Intestinal absorption and postabsorptive metabolism of linoleic acid in rats with short-term bile duct ligation

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Minich, Deanna M., Rick Havinga, Frans Stellaard, Roel J. Vonk, Folkert Kuipers, and Henkjan J. Verkade. Intestinal absorption and postabsorptive metabolism of linoleic acid in rats with short-term bile duct ligation. Am J Physiol Gastrointest Liver Physiol 279: G1242–G1248, 2000.—We investigated in bile duct-ligated (BDL) and sham-operated control rats whether the frequent presence of essential fatty acid deficiency in cholestatic liver disease could be related to linoleic acid malabsorption, altered linoleic acid metabolism, or both. In plasma of BDL rats, the triene-to-tetraene ratio, a biochemical marker for essential fatty acid deficiency, was increased compared with controls (0.024 ± 0.004 vs. 0.013 ± 0.001; P < 0.05). Net and percentage of dietary linoleic acid absorbed were decreased in BDL rats compared with control rats (1.50 ± 0.16 mmol/day and 81.3 ± 3.3% vs. 2.08 ± 0.07 mmol/day and 99.2 ± 0.1%, respectively; each P < 0.001). At 24 h after [13C]linoleic acid administration, BDL rats had a similar ratio of plasma [13C]arachidonic acid to plasma [13C]linoleic acid concentration compared with control rats. Δ6-Desaturase activity was not significantly different in hepatic microsomes from control or BDL rats. At 3 h after [13C]linoleic acid administration, plasma appearance of [13C]linoleic acid and cumulative expiration of 13CO2 were decreased in BDL rats, compared with controls (by 54% and 80%, respectively). The present data indicate that the impaired linoleic acid status in cholestatic liver disease is mainly due to decreased net absorption and not to quantitative alterations in postabsorptive metabolism.

ESSENTIAL FATTY ACIDS (EFAs) and their polyunsaturated fatty acid (PUFA) metabolites are major components of structural lipids in all tissues and modulate cell membrane fluidity and function. The availability of long-chain PUFA (LCPUFA; >18 carbon atoms), such as arachidonic acid [20:4(n-6)], is important for early human growth and for the production of eicosanoids, which mediate immune and vascular functions (6, 23).

A suboptimal status of EFAs and LCPUFAs is frequently noted in patients with impaired bile formation, such as cholestatic liver disease (1, 9, 10, 24). Low plasma concentrations of these fatty acids have been speculated to contribute to the morbidity and mortality of these patients.

Theoretically, impaired status of EFAs and LCPUFAs in plasma may be related to malabsorption, increased metabolism (i.e., desaturation and elongation, β-oxidation), and/or tissue redistribution of these fatty acids. Because patients with cholestatic liver disease have impaired bile secretion into the intestinal lumen, it would seem reasonable that they malabsorb a substantial amount of their dietary lipids. Kobayashi et al. (14) have reported that dietary lipid absorption was reduced to 30% in patients with biliary atresia. Recently, we (19) found that rats with permanent biliary drainage had decreased percentages of linoleic acid absorption; however, by ingesting more diet (~ 40%), they compensated for fecal losses of linoleic acid. As a result, their net uptake of dietary linoleic acid was not different from control rats (19). In addition to impaired bile formation, the pathophysiology of cholestasis is characterized by retention of bile-destined compounds such as bile salts in the body (11). It may be that the retention of bile compounds contributes to impaired status of EFAs and LCPUFAs in cholestasis, for example, by enhancing EFA metabolism (preferential oxidation, increased elongation/desaturation). Indirect indications that the efficacy of hepatic elongation/desaturation is altered during cholestatic liver disease in infants were recently reported by Socha et al. (24, 25). It could be conceived that linoleic acid is metabolically more readily utilized for energy supply, i.e., β-oxidized, under conditions of impaired fat absorption.

