Essential fatty acid deficiency in mice impairs lactose digestion

S. Lukovac,* E. L. Los,* F. Stellaard, E. H. H. M. Rings, and H. J. Verkade
Pediatric Gastroenterology, Department of Pediatrics, Beatrix Children’s Hospital, Groningen University Institute for Drug Exploration (GUIDE), Center for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Center Groningen, The Netherlands

Submitted 21 February 2008; accepted in final form 19 July 2008

Lukovac S, Los EL, Stellaard F, Rings EH, Verkade HJ.
Essential fatty acid deficiency in mice impairs lactose digestion. Am J Physiol Gastrointest Liver Physiol 295: G605–G613, 2008. First published July 24, 2008; doi:10.1152/ajpgi.90206.2008.—Essential fatty acid (EFA) deficiency in mice induces fat malabsorption. We previously reported indications that the underlying mechanism is located at the level of the intestinal mucosa. We have investigated the effects of EFA deficiency on small intestinal morphology and function. Mice were fed an EFA-deficient or control diet for 8 wk. A 72-h fat balance, the EFA status, and small intestinal histology were determined. Carbohydrate absorbptive and digestive capacities were assessed by stable isotope methodology after administration of [U-13C]glucose and [1-13C]lactose. The mRNA expression and enzyme activity of lactase, and concentrations of the EFA linoleic acid (LA) were measured in small intestinal mucosa. Mice fed the EFA-deficient diet were markedly EFA-deficient with a profound fat malabsorption. EFA deficiency did not affect the histology or proliferative capacity of the small intestine. Blood [13C]glucose appearance and disappearance were similar in both groups, indicating unaffected monosaccharide absorption. In contrast, blood appearance of [13C]glucose, originating from [1-13C]lactose, was delayed in EFA-deficient mice. EFA deficiency profoundly reduced lactase activity (−58%, P < 0.01) and mRNA expression (−55%, P < 0.01) in mid-small intestine. Both lactase activity and its mRNA expression strongly correlated with mucosal LA concentrations (r = 0.77 and 0.79, respectively, P < 0.01). EFA deficiency in mice inhibits the capacity to digest lactose but does not affect small intestinal histology. These data underscore the observation that EFA deficiency functionally impairs the small intestine, which in part may be mediated by low LA levels in the enterocytes.

carbohydrate digestion; lactase; small intestine

ESSENTIAL FATTY ACID (EFA) deficiency can occur in cholestatic liver diseases as a consequence of fat malabsorption (5, 34). However, EFA deficiency itself also induces fat malabsorption (24, 45). The underlying mechanism of EFA deficiency-induced fat malabsorption remains unclear. Absorption of fat involves lipolysis, solubilization, intestinal translocation from the lumen into the mucosa, chylomicron assembly, and transport into the lymph (14, 28). Previous studies in EFA-deficient mice have indicated that impaired lipolysis or bile formation do not cause the fat malabsorption in EFA deficiency (45). However, studies in rats show that EFA deficiency alters both the intraluminal and intracellular phases of fat absorption (24). This implies that the effects of EFA deficiency on mucosal phases of fat absorption could be species dependent.

Recently, we reported data to suggest that EFA deficiency in mice affects fat absorption at the level of the small intestinal mucosa (45). However, it has not been proven that EFA deficiency impairs the mucosal phase of fat absorption. On the basis of previous findings, we hypothesize that EFA deficiency functionally impairs the small intestine.

In contrast to fat absorption, the absorption of di- and monosaccharide carbohydrates exclusively depends on mucosal function (36). The monosaccharide glucose is actively transported across the brush border membrane in the small intestine by the brush-border transporter sodium-dependent glucose transporter (SGLT1) (18, 36). The disaccharide lactose is first hydrolyzed by the mucosal membrane-anchored lactase-phlorizin hydrolase (lactase, LPH) into glucose and galactose before their active transport across the brush border by SGLT1 (17, 18). Besides being an important enzyme in lactose hydrolysis, lactase is a marker of enterocyte differentiation (32). Throughout development, total intestinal lactase activity remains similar to that found in newborns (4). If EFA deficiency affects lactase expression and activity in the small intestine, even slight changes should easily be detectable in adult EFA-deficient mice. For this reason, lactase is a good marker for functional assessment of the small intestine in adult animals.

