.

In contrast, increasing concentrations of the two major members of the n–7 family, palmitoleic acid (16:1n–7) and vaccenic acid (18:1n–7), increased total n–7 fatty acids from 2.5% to 2.7%, 2.9%, and 3.4% as fat malabsorption was aggravated (groups 1–4, P < 0.001; Table 2).

A close inverse correlation (r = 0.91, P < 0.001) between nonessential and essential unsaturated fatty acids was detected (Figure 2). In comparison, there was no significant correlation between 20:3n–9 and 20:4n–6, the respective numerator and

| Table 1 | Patient characteristics
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<tbody>
<tr>
<td></td>
<td>Fat malabsorption</td>
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<tr>
<td></td>
<td>Group 1, 0–10%</td>
<td>Group 2, 10–25%</td>
<td>Group 3, 25–50%</td>
<td>Group 4, &gt;50%</td>
</tr>
<tr>
<td>Age (y)</td>
<td>33.5 (28.5–45.5)</td>
<td>48.0 (41.8–51.5)</td>
<td>51.5 (42.0–61.5)</td>
<td>52.0 (41.5–61.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 (19.5–24.4)</td>
<td>22.8 (20.6–25.8)</td>
<td>22.1 (19.4–24.2)</td>
<td>21.5 (19.3–24.6)</td>
</tr>
<tr>
<td>Home diet energy (MJ/d)</td>
<td>9.7 (8.0–12.1)</td>
<td>8.8 (7.3–11.2)</td>
<td>10.4 (7.6–14.0)</td>
<td>9.8 (7.9–14.6)</td>
</tr>
<tr>
<td>Hospital diet energy (MJ/d)</td>
<td>9.3 (8.2–10.4)</td>
<td>9.1 (8.1–10.3)</td>
<td>9.3 (8.7–11.4)</td>
<td>8.7 (8.7–9.8)</td>
</tr>
<tr>
<td>Home diet fat (g/d)</td>
<td>93 (69–113)</td>
<td>87 (71–118)</td>
<td>86 (71–124)</td>
<td>90 (69–152)</td>
</tr>
<tr>
<td>Hospital diet fat (g/d)</td>
<td>76 (71–78)</td>
<td>72 (69–77)</td>
<td>76 (70–79)</td>
<td>76 (76–78)</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>8.2 (7.9–8.8)</td>
<td>8.4 (8.1–9.0)</td>
<td>8.3 (7.9–8.6)</td>
<td>8.5 (7.9–8.6)</td>
</tr>
<tr>
<td>(g/L)</td>
<td>127 (122–136)</td>
<td>130 (126–140)</td>
<td>129 (122–133)</td>
<td>132 (122–133)</td>
</tr>
<tr>
<td>Sedimentation (arbitrary units)</td>
<td>10 (5–26)</td>
<td>12 (6–22)</td>
<td>9 (6–20)</td>
<td>18 (14–28)</td>
</tr>
<tr>
<td>Albumin (mmol/L)</td>
<td>674 (622–709)</td>
<td>625 (574–690)</td>
<td>666 (626–693)</td>
<td>635 (585–695)</td>
</tr>
<tr>
<td>(g/L)</td>
<td>44.5 (41.1–46.8)</td>
<td>41.3 (37.9–45.5)</td>
<td>44.0 (41.3–45.7)</td>
<td>41.9 (38.6–45.9)</td>
</tr>
</tbody>
</table>

1 Median; 25th and 75th percentiles in parentheses.
2 Significantly different by group, P < 0.001 (Kruskal-Wallis one-way ANOVA on ranks).
denominator of the Holman index ($r = 0.06, P > 0.2$; Figure 3).