In the present study, we aimed to simulate the clinical condition of cholestasis by using an experimental animal model, the bile duct-ligated (BDL) rat, which is characterized both by intestinal bile deficiency and by accumulation of biliary compounds in the body. The absorption and metabolism of linoleic acid was investigated in short-term (1 wk) BDL rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (Central Animal Laboratory, Groningen, The Netherlands), weighing 250–350 g (mean ± SD: 277 ± 37 g), were kept in an environmentally controlled...
facility with diurnal light cycling and free access to diet (standard rodent chow, Hope Farms, Woerden, The Netherlands) and tap water. Experimental protocols were approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen.

Labeled substrates. [1-13C]palmitic acid and [U-13C]linoleic acid were purchased from IsoTech. Both stable isotopes were >99% enriched.

Experimental procedures. Rats were individually housed in metabolic cages and fed a high-fat diet (55 en% lipid, 4.283 kcal/kg diet; major long-chain fatty acid composition as measured by gas chromatography: 16:0, 31.5%; 18:0, 7.3%; 18:1(n-9), 31.2%; and 18:2(n-6), 30.0% (Hope Farms). After 1 wk of feeding, rats were equipped with permanent catheters in jugular vein and duodenum, as described by Kuipers et al. (17). Bile duct ligation was performed on one group of rats (n = 6), and the other group was sham operated (n = 6). The experimental model allows for physiological studies in unanesthetized rats with bile duct ligation without the interference of stress or restraint. Animals were allowed to recover from surgery for 7 days.

On day 7, 1.67 ml lipid/kg body wt was slowly administered as a bolus via the duodenal catheter to one-half of the rats in each group. Medium-chain triglyceride oil was included in the bolus to have a sufficient amount to allow a reliable, reproducible delivery. Specifically, the lipid bolus was composed of olive oil [25% vol/vol; fatty acid composition: 16:0, 14%; 18:1(n-9), 79%; and 18:2(n-6), 8%] and medium-chain triglyceride oil (75% vol/vol; composed of extracted coconut oil and synthetic triglycerides; fatty acid composition: 6:0, 2%; 8:0, 50–65% maximum; 10:0, 30–45%; 12:0, 3% maximum) and contained 6.7 mg [U-13C]linoleic acid and 6.7 mg [1-13C]palmitic acid/kg body wt. The lipid bolus represented 10% of the daily lipid intake for control and BDL rats. Blood samples (0.2 ml) were taken from the jugular cannula at baseline and hourly for 6 h after administration of the label and were collected into tubes containing heparin. A blood sample for quantification of [13C]linoleic acid and [13C]arachidonic acid was taken at 24 h after label administration. Plasma was separated by centrifugation (10 min, 2,000 rpm, 4°C) and stored at −20°C until further analysis. Feces were collected in 24-h fractions starting 24 h before label administration and ending 48 h afterward (72 h total). Feces samples were stored at −20°C before analysis. Diet ingestion was documented for 72 h by daily weighing of the diet container. On the third day after bolus administration, the heart was punctured to obtain a large blood sample from all rats (2 ml) for analysis of biochemical markers for cholestasis [alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, bile salts]. Livers were excised from the rats that participated in the bolus experiment, weighed, and placed directly in −80°C until further analysis. Livers from the rats that did not participate in the bolus experiment were removed and weighed, followed immediately by homogenization and microsome isolation (22). Microsome samples were stored at −80°C, until determination of hepatic fatty acid elongation and desaturation activity.