EFAs are structural components of membrane phospholipids. Enterocyte membrane phospholipids are particularly rich in linoleic acid (LA, C18:2n-6) (22), which is necessary for modulations of a wide variety of biological functions and for physiochemical adaptations of the membrane lipid matrix to alterations in membrane fluidity (20). The lipid matrix influences the conformation and function of proteins embedded in the inner and/or outer leaflet of the membrane (41). Recently, an additional role of EFAs in alterations of bilayer elastic properties and lipid composition in lipid rafts have been reported (3, 25). Through activation of peroxisome proliferator-activated receptors (PPARs), EFAs can regulate transcriptional activity of several genes, including that of those involved in fatty acid transport and metabolism (12, 33).

In the present study, we characterized the effects of EFA deficiency on small intestinal morphology and function in mice. Korotkova et al. (22) have shown that EFA deficiency affects the fatty acid composition in the phospholipids of the rat small intestinal mucosa by decreasing jejunal concentrations of linoleic acid. However, no studies have been performed on the effect of EFA deficiency on the small intestinal function concerning carbohydrate absorption. We assessed the absorption of glucose, a major source of metabolic energy for mammalian cells (1), and the expression and activity of the
lactase enzyme, as appropriate functional markers of the small intestine, in a previously developed and characterized murine model of EFA deficiency (45). We applied stable isotope methodology (35) since this approach allows extension to similar studies in patients with EFA deficiency, cholestasis, or other forms of malabsorption (11, 21, 42). [U-13C]glucose and [1-13C]lactose were administered to EFA-deficient and control mice. Blood appearance of labels derived from administered glucose ([13C6]glucose) and lactose ([13C]glucose) into the blood glucose fraction was subsequently quantified. We also determined the activity and expression of lactase, as well as the concentration of LA, in the mucosa along the proximal-to-distal axis of the small intestine. To determine whether EFA deficiency specifically affects lactase activity or disaccharide activity in general, we additionally measured the activity of another disaccharide, sucrase.

Our data show that EFA deficiency is associated with impaired lactose digestion in mice. This functional observation is specific for lactase and corresponds with lower lactase mRNA expression and enzyme activity in the mid-small intestine of EFA-deficient mice. All together, these findings support the idea that EFA deficiency functionally impairs the small intestine.

MATERIALS AND METHODS

Mice and Housing

Wild-type mice with a free virus breed background were obtained from Harlan (Horst, The Netherlands). Male mice (25–35 g) were housed in a light-controlled (lights on 6:00 AM to 6:00 PM) and temperature-controlled facility and were allowed tap water and chow (AB Diets, Woerden, The Netherlands) ad libitum. The experimental protocol was approved by the Ethics Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen, The Netherlands.

Materials

[U-13C]glucose and [1-13C]lactose were obtained from Isotec (Miamisburg, OH) with isotopic enrichments of 99%. Unlabeled lactose was obtained from Fluka (Buchs, Switzerland).

Experimental Diets

Similar to previous studies, we used high-fat EFA-deficient and EFA-sufficient (control) diets (16 wt% and 34 energy% fat), to mimic more closely the human diet composition (45). The diets were custom synthesized by Arie Bloks (Woerden, The Netherlands); diet codes were EFA-deficient no. 4141.08 and EFA-sufficient no. 4141.07). The EFA-deficient diet contained 64 mol% palmitic acid (C16:0), 18 mol% stearic acid (C18:0), 13 mol% oleic acid (C18:1n-9), and 5 mol% LA (C18:2n-6). The isocaloric EFA-sufficient diet contained 36 mol% C16:0, 5 mol% C18:0, 31 mol% C18:1n-9, and 29 mol% C18:2n-6. Fatty acid contents of the diets were analyzed by extracting, hydrolyzing, and methylating total dietary fatty acids as described by Muskiet et al. (29), and subsequent separation and quantification of fatty acid methyl esters was performed by gas chromatography as described previously (45).