The correlation between length of remaining small intestine and 18:2n-6 was poor although significant ($r = 0.45, P < 0.05$). No difference was detected between the relation of length of remaining small intestine and 18:2n-6 concentration in patients with Crohn disease and patients who had small intestinal resections from other causes (Crohn disease: linear correlation, $\tau \pm SD$, intercept 1 length = 0 cm, 13 ± 2%, slope $\alpha_1 = 0.03 \pm 0.01, r = 0.38$; and other causes: linear correlation, intercept 2 length = 0 cm, 13 ± 1%, slope $\alpha_2 = 0.02 \pm 0.01, r = 0.55, P_{\text{intercept } 1-2} = 0.85, P_{\alpha_1-\alpha_2} = 0.75$).

In this study the mean Holman index was 0.05 and the upper limit of the 95% CI was 0.2. Indexes > 0.2 were found in 2 of the 112 patients: 1 in group 1, 0 in group 2, 2 in group 3, and 4 in group 4 had signs of EFAD as evidenced by a Holman index > 0.2. Of the seven patients, one (no. 1) had no malabsorption of fat but had just stopped lactating, two (nos. 3 and 6) reported home energy intake below estimated basic metabolic rate, four (nos. 2, 3, 5, and 6) compiled strictly with the recommended low-fat diet and had a daily fat intake < 50 g and two (nos. 6 and 7) had an extreme malabsorption of fat (~90%) and had previously received parenteral nutrition.

The outcome according to the various evidences of EFAD is shown in Table 3. The lower limit of the 95% CI of the overall ratio between the $n-7$ and $n-9$ fatty acids and the $n-3$ and $n-6$ fatty acids in the group without malabsorption (group 1) selected 16 patients, 4% (2/52) in group 1, 0% (0/21) in group 2, 29% (7/24) in group 3, and 47% (7/15) in group 4, as having biochemical evidence of EFAD. A further narrowing of criteria to the 99% CI selected 13 patients as deficient: 2% (1/52) in group 1, 0% (0/21) in group 2, 25% (6/24) in group 3, and 40% (6/15) in group 4. The 18:2n-6 concentration per se as a marker of EFAD indicated at the 95% level that 21 patients were at risk, 2% (1/52) in group 1, 5% (1/21) in group 2, 38% (9/24) in group 3, and 67% (10/15) in group 4, whereas a cutoff at the lower limit of the 99% CI reduced the number of patients with biochemical signs of EFAD to 13, 2% (1/52) in group 1, 0% (0/21) in group 2, 29% (7/24) in group 3, and 33% (5/15) in group 4 (Table 3).