In a second experiment, metabolic oxidation of [13C]linoleic acid was determined after its enteral administration in an olive oil bolus to control and BDL rats. From the night before the test, rats were fasted and put in metabolic cages. The following morning they were placed in an airtight container (volume ~4.5 l) through which CO2-free air was passed at a continuous flow of 1,000 ml/min. The air leaving the metabolic cage was diverted to a CO2 monitor (Capnograph IV, Gould Medical, Bilthoven, The Netherlands) for measuring percentage of total CO2 in the breath, and in the case of breath sampling, to 10-ml test tubes (Extemators, Labco Limited, High Wycombe, United Kingdom). The rats were placed in the cage at least 30 min before administration of bolus, to allow adaptation and collection of background breath samples. After the background sample was taken, the lipid bolus was administered by gavage. The bolus consisted of olive oil (0.75 ml/kg body wt) and [13C]linoleic acid (7.5 mg/kg body wt). After administration of the bolus, 1-min breath samples were collected in duplicate every 15 min for a 3-h period.

Analytical techniques. Plasma ALT, AST, and bilirubin were determined by routine clinical procedures. Plasma bile salts were measured enzymatically (17). Lipids from plasma (n = 6/group) were extracted and methylated according to Lepage et al. (18). After freeze-drying and mechanical homogenization, aliquots of diet and feces were subjected to the same procedure (18). Duplicate aliquots of liver homogenate (n = 3/group) were extracted (2) and methylated (18) as described previously. Resulting fatty acid methyl esters from all biological samples were analyzed by gas chromatography to quantify total and individual major fatty acids and, for plasma, liver and feces, by gas chromatography–combustion-isotope ratio mass spectrometry (GC-C-IRMS) to measure the 13C enrichment of palmitic and linoleic acids and linoleic acid metabolites.

Gas liquid chromatography. Fatty acid methyl esters in plasma were separated and quantified by gas liquid chromatography as detailed by Kalivianakis et al. (13) using heptadecanoic acid (17:0) as internal standard.

GC-C-IRMS. 13C enrichment of palmitic, linoleic, and arachidonic methyl esters was determined by using a Finnigan MAT Delta S IRMS interfaced to a Varian 3400 gas chromatograph via a capillary oxidation furnace (Finnigan MAT, Bremen, Germany), according to the method described by Minich et al. (20).

IRMS. 13C enrichment in aliquots of breath samples was determined by means of continuous flow isotope ratio mass spectrometry (Finnigan Breath MAT). The 13C abundance of breath CO2 was expressed as the difference from the reference standard Pee Dee Belemnite limestone (δ13CpDB, %). The proportion of 13C label excreted in breath CO2 was expressed as the cumulative percentage of administered 13C label recovered per indicated time point.

Δ6-Desaturase assay. The metabolism of [13C]linoleic acid to [13C]arachidonic acid was measured in vivo using the ratio between [13C]arachidonic acid and [13C]linoleic acid in plasma at 24 h after [13C]linoleic acid bolus administration. Δ6-Desaturase activity was measured in hepatic microsomes from BDL and control rats in vitro. Hepatic microsomes were isolated in conformance with the procedures described by Smit et al. (22). The conditions for the (unlabeled) desaturation assay were described by Su and Brenna (26), using incubation media made according to de Antuerno et al. (5). After isolation of hepatic microsomes, the activity of Δ6-Desaturase was determined by measuring the conversion of linoleic acid [18:2(n–6)] to gamma-linolenic acid [18:3(n–6)], during 1 h in a shaking water bath at 37°C. The reaction was terminated by addition of 10% (wt/vol) potassium hydroxide in ethanol, after which lipids were extracted, methylated, and analyzed by gas chromatography, as described above. Δ6-Desaturase activity was expressed as the change in mass of 18:3(n–6) calculated directly from the quantitative gas chromatography results. Results obtained from a blank, non-incubated sample were subtracted from those from the incubated sample.

Calculations. The triene-to-tetraene ratio was calculated for plasma and liver by dividing the concentration of
20:3(n-9) by that of 20:4(n-6). In plasma and liver, n-6 fatty acid status was calculated using the sum of the area of major fatty acids (>90%) [16:0, 16:1(n-7), 18:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), 18:3(n-6), 20:3(n-6), 20:3(n-9), and 20:4(n-6)] and then expressing the area of each individual n-6 fatty acid as a percentage of the total amount. Absorption of major dietary fatty acids (palmitic, stearic, oleic, linoleic acids) and of [13C]palmitic and linoleic acids was measured using balance techniques as described by Minich et al. (19).