Experimental Procedures

Induction of EFA deficiency. Mice were fed standard laboratory chow containing 6 weight% fat from weaning, and were switched to EFA-deficient or control diet at 8 wk of age. After 8 wk of EFA-deficient or control diet, fat absorption was assessed by measuring food intake and collecting feces for 72 h. Mice underwent a glucose/lactose absorption test with [U-13C]glucose and [1-13C]lactose (details see below) (39, 46). After the test, the mice were anesthetized and euthanized by obtaining a large blood sample through cardiac puncture for determination of erythrocyte EFA-status by the triene/tetraene (C20:3n-9/C20:4n-6) ratio (45). The small intestine was excised, flushed with ice-cold PBS, and divided into a proximal, mid-, and distal segment of similar size. Smaller parts from the middle of each small intestinal segment were harvested for histology and gene expression. Another part of the proximal, mid-, and distal small intestine was opened lengthwise, and the mucosa was removed by scraping the luminal surface with a glass coverslip. Mucosa was homogenized in buffer (see below for details) and used for the determination of enzyme activity, proteins, and LA concentrations in mucosal phospholipids.

Glucose/lactose absorption. Glucose absorption and lactose digestion were determined by a combined [U-13C]glucose/[1-13C]lactose absorption test. After an overnight fast, mice received 0.5 mg [U-13C]glucose, 5 mg [1-13C]lactose, and 5 mg naturally enriched lactose in 300 μl of PBS via gastric gavage. Before and at time points 15, 30, 45, 60, 90, 120, and 180 min after administration, blood samples were obtained by blood spot technique from the tail for determination of blood concentrations of (total) glucose, [13C6]glucose (glucose originating from [U-13C]glucose), and [13C]glucose (originating from [1-13C]lactose) (39). For reasons of clarity, we will address “blood” [13C]glucose and [13C]glucose as “plasma” in RESULTS and DISCUSSION.

Analytical Methods

Lipid absorption, triene/tetraene ratio, erythrocyte fatty acid concentrations, blood glucose, and serum insulin concentrations. Lipid absorption, erythrocyte fatty acid concentrations, and triene/tetraene ratio were determined as described previously (27, 45). Blood glucose levels were measured with a Lifescan EuroFlash glucose meter (Lifescan Benelux, Beere, Belgium). Insulin was measured in a solid phase two-site enzyme immunoassay in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule (ultrasensitive mouse insulin kit; Mercodia, Uppsala, Sweden).

Histology and villus length along the small intestinal axis. Morphology of proximal, mid-, and distal small intestine was assessed by hematoxylin/eosin staining of formalin-fixed material. Proliferating cells were detected by staining of nuclear Ki-67 antigen. Morphometrical analysis of small intestinal samples was performed as described by evaluation of approximately five vertically oriented villi per intestinal segment of four to six animals per group. The digitized images were evaluated at ×10 magnification using the calibrated image analysis system (Leica Quantimet 570 C; Leica Qwin Pro V 2.8). The epithelial surface lining was demarcated and measured as a parametrical length, whereby one pixel was equal to 0.544 μm.

Glucose/lactose absorption. The analysis of [13C]glucose and [13C]lactose concentrations from blood spots was performed according to Van Dijk et al. (39) by gas chromatography-mass spectrometry (SSQ700; ThermoFischer, Breda, The Netherlands).