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**Table 2**

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Group 1, 0–10% (n = 52)</th>
<th>Group 2, 10–25% (n = 21)</th>
<th>Group 3, 25–50% (n = 24)</th>
<th>Group 4, &gt;50% (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% by wt of total fatty acids</td>
<td>$P^2$</td>
<td></td>
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<tr>
<td><strong>Saturated</strong></td>
<td></td>
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</tr>
<tr>
<td>14:0</td>
<td>0.3 (0.2–0.3)</td>
<td>0.3 (0.3–0.4)</td>
<td>0.4 (0.3–0.4)</td>
<td>0.4 (0.2–0.4)</td>
</tr>
<tr>
<td>15:0</td>
<td>0.2 (0.1–0.2)</td>
<td>0.2 (0.1–0.2)</td>
<td>0.2 (0.2–0.2)</td>
<td>0.2 (0.2–0.2)</td>
</tr>
<tr>
<td>16:0</td>
<td>27.1 (25.6-28.2)</td>
<td>28.2 (26.6-29.3)</td>
<td>28.8 (27.1-29.9)</td>
<td>28.8 (27.8-30.7)</td>
</tr>
<tr>
<td>17:0</td>
<td>0.4 (0.3–0.4)</td>
<td>0.3 (0.3–0.4)</td>
<td>0.4 (0.3–0.5)</td>
<td>0.3 (0.2–0.4)</td>
</tr>
<tr>
<td>18:0</td>
<td>13.8 (12.6–14.8)</td>
<td>13.2 (12.3–14.0)</td>
<td>13.1 (11.7–14.2)</td>
<td>12.8 (11.5–14.2)</td>
</tr>
<tr>
<td>Total saturated</td>
<td>42.1 (41.1–43.2)</td>
<td>42.9 (41.6–43.6)</td>
<td>43.4 (42.7–44.1)</td>
<td>44.2 (41.7–44.7)</td>
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<tr>
<td><strong>Unsaturated</strong></td>
<td></td>
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<tr>
<td>16:1n-7</td>
<td>0.7 (0.6–0.9)</td>
<td>0.8 (0.7–1.1)</td>
<td>1.4 (0.6–1.7)</td>
<td>1.5 (1.0–2.1)</td>
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<tr>
<td>18:1n-7</td>
<td>1.5 (1.4–1.7)</td>
<td>1.5 (1.5–1.7)</td>
<td>1.7 (1.6–2.0)</td>
<td>1.9 (1.7–3.4)</td>
</tr>
<tr>
<td>20:3n-7</td>
<td>0.3 (0.2–0.3)</td>
<td>0.3 (0.2–0.3)</td>
<td>0.3 (0.2–0.3)</td>
<td>0.2 (0.2–0.3)</td>
</tr>
<tr>
<td>Total n-7</td>
<td>2.5 (2.3–2.8)</td>
<td>2.7 (2.4–3.1)</td>
<td>2.9 (2.3–4.1)</td>
<td>3.4 (3.0–5.8)</td>
</tr>
<tr>
<td>16:1n-9</td>
<td>0.2 (0.2–0.2)</td>
<td>0.2 (0.2–0.2)</td>
<td>0.2 (0.2–0.3)</td>
<td>0.2 (0.2–0.3)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>11.6 (10.8–13.0)</td>
<td>11.9 (11.0–12.9)</td>
<td>12.8 (11.3–14.7)</td>
<td>12.9 (11.0–16.0)</td>
</tr>
<tr>
<td>20:3n-9</td>
<td>0.2 (0.2–0.2)</td>
<td>0.2 (0.2–0.2)</td>
<td>0.2 (0.2–0.2)</td>
<td>0.2 (0.2–0.2)</td>
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<tr>
<td>22:1n-9</td>
<td>0.2 (0.1–0.2)</td>
<td>0.2 (0.1–0.2)</td>
<td>0.2 (0.2–0.4)</td>
<td>0.3 (0.2–0.4)</td>
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<tr>
<td>Total n-9</td>
<td>12.4 (11.7–13.9)</td>
<td>12.8 (11.6–14.1)</td>
<td>13.8 (12.5–16.8)</td>
<td>14.1 (12.0–18.3)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>21.7 (19.2–23.6)</td>
<td>19.4 (16.6–21.5)</td>
<td>16.4 (12.9–21.6)</td>
<td>13.4 (10.8–16.7)</td>
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<tr>
<td>18:3n-3</td>
<td>0.1 (0.1–0.1)</td>
<td>0.1 (0.1–0.2)</td>
<td>0.1 (0.1–0.2)</td>
<td>0.1 (0.1–0.2)</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>2.8 (2.3–3.4)</td>
<td>2.8 (2.6–3.2)</td>
<td>3.0 (2.3–3.7)</td>
<td>3.4 (3.3–4.1)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>8.1 (7.1–9.0)</td>
<td>9.2 (8.0–10.6)</td>
<td>8.4 (7.5–9.7)</td>
<td>7.8 (6.9–9.0)</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>0.3 (0.3–0.4)</td>
<td>0.3 (0.3–0.4)</td>
<td>0.3 (0.3–0.4)</td>
<td>0.3 (0.3–0.5)</td>
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<tr>
<td>22:5n-6</td>
<td>0.1 (0.1–0.2)</td>
<td>0.2 (0.1–0.2)</td>
<td>0.2 (0.1–0.3)</td>
<td>0.2 (0.2–0.4)</td>
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<tr>
<td>Total n-6</td>
<td>33.4 (31.3–35.2)</td>
<td>32.0 (30.6–34.1)</td>
<td>30.0 (25.0–32.8)</td>
<td>25.8 (23.2–31.7)</td>
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<tr>
<td><strong>Total unsaturated</strong></td>
<td></td>
<td></td>
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<tr>
<td>55.7 (54.1–56.5)</td>
<td>55.2 (54.1–55.8)</td>
<td>54.5 (52.9–55.1)</td>
<td>45.4 (42.0–53.1)</td>
<td></td>
</tr>
<tr>
<td>$n-7 + n-9$</td>
<td>0.37 (0.35–0.44)</td>
<td>0.40 (0.36–0.45)</td>
<td>0.45 (0.36–0.64)</td>
<td>0.50 (0.40–0.84)</td>
</tr>
<tr>
<td>Holman index</td>
<td>0.03 (0.02–0.03)</td>
<td>0.03 (0.01–0.04)</td>
<td>0.05 (0.03–0.13)</td>
<td>0.08 (0.04–0.2)</td>
</tr>
</tbody>
</table>