Statistics. Values represent means ± SE for the indicated number of animals per group. Using SPSS version 6.0 statistical software (Chicago, IL), we calculated significance of differences with the two-tailed Student’s t-test for normally distributed, unpaired data or a Mann-Whitney U-test for data that were not normally distributed. Variance among data was determined using Levene’s test for equality of variances. P < 0.05 was considered significant.

RESULTS

Body weight and food ingestion. Between BDL and control rats, there were no significant differences in body wt (277.5 ± 14.9 g vs. 290.3 ± 12.2 g, respectively) or in amount of food ingested (recorded values for 3 days: 14.2 ± 1.0 vs. 16.3 ± 0.6 g, respectively).

Cholestatic markers in plasma. Compared with control rats, BDL rats had increased activities in plasma of the liver enzymes ALT (34.0 ± 3.0 vs. 74.5 ± 10.3 U/l, P < 0.01) and AST (98.0 ± 7.0 vs. 273.7 ± 59.0 U/l, P < 0.05), and higher concentrations of total bilirubin (4.2 ± 0.2 vs. 172.3 ± 10.7 µmol/l, P < 0.01) and bile salts (9.4 ± 2.0 vs. 229.5 ± 20.8 µmol/l, P < 0.01), respectively, in accordance with the presence of cholestasis. Liver weights were similar between BDL and control rats (13.9 ± 1.0 vs. 11.3 ± 0.4 g; not significant).

Absolute and relative fatty acid concentrations in plasma and in liver. Total plasma lipid analysis revealed a similar cumulative concentration of major fatty acids between BDL and control rats (7.11 ± 0.18 vs. 6.90 ± 0.33 mM; not significant). Plasma concentrations of the individual fatty acids, palmitic, linoleic, and arachidonic acid, were similar between both groups (data not shown). However, stearic acid plasma concentration decreased and oleic acid plasma concentration increased in BDL vs. control rats (stearic acid, 0.79 ± 0.05 vs. 1.04 ± 0.04 mM; P < 0.01; oleic acid, 1.39 ± 0.06 vs. 0.98 ± 0.07 mM, respectively; P < 0.01). Accordingly, the molar percentages (Fig. 1) for stearic acid and oleic acid were decreased and increased, respectively, in plasma of BDL rats compared with that of control rats (11.08 ± 0.24% vs. 15.13 ± 0.40%, P < 0.001; 19.63 ± 0.71% vs. 14.22 ± 0.71%, P < 0.001).

Yet, both values were still considerably below 0.2, the generally accepted threshold value for EFA deficiency in humans (12). Relative fatty acid concentrations in liver were similar between control and BDL rats for all major fatty acids (data not shown).

Dietary fatty acid balance profile. In Table 1, dietary fatty acid balance data (ingestion, fecal excretion, and net absorption) for control and BDL rats are shown. BDL rats excreted significantly more fatty acids into feces compared with control rats (P < 0.001). The overall lipid absorption percentage was significantly decreased in BDL rats compared with control rats (53.7 ± 5.0% vs. 94.2 ± 0.6%; P < 0.001). On comparison of the percent uptake of individual fatty acids from the diet (Fig. 2), the absorption of saturated fatty acids (palmitic acid, stearic acid) appeared considerably more affected by bile duct ligation than that of unsaturated species (oleic acid, linoleic acid). Given the similar amounts of diets ingested by the two groups of rats, BDL rats had significantly decreased net fatty acid absorption (Table 1, P < 0.001).