Disaccharidase activity assay in mucosal homogenates. A portion of small intestinal mucosa (proximal, mid, and distal) was homogenized with PBS buffer containing protease inhibitors (Roche, Indianapolis, IN) to make 4% homogenates for use in enzyme activity assay. Enzyme activity level of lactase and sucrase were measured in freshly scraped intestinal mucosa as described previously by Dahlqvist (9). Activity was normalized to protein levels, measured by the bicinchoninic acid method as described by the manufacturer (Pierce, Rockford, IL).

Measurement of mRNA expression by real-time PCR. mRNA expression of lactase and sucrase isomaltase was measured in proximal, mid-, and distal small intestine by real-time PCR as described previously (15). In addition, mRNA expression levels of intestine-
specific transcription factors (Cdx-2, Gata-4, and Hnf-1α) were measured by real-time PCR in the mid part of the small intestine. PCR results were normalized to β-actin mRNA levels. The sequences of the primers and probes are listed in Table 1.

**LA determination in phospholipids of intestinal mucosa.** Thirty milligrams of intestinal mucosa were homogenized in 200 μl of 0.9% NaCl, and lipids were extracted according to Bligh and Dyer (2) after the addition of the fatty acid internal standard (C17:0) and antioxidant butylated hydroxytoluene. Lipid extracts were fractionated into phospholipids and other lipids using TLC (20 × 20 cm, Silica gel 60 F254; Merck, Darmstadt, Germany) with hexane/diethyl ether/acetic acid (80:20:1, vol:vol:vol) as running solvent. Phospholipid spots were scraped, and phospholipids were extracted by methanol/chloroform. Phospholipid LA ratio was determined according to Muskiet et al. (29) as described previously (43).

**Statistical Analysis**

Values represent means ± SD for the indicated number of mice per group. Using SPSS version 12.0.2 statistical software (Chicago, IL), we calculated significance of differences with the Mann-Whitney U-test. P values <0.05 were considered statistically significant. Correlations between the linoleic concentrations in the mucosa of the mid-small intestine and mRNA expression and enzyme activity of lactase and sucrase were determined by means of linear regression and are expressed as nonparametric Spearman correlation coefficient (SPSS version 14.0). Differences between means were considered significant at the level of P < 0.01.

**RESULTS**

Body weight and food ingestion were assessed every 2 wk, and there were no significant differences in basal or final body weight, nor in food intake between EFA-deficient and control mice (data not shown).

**Pronounced EFA deficiency of EFA-deficient mice.** After 8 wk of treatment, in mice fed the EFA-deficient diet, the triene/tetraene ratio in red blood cell membranes was strongly increased compared with the control group (0.23 ± 0.06 vs. 0.01 ± 0.00, respectively; P < 0.01). Fatty acid profile in erythrocyte lipids is very similar to those obtained in our previous studies in mice (44). They revealed a severe decrease in n-6 family of EFAs, accompanied by an increase in concentrations of nonessential fatty acids of the n-7 and n-9 families (fig. 1B). Fat balance during 72 h revealed a decreased total fat absorption in EFA-deficient compared with control mice (81% vs. 99%, respectively; P < 0.01; Fig. 1A). The absorption of saturated fatty acids, palmitic (C16:0) and stearic (C18:0) acids, was affected to a greater extent than that of the unsaturated fatty acids oleic (C18:1n-9) and linoleic acid (C18:2n-6). Together, these observations indicated that the mice fed the EFA-deficient diet had profound EFA deficiency after 8 wk on the experimental diet (45).

**EFA deficiency in mice is not associated with alterations in intestinal morphology.** Hematoxylin/eosin (data not shown) and Ki-67 staining of the three segments of the small intestine revealed no clear differences in morphology or proliferative capacity between EFA-deficient and control mice (Fig. 2A). The villus lengths were similar in EFA-deficient and control mice, as determined by morphometrical measurements (Fig. 2B).