1 Median: 25th and 75th percentiles in parentheses.
2 ANOVA.
ESSENTIAL FATTY ACIDS AND FAT MALABSORPTION

All but 1 (no. 3, Table 4) of the 7 patients with Holman indexes $> 0.2$ were included in the group of 12 patients with concentrations of 18:2$n-6$ below the lower limit of the 99% CI and in the 13 patients with ratios of $n-7 + n-9$ to $n-3 + n-6$ fatty acids above the upper limit of the 99% CI. Ten of the 12 patients with low concentrations of 18:2$n-6$ were likewise included among the 13 patients with raised ratios of $n-7 + n-9$ to $n-3 + n-6$.

**DISCUSSION**

In this study, fat malabsorption decreased serum concentrations of the essential fatty acids 18:2$n-6$ and 18:3$n-3$. A low absorption of essential fatty acids from the $n-6$ and $n-3$ families was paralleled by increased endogenous production of unsaturated fatty acids from the $n-9$ and $n-7$ families, thus maintaining total concentrations of unsaturated fatty acids.

The choice of biochemical indicators of EFAD and the definition of reference intervals is troublesome because of the inconsistent connection between laboratory indexes and clinical symptoms. In this study a Holman index $> 0.2$ was the most conservative criterion, indicating only seven patients with biochemical evidence of EFAD. The correlation between 20:

$$3n-9 \text{ and } 20:4n-6 \text{ was nevertheless poor. The findings of Färrkkilä et al (7) that } 20:3n-9 \text{ increased with severity of malabsorption in contrast with concentrations of } 20:4n-6 \text{ was also reproduced in our study (Table 2), which obviously disturbed the inverse correlation between the two acids (Figure 3) and confused the issue of whether the Holman index is more useful than other ratios or concentrations of fatty acids, eg, the 95% or 99% CIs of } 18:2n-6. \text{ Use of the } 95\% \text{ CIs from the patients without steatorrhea (group 1) leaves a large group of patients with steatorrhea who have abnormal serum lipid fatty acid composition suggestive of EFAD. Systematic registration of clinical manifestations of EFAD was not included in this study, which leaves the significance of the laboratory findings to be evaluated clinically, although it is tempting to hypothesize that biochemical evidence of EFAD might be in a subclinical state for years and possibly precedes the development of clinical manifestations.}

Siguel et al (9) examined 10 patients with evidence of fat malabsorption and inflammatory bowel disease who had required recent parenteral nutrition and compared them with healthy control subjects. As in our study, they found evidence of EFAD to be decreased concentrations of 18:2$n-6$ and ratios of essential fatty acids to nonessential fatty acids. Significant inverse correlations between essential fatty acids and monounsaturated fatty acids were noted. However, correlations with fat malabsorption were not identified.