The quantitatively different effects of bile duct ligation on the intestinal absorption of saturated and unsaturated fatty acids were also investigated by absorp-

### Table 1. Dietary fatty acid balance data for control and BDL rats

<table>
<thead>
<tr>
<th>Ingestion, mmol/day</th>
<th>Fecal Excretion, mmol/day</th>
<th>Net Absorption, mmol/day</th>
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<tbody>
<tr>
<td></td>
<td>Con</td>
<td>BDL</td>
</tr>
<tr>
<td>16:0</td>
<td>2.21 ± 0.07</td>
<td>1.93 ± 0.17</td>
</tr>
<tr>
<td>18:0</td>
<td>0.51 ± 0.02</td>
<td>0.45 ± 0.04</td>
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<tr>
<td>18:1</td>
<td>2.28 ± 0.06</td>
<td>2.00 ± 0.18</td>
</tr>
<tr>
<td>18:2</td>
<td>2.10 ± 0.07</td>
<td>1.83 ± 0.16</td>
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</table>

Values are means ± SE of control (Con) and bile duct-ligated (BDL) rats (n = 6/group). Mean values represent the average of 3 days/rat. Included in these 3 days is the experimental day in which the lipid bolus was administered (n = 3/group). *P < 0.001.
tion experiments using stable isotope-labeled palmitic acid and linoleic acid. After intraduodenal administration, fecal excretion of [13C]palmitic acid was significantly greater in BDL rats compared with control rats (4.3 ± 0.5 vs. 1.4 ± 0.4 μmol/48 h; P < 0.05). In contrast to [13C]palmitic acid, no quantitative differences between control and BDL rats was found in fecal excretion of [13C]linoleic acid (0.4 ± 0.3 vs. 0.5 ± 0.1 μmol/48 h; not significant) or in its net absorption (5.9 ± 0.2 vs. 5.7 ± 0.4 μmol/48; not significant). The percentage of [13C]palmitic acid absorbed was significantly decreased in BDL rats compared with control rats (38.6 ± 13.0% vs. 81.6 ± 5.8%; P < 0.05); however, there was no difference in the percentage of [13C]linoleic acid absorbed between the groups (91.9 ± 1.8% vs. 94.2 ± 4.4%, BDL vs. control).

Plasma [13C]-fatty acid concentrations. Absorption kinetics of saturated and unsaturated fatty acids were studied by determining the appearance of [13C]-fatty acids in plasma after their duodenal administration to control and BDL rats (Fig. 3). In control rats, plasma [13C]linoleic acid and [13C]palmitic acid concentrations increased within 1 h, reaching apparent maximum values of 0.082 ± 0.014 and 0.068 ± 0.009% dose/ml plasma at 6 and at 4 h after bolus administration, respectively. On bile duct ligation, plasma [13C]-fatty acid concentrations for either [13C]palmitic acid or [13C]linoleic acid were significantly lower than in controls (P < 0.001). Area under the curve (AUC) for the plasma appearance of [13C]linoleic acid was significantly lower in BDL rats than in controls (after 3 h: 0.031 ± 0.019 vs. 0.157 ± 0.080% administered dose·h⁻¹·ml⁻¹, respectively; P < 0.05). A similar, equally significant difference between BDL and control rats was observed for the AUC for the plasma appearance of [13C]palmitic acid (Fig. 3B).

Metabolism of [13C]linoleic acid. Cholestasis by bile duct ligation may not only affect linoleic acid status via its intestinal absorption of EFA, it could also induce alterations in EFA metabolism. The metabolism of linoleic acid can be largely discriminated into its elongation and desaturation to LCPUFA and its β-oxidation. An in vivo estimation of elongation and desaturation activity during bile duct ligation was obtained by measuring plasma concentrations of [13C]arachidonic acid and [13C]linoleic acid at 24 h after intraduodenal administration of [13C]linoleic acid. At 24 h after [13C]linoleic acid administration, BDL rats had a similar ratio of plasma [13C]arachidonic acid to plasma [13C]linoleic acid concentration compared with control rats (0.53 ± 0.19 vs. 0.60 ± 0.13; not significant). In accordance with these in vivo data, Δ⁶-desaturase activity in vitro appeared not significantly different in hepatic microsomes from control or BDL rats (32.9 ± 5.7 vs. 32.9 ± 9.2 pmol·min⁻¹·mg protein⁻¹, n = 5 and 4/group, respectively).