**EFA deficiency is associated with delayed glucose clearance.** Basal blood glucose concentrations were similar in EFA-deficient and control mice. After intragastric administration of the glucose/lactose bolus, glucose concentrations rap-

**Table 1.** The PCR primers and TaqMan Probes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pgc-1a</td>
<td>AGCGTACTCTGACGCTGCA</td>
<td>CGACGCTGATCCAGATTTG</td>
</tr>
<tr>
<td>Sirt1</td>
<td>CTGTCGAGGAAGATTTGCTG</td>
<td>TCTCCGGAGTCCATCACAATG</td>
</tr>
<tr>
<td>Sirt2</td>
<td>GTCCCTGTATGCTCTGGTCGTACCAC</td>
<td>TGTCCCTGTATGCCTCTGGTCGTACCAC</td>
</tr>
<tr>
<td>Hnf-1α</td>
<td>AGGATGCGCCCCATCAAGGA</td>
<td>ACTGTCATCTCACTATGGGACAT</td>
</tr>
<tr>
<td>Lactase</td>
<td>GTCCCTAGGAAGCCAAGTGAAA</td>
<td>CTCCTGATGGTGATGTATCGACTA</td>
</tr>
<tr>
<td>Sucrase-Isomaltase</td>
<td>CTGTCATCTCACTATGGGACAT</td>
<td>CTGTCATCTCACTATGGGACAT</td>
</tr>
<tr>
<td>Cdx-2</td>
<td>AGGATGCGCCCCATCAAGGA</td>
<td>ACTGTCATCTCACTATGGGACAT</td>
</tr>
<tr>
<td>Gata-4</td>
<td>GTCCCTAGGAAGCCAAGTGAAA</td>
<td>CTCCTGATGGTGATGTATCGACTA</td>
</tr>
<tr>
<td>Hnf-1α</td>
<td>AGGATGCGCCCCATCAAGGA</td>
<td>ACTGTCATCTCACTATGGGACAT</td>
</tr>
</tbody>
</table>

Linoleic acid (LA) determination in phospholipids of intestinal mucosa. Thirty milligrams of intestinal mucosa was homogenized in 200 μl of 0.9% NaCl, and lipids were extracted according to Bligh and Dyer (2), after the addition of the fatty acid internal standard (C17:0) and antioxidant butylated hydroxytoluene. Lipid extracts were fractionated into phospholipids and other lipids using TLC (20 × 20 cm, Silica gel 60 F254) with hexane/diethyl ether/acetic acid (80:20:1, vol:vol:vol) as running solvent. Phospholipid spots were scraped, and phospholipids were extracted by methanol/chloroform. Phospholipid LA ratio was determined according to Muskiet et al. (29) as described previously (43).
idly increased in control mice, with a maximum concentration at 30 min after administration (Fig. 3A). In EFA-deficient mice, the increase in blood glucose levels was similar to that in control mice up to 30 min but then continued to increase, reaching a maximum concentration at 60 min. The glucose concentrations between 60 and 180 min were slightly but significantly higher in EFA-deficient mice compared with controls (10–15%, P < 0.05). Accordingly, the area under the curve was higher for the EFA-deficient mice compared with controls (15%, P < 0.05, data not shown). On the basis of the apparently delayed glucose clearance, we determined insulin concentrations at the end of the experiment (at 180 min). In EFA-deficient mice, insulin concentrations were significantly higher than in control mice (0.55 ± 0.10 μg/ml vs. 0.35 ± 0.02 μg/ml, respectively, P < 0.01).

Similar glucose absorption but delayed lactose digestion in EFA-deficient mice. To assess the competence of monosaccharide absorption in EFA deficiency, we determined plasma appearance of [13C6]glucose (Fig. 3B). After the administration of the bolus, plasma [13C6]glucose concentration rapidly increased with a maximum at 45 min for both groups. After 45 min, [13C6]glucose rapidly disappeared until 120 min, after which the rate of disappearance decreased in both EFA-deficient and control mice. Thus the plasma [13C6]glucose appearance and disappearance were similar in EFA-deficient and control mice, supporting unaffected monosaccharide absorption in the former.