Färrkkilä et al (7) examined 31 outpatients with ileal resections on their customary home diet; they were divided into two subgroups according to the presence ($> 7 \text{ g/d, } 23.6 \pm 20.7 \text{ g/d}$) or absence of fat malabsorption ($4.8 \pm 1.6 \text{ g/d}$). Hence, the design of this study was similar to ours except for fewer patients. They used a patient control group and found a significant decrease in the concentration of 18:2$n-6$ in serum triacylglycerol and cholesterol esters. Changes in phospholipid 18:2$n-6$ were not significant, possibly because of the smaller number of patients, which did not allow differentiation according to severity of malabsorption. However, there was an inverse correlation of fecal fat excretion with phospholipid 18:2$n-6$ ($r = -0.513, P < 0.001$) and a direct correlation with most of the saturated (14:0 and 16:0) and nonessential unsaturated fatty acids (16:1$n-7$, 18:1$n-9$, and 20:3$n-9$), in accordance with our results. Laboratory criteria of EFAD were suggested to be low cholesterol ester 18:2$n-6$, an increased Holman index ($> 0.16$) for the phospholipid fraction, increased

**FIGURE 2.** Relation between the sum of nonessential $n-7$ and $n-9$ fatty acids and the sum of essential $n-6$ and $n-3$ fatty acids. ●, Holman index $> 0.2$.

**TABLE 3**

Outcome according to evidence of essential fatty acid deficiency (EFAD)

<table>
<thead>
<tr>
<th>Fat malabsorption</th>
<th>EFAD indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Holman index $&gt;$ 0.2</td>
<td>1</td>
</tr>
<tr>
<td>n-7 + n-9/n-6 + n-3 $&gt; 0.59$</td>
<td>2</td>
</tr>
<tr>
<td>n-7 + n-9/n-6 + n-3 $&gt; 0.64$</td>
<td>1</td>
</tr>
<tr>
<td>18:2n-6 $&lt; 15.0$</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 13.0$</td>
<td>1</td>
</tr>
</tbody>
</table>

1 95% cutoff based on group 1.
2 99% cutoff based on group 1.

3n-9 and 20:4n-6 was nevertheless poor. The findings of Färrkkilä et al (7) that 20:3n-9 increased with severity of malabsorption in contrast with concentrations of 20:4n-6 was also reproduced in our study (Table 2), which obviously disturbed the inverse correlation between the two acids (Figure 3) and confused the issue of whether the Holman index is more useful than other ratios or concentrations of fatty acids, eg, the 95% or 99% CIs of 18:2n-6. Use of the 95% CIs from the patients without steatorrhea (group 1) leaves a large group of patients with steatorrhea who have abnormal serum lipid fatty acid composition suggestive of EFAD. Systematic registration of clinical manifestations of EFAD was not included in this study, which leaves the significance of the laboratory findings to be evaluated clinically, although it is tempting to hypothesize that biochemical evidence of EFAD might be in a subclinical state for years and possibly precedes the development of clinical manifestations.

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**FIGURE 3.** Relation between the fatty acids of the Holman index: eicosatrienonic acid (20:3$n-9$) and arachidonic acid (20:4$n-6$). ●, Holman index $> 0.2$.
20:3n-9 concentration, and increased ratio of dihomogamma-
linolenic acid + 20:4n-6 to 18:2n-6 with cutoffs being the lower or upper limits of 95% CIs in gastrointestinal patients
without fat malabsorption. Almost one-third of the 19 patients with fat malabsorption met these criteria for EFAD, yet no clinical evidence of EFAD was documented. In accordance with these data, we found that 20 of 60 patients with steatorrhea had 18:2n-6 concentrations in the phospholipid fraction below the lower limit of the 95% CI for the patient group without steatorrhea. However, differentiation between mild, moderate, and severe fat malabsorption was crucial, as indicated by the rarely encountered changes in serum fatty acids in the group of patients who malabsorbed < 25% of intake and the common changes in the majority of patients who malabsorbed > 50% of dietary fat (Table 2).