The oxidation of [13C]linoleic acid was measured in control and BDL rats by quantitation of [13CO₂] expiration after its enteral administration with an olive oil bolus. The cumulative amount of [13CO₂] expired after 3 h was decreased by 54% in BDL rats compared with
control rats (10.7 ± 1.5 vs. 23.4 ± 5.7% administered dose, respectively; Fig. 4). It would be optimal if the cumulative amount of \([^{13}C]\)linoleic acid oxidized at a specific time point could be directly related to the amount of the parent compound taken up from the intestine. The latter information, however, could not be obtained in the applied experimental setup, because it would require the detailed analysis of label left in the intestinal lumen at the indicated time points. An indirect estimate from the amount absorbed can be derived from the AUC of the plasma concentrations of \([^{13}C]\)linoleic acid (Fig. 3A). In BDL rats, the AUC for 0–3 h for plasma \([^{13}C]\)linoleic acid concentrations was decreased by 80%, compared with controls (Fig. 3A). The comparison between the indirect estimates of the amount of \([^{13}C]\)linoleic acid absorbed (~80% at 3 h) with the cumulative amounts oxidized (~54% at 3 h) indicates that bile duct ligation does not profoundly affect the fraction of the absorbed linoleic acid molecules that are oxidized within 3 h after administration.

**DISCUSSION**

The association between cholestatic liver disease and impaired status in plasma of EFA and LCPUFA is well known. However, the pathophysiological relationship between the two has remained unclear. From the aspect of cholestatic liver disease, it is not clear whether its effects on EFA and LCPUFA status are due to the decreased availability of intestinal bile, the (toxic) accumulation of compounds normally secreted into bile (e.g., bile salts) in the body, or both. Thus it has remained unresolved whether the mechanism(s) behind impaired EFA status involves decreased intestinal absorption of EFA, increased metabolism, or both. In the present study, we aimed to determine the influence of cholestatic liver disease on the intestinal absorption and metabolism of the major EFA, linoleic acid, in an animal model. One-week-old BDL rats had a significantly decreased percent absorption of dietary fat (54%). BDL rats did not ingest more food to compensate for their increased fecal lipid excretion, and the net uptake of dietary linoleic acid was decreased by ~28% compared with control rats. Our present data also indicate that postabsorptive metabolism of linoleic acid (elongation/desaturation, oxidation) is not profoundly affected in short-term (1 wk) BDL rats, compared with control rats.

The cholestatic condition is characterized both by decreased availability of intestinal bile and by (toxic) accumulation in the body of compounds that are normally secreted into bile, such as bile salts. The present data, in combination with our previous study (19), allow us to qualify the contributions of each of these two pathophysiological processes to EFA absorption during cholestasis. We (19) recently determined linoleic acid absorption in a rat model of chronic bile diversion. Bile diversion results in intestinal bile deficiency but, in contrast to bile duct ligation or cholestasis, it is not associated with the toxic accumulation of bile compounds in the body. In bile-diverted rats, the percentage of dietary linoleic acid absorption from the diet was 78% (19), a value very similar to the percent absorption presently found in BDL rats (82%). From these quantitative data, it can be inferred that the intestinal bile deficiency per se, either with (bile duct ligation) or without (bile diversion) toxic accumulation of bile components in the body, is responsible for the impaired efficiency of linoleic acid absorption.