To measure the competence of disaccharide digestion and absorption, we determined plasma appearance of [13C]glucose,
originating from the administered [1-13C]lactose (Fig. 3C). [13C]glucose reached a maximum concentration in control mice at 45 min after bolus administration. [1-13C]glucose disappeared from the blood within the next 2 h, with the slowest disappearance during the last hour after the bolus administration. Plasma appearance of [13C]glucose in EFA-deficient mice, however, increased to a slower extent and reached its maximal concentration at ~60 min after the bolus administration. In addition, the peak of [13C]glucose absorption in EFA-deficient mice was lower compared with control mice. Thus the lactose uptake was delayed in EFA-deficient compared with control mice.

Specific decrease in mRNA expression and activity of lactase in mid-small intestine of EFA-deficient mice. Measurement of the enzyme activity of lactase along the proximal-to-distal axis of the small intestine revealed a lower activity in the mucosa of the mid part of the small intestine of EFA-deficient mice (Fig. 4A). The decreased lactase activity corresponded with lower mRNA levels of lactase, as shown by quantitative PCR (Fig. 4B). EFA deficiency was not associated with decreased activity [3.6 ± 1.6 vs. 2.8 ± 0.7 nmol/µg protein in controls, not significant (NS)] or mRNA expression (0.9 ± 0.1 vs. 0.7 ± 0.1 in controls, NS) of another disaccharidase in the mid-small intestine, sucrase, indicating a distinct specificity of EFA deficiency on lactase.

We determined whether reduced lactase mRNA expression levels were regulated by the transcription factors at the transcriptional level. The mRNA expression of transcription factors involved in regulation of the lactase mRNA expression, namely Cdx-2, Gata-4, and Hnf-1α (Fig. 4C), was not different between EFA-deficient and control animals. This indicates that the regulation of lactase is at least not regulated at the transcriptional level of the transcription factors Cdx-2, Gata-4 and Hnf-1α.

Decreased lactase activity and mRNA expression are associated with low LA concentrations in the mid-small intestine. EFAs are involved in regulation of membrane fluidity and alterations in membrane lipid matrix. Therefore, it has been proposed that EFAs indirectly influence normal conformation and functioning of the proteins embedded in the membrane (41). For this reason, we tested whether lactase activity in the mid segment of the small intestine correlated with LA levels. LA concentrations were highest in the mid part of the small intestinal mucosa of EFA-deficient compared with control mice (26 mol% vs. 16 mol%, respectively, *P < 0.01) (Fig. 5A). LA concentrations in proximal and distal part were similar in both groups. In the mid-small intestine, LA concentrations positively correlated with lactase activity (r = 0.77, *P < 0.01) and with mRNA expression of lactase (r = 0.79, *P < 0.01) (Fig. 5, B and C, respectively). However, decreased mRNA levels of lactase clearly indicate that the intestinal impairment cannot exclusively be the result of alterations in membrane composition and fluidity. There was no relationship, however, between LA concentrations in the mid-small intestine, on the one hand, and activity (r = −0.08, NS) or mRNA expression (r = −0.03, NS) of sucrase, on the other hand (Fig. 5, D and E, respectively).

Fig. 3. A: total blood glucose response in EFA-deficient and control mice measured at different time points (7.5, 15, 30, 45, 60, 90, 120, and 180 min) after the intragastric glucose/lactose bolus. B: plasma appearance of [13C6]glucose originating from the administered [U-13C]glucose in EFA-deficient and control mice after an intragastric administration of glucose/lactose bolus. C: plasma appearance of [13C]glucose originating from the administered [1-13C]lactose in EFA-deficient and control mice after an intragastric administration of glucose/lactose bolus. Data represent means ± SD of 7 mice per group. *P < 0.05 for EFA-deficient vs. control mice.
DISCUSSION