In theory, the risk of developing EFAD depends on the
demand for essential fatty acids, amount and availability of
dependent stores, amounts of 18:2n-6 in the diet, and the
ability to absorb ingested fat. Adults of normal weight accumu-
late 15-25% of body weight as fat (33), of which 8-12% is
18:2n-6 (34), which is equal to 0.8-2 kg 18:2n-6. Infants
mobilize fatty acids early for energy needs when faced with
deficient dietary intake or absorption, which, combined with
the requirements of growth, renders infants more susceptible to
EFAD (35). Newborns usually develop biochemical evidence of
EFAD within 2 wk when kept on a fat-free diet (36), but in
older children and adults body reserves of essential fatty acids
prolong the period before EFAD is evident. The requirements
for essential fatty acids are increased during lactation (37),
which may explain the biochemical evidence of EFAD in
patient no. 1 (Table 4). Patients receiving fat-free parenteral
nutrition are especially vulnerable to EFAD and biochemical
evidence of EFAD has been described within 2 wk of begin-
ning the treatment in critically ill postoperative patients (2, 38).
Continuous infusion of glucose blocks adipose tissue lipolysis
and outflow of 18:2n-6 secondary to high insulin concentra-
tions (39), and patients with a moderate oral intake treated
intravenously with hypertonic glucose and amino acids may be
as prone to develop EFAD as patients receiving total parenteral
nutrition (40).

In this study fat absorption was a better marker of small
intestinal quality than length of remaining small intestine. No
difference could be detected between patients with Crohn dis-
ease and patients with small intestinal resections from other
causes for correlation between length of remaining small in-
testine and fat absorption or concentration of 18:2n-6. Daily
requirements for 18:2n-6 are unknown. Estimates vary be-
tween 0.5% and 7% of total energy intake and most recom-
endations are minimums of 3-4% of energy (41), but the
restricted fat intake often recommended by gastroenterologists
in the treatment of severe fat malabsorption may further worsen
body supplies of essential fatty acids.

This study showed changes in plasma fatty acids usually
taken as signs of EFAD in patients not receiving parenteral
nutrition who had a certain severity of fat malabsorption equiv-
alent to fecal excretions > 25% (~20 g/d) of dietary intake. In
contrast, patients with a malabsorption of fat < 25% were
apparently not at risk even though classified as malabsorbers
according to the common criterion of an excretion of > 10% fat
(7-8 g/d).

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and Birthe Stenbaek Hansen was greatly appreciated.

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TABLE 4

Characteristics of patients with a Holman index >0.2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Patient number</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Sex (y)</td>
<td>30</td>
<td>73</td>
<td>58</td>
<td>59</td>
<td>52</td>
<td>42</td>
<td>56</td>
</tr>
<tr>
<td>Age (y)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.8</td>
<td>19.2</td>
<td>27.0</td>
<td>16.9</td>
<td>19.3</td>
<td>23.0</td>
<td>17.8</td>
</tr>
<tr>
<td>BMR (MJ/d)</td>
<td>6.0</td>
<td>5.4</td>
<td>6.3</td>
<td>5.4</td>
<td>5.5</td>
<td>7.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Diagnosis^2</td>
<td>UC + L</td>
<td>CD</td>
<td>CD</td>
<td>RE</td>
<td>CD</td>
<td>MT</td>
<td></td>
</tr>
<tr>
<td>Remaining small intestine (cm)</td>
<td>350</td>
<td>100</td>
<td>200</td>
<td>215</td>
<td>330</td>
<td>170</td>
<td>50</td>
</tr>
<tr>
<td>Remaining colon (%)</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Home diet energy (MJ/d)</td>
<td>9.5</td>
<td>13.5</td>
<td>5.6</td>
<td>10.3</td>
<td>7.7</td>
<td>7.7</td>
<td>14.1</td>
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<td>Home diet fat (g/d)</td>
<td>100</td>
<td>40</td>
<td>39</td>
<td>90</td>
<td>49</td>
<td>35</td>
<td>89</td>
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<td>Fat malabsorption (%)</td>
<td>7</td>
<td>28</td>
<td>34</td>
<td>54</td>
<td>69</td>
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<td>88</td>
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<td>no</td>
<td>no</td>
<td>no</td>
<td>February 1992</td>
<td>March 1989</td>
</tr>
</tbody>
</table>

^1 Basal metabolic rate.
^2 UC, ulcerative colitis; CD, Crohn disease; RE, radiation enteritis; MT, mesenteric thrombosis; L, recent lactation.