Our previous studies suggested that EFA deficiency in mice affects fat absorption at the level of the small intestinal mucosa (45). We now explored the effects of EFA deficiency in mice on mucosal histology and physiological function of the small intestine, carbohydrate digestion, and absorption. Our data demonstrate that EFA deficiency is not only associated with fat malabsorption but also with impaired lactose digestion in the murine model of EFA deficiency. The effect of EFA deficiency is mainly on lactase mRNA transcription or stability since the impaired lactose digestion coincided with an ~50% reduced lactase activity and mRNA expression in mid-small intestine of EFA-deficient mice. Interestingly, the reduction in enzyme activity during EFA deficiency was specific for lactase. In addition, intestinal lactase activity and mRNA expression strongly correlated with mucosal LA concentrations, which were depressed in EFA deficiency, particularly in mid-intestine.

Our findings seem to be in concordance with a study of Clark et al. (8), which showed the most marked delay in fat transport during EFA deficiency in the mid portion of the small intestine. We analyzed whether regional differences in the severity of EFA deficiency could contribute to the observations. However, the severity of EFA deficiency was similar in the different parts of the small intestine of EFA-deficient mice (data not shown). On the basis of the pathophysiology, we suggest two possibilities for this phenomenon: EFA deficiency in the midintestine is associated with either increased (local) oxidation of LA or with increased turnover/export of LA. However, further studies need to be performed to investigate the specific effect of EFA deficiency on fatty acid composition and lactase activity in the mid-small intestine.

As expected from previous studies, our murine model of EFA deficiency was clearly deficient, as indicated by elevated triene/tetraene ratios in erythrocytes and fat malabsorption. In addition, we measured the bile production by the collection of bile via gallbladder cannulation as described previously (19). Conforming with previous observations, the mice fed the EFA-deficient diet have increased bile flow, biliary bile salt, and phospholipid secretion rates (data not shown), as well as higher plasma levels of triene/tetraene ratio (0.55 ± 0.20 vs. 0.01 ± 0.00, \( P < 0.01 \)), compared with control mice (45). Although one would expect that EFA deficiency-associated fat malabsorption would lead to a lower body weight, we did not observe this in our present or previous studies (45). Therefore, we assume that prolongation of the EFA-deficient state would indeed be expected to result in lower body weight.

EFA deficiency in mice did not affect morphology or proliferative capacity of the small intestine. As far as we know, our study is the first to describe the effects of EFA deficiency on the intestinal morphology in mice. Christon et al. have shown that low dietary LA levels were associated with alterations in villi and crypt sizes in rats (7). We did not observe differences in villus length between EFA-deficient and control mice in the proximal-to-distal axis of the small intestine. These results indicate that EFA deficiency-associated malabsorption of fats and disaccharides is not associated with morphological alterations in small intestine of mice.

To assess small intestinal function in EFA-deficient mice, we studied carbohydrate absorption using stable isotope methodology (39). The advantage of stable isotope methodology is that it can easily be extrapolated to patient studies (11, 21). EFA-deficient mice had higher total blood glucose levels from 60 min after the administration of the glucose/lactose bolus. High total blood glucose levels could theoretically be ex-
plained by lower blood glucose clearance (slower postprandial uptake of glucose by the peripheral tissues), rather than by disturbed intestinal absorption. This finding is associated with higher insulin concentrations at the end of the experiment in EFA-deficient mice, which is not the result of an impaired glucose disposal, as this appears to be normal. This observation is in accordance with previous studies suggesting a relationship between EFA deficiency and insulin resistance (10). However, it is not clear what the exact reason is for higher insulin levels in EFA-deficient mice. We cannot exclude that the increased content of saturated fats in the EFA-deficient diet contributes to this phenomenon independently from EFA deficiency (23).

Measurement of the absorption of [13C6]glucose, originating from the administered [U-13C]glucose, revealed similar appearance and disappearance of the labeled glucose in both groups. This observation indicates that EFA deficiency does not affect the monosaccharide glucose absorption in mice. The plasma appearance of [13C]glucose originating from lactose, however, was significantly delayed in EFA-deficient mice. The discrepancy in the effect of EFA deficiency on glucose and lactose absorption could be explained by the diverse intestinal fates of these carbohydrates. Unlike glucose, which is directly transported by the glucose transporters across the brush border membrane of the enterocyte, lactose first needs to be hydrolyzed by the enzyme lactase (18, 36). To investigate whether our functional results corresponded with altered lactase activity or expression, we measured these parameters in EFA-deficient and control mice. Lactase is a critical disaccharidase during early postnatal life and a sensitive intestinal marker for functional changes occurring in the small intestine of the adult animal. Its activity relatively decreases during weaning to low adult levels; thus the total lactase activity remains the same during the adulthood (31, 32). The delayed lactose digestion corresponded with an approximate 50% reduction in both lactase activity and mRNA expression in EFA-deficient mice. The mRNA levels of relevant transcription factors for lactase mRNA expression were unaffected in EFA-deficient mice. Because of the unaltered mRNA levels of the transcription factors, we conclude that the regulation of lactase is at least not regulated at the transcriptional level of the transcription factors Cdx-2, Gat-4, and Hnf-1α.
To assess the specificity of the reduced enzyme activity associated with EFA deficiency for lactase, we measured the enzyme activity of another disaccharidase, sucrase. Enzyme activity of sucrase was not decreased in the three parts of the small intestine of EFA-deficient mice. These data clearly demonstrated that EFA deficiency does not generally affect disaccharidase function and activity but rather specifically affects the mRNA expression and activity of lactase.

Under physiological conditions, phospholipids of the small intestinal mucosa contain considerable amounts of LA (C18:2n-6) and its long-chain polyunsaturated fatty acid metabolite arachidonic acid (C20:4n-6) (16). During EFA deficiency, LA levels are decreased in intestinal mucosa (13, 22). We observed LA deficiency in mucosal phospholipids, particularly in the mid part of the small intestine, which strongly correlated with reduced lactase activity and mRNA expression. It is tempting to speculate that low LA levels in phospholipids of cellular membranes lead to structural and physiological changes in the lipid membrane. A study in pigs (6) suggested that EFA deficiency reduces membrane fluidity and affects membrane protein behavior in the enterocyte membranes. Theoretically, these structural changes in the cellular membrane of the enterocytes during EFA deficiency could also be the cause of functional alterations in the membrane anchoring of lactase. However, since not only lactase activity but also its mRNA expression was decreased in EFA deficiency and since the lactase hydrolytic portion of the enzyme is at a considerable distance from the membrane (40), it is likely that altered membrane fluidity is not the (single) factor involved.

It remains unclear how EFA deficiency specifically affects lactase; several factors could be involved. Theoretically, EFA deficiency could inhibit the differentiation of the enterocytes, accompanied by reduced expression of lactase (32). Lactase and sucrase genes have different mechanisms of transcriptional regulation, which could lead to differential transcription during EFA deficiency (37, 38). Alternatively, under certain conditions, for example, during malnutrition (30), stability of lactase mRNA is more profoundly decreased than that of sucrase. Our present results do not allow us to discriminate between these theoretical options.

Our present results indicate that EFA deficiency has functional consequences for small intestinal function in mice, and it provides indirect support for the hypothesis that reduced mucosal function is involved in fat malabsorption in EFA deficiency. EFA deficiency in (pediatric) cholestatic mice, and it provides indirect support for the hypothesis that functional consequences for small intestinal function in the theoretical options.

Acknowledgments

The authors thank Rick Havinga, Ingrid Martini, Juul Baller, Theo Boer, Henk Wolters, and Renze Boverhof for excellent technical assistance and helpful suggestions.

Grants

This study was supported by the Dutch Digestive Foundation (MLDS). E.H.H.M. Rings is supported by a fellowship of the Royal Netherlands Academy of Arts and Sciences (KNAW).

References