The percent linoleic acid absorption from the diet differed for the various fatty acid species. Absorption percentage and net uptake decreased in BDL rats in the following order: linoleic acid > oleic acid > palmitic acid > stearic acid. These results are in accordance with those of Demarne et al. (8), who reported a decrease in the percentage of dietary palmitic acid and stearic acid absorption relative to that of oleic acid and linoleic acid in 10-day-old BDL rats fed diets containing 10% peanut oil. The selectivity of fatty acid absorption in bile duct ligation was confirmed by the decreased fecal excretion and, accordingly, increased net absorption of intraduodenally administered \([^{13}C]\)linoleic acid compared with \([^{13}C]\)palmitic acid in BDL rats.

**Table 2.** \(^{13}C\)-fatty acid balance data for control and BDL rats

<table>
<thead>
<tr>
<th></th>
<th>Amount Administered, mmol/day</th>
<th>Fecal Excretion, mmol/48 h</th>
<th>Net Absorption, mmol/48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>BDL</td>
<td>Con</td>
</tr>
<tr>
<td>16:0</td>
<td>7.24 ± 0.25</td>
<td>7.19 ± 0.60</td>
<td>1.66 ± 0.50</td>
</tr>
<tr>
<td>18:2</td>
<td>6.25 ± 0.22</td>
<td>6.20 ± 0.51</td>
<td>0.52 ± 0.36</td>
</tr>
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</table>

Values are means ± SE of control and BDL rats (n = 3/group). *P < 0.05.
Despite the relative preservation of linoleic acid absorption, however, its quantitative uptake (in mmol/day) was reduced in BDL rats compared with controls. Whereas bile-deficient rats in our previous study (19) managed to maintain a net similar quantitative uptake of linoleic acid, at least in part by the ingestion of increased amounts of food, BDL rats appear not to compensate for the decreased efficiency of fat absorption. It was demonstrated previously (7) that appetite and chemosensory function are altered in human liver disease, although the underlying mechanisms have not been elucidated. It is tempting to speculate that the present observations in rats refer to an analogous phenomenon.

In addition to fecal balance data, the appearance of $^{13}$C-fatty acids in plasma was measured after their intraduodenal administration. The plasma concentrations of either $[^{13}]$linoleic acid or $[^{13}]$palmitic acid were significantly decreased in BDL rats compared with controls. Given the relative preservation of linoleic acid absorption measured by balance techniques (Table 2), it can be speculated not only that the absorption is not limited to the proximal segments of the intestine of BDL rats but also that more distal segments are involved in its absorption, giving rise to altered plasma kinetics (3, 4). To exclude the (theoretical) possibility that absorbed linoleic acid would be distributed differently over the various lipid classes in plasma (for example, triacylglycerols, phospholipids, free fatty acids), an analytical method was applied in which all fatty acids present in plasma, either esterified or unesterified, were determined simultaneously in a pooled fashion.

As stated above, a quantitatively altered metabolism (i.e., desaturation/elongation, $\beta$-oxidation) could contribute to the frequently observed impaired EFA and LCPUFA status in cholestatic patients. Socha et al. (24) suggested that the hepatic microsomal desaturase/elongase activity needed for LCPUFA synthesis is altered in patients with cholestatic liver disease. In the presently used rat model of bile duct ligation, the conversion of $[^{13}]$linoleic acid to $[^{13}]$arachidonic acid appeared to be not different quantitatively compared with control rats. At 24 h after its duodenal administration, the ratio between $[^{13}]$arachidonic acid and $[^{13}]$linoleic acid concentrations in plasma was similar in BDL and control rats. In agreement with these in vivo data, $\Delta^6$-desaturase activity was not significantly different as determined in hepatic microsomes isolated from BDL and control rats. It is possible that the cholestatic (BDL) condition affects the EFA status by quantitatively altering the utilization of linoleic acid for energy purposes, i.e., $\beta$-oxidation. After administration of $[^{13}]$linoleic acid to BDL rats, however, the plasma appearance and cumulative expiration of $^{13}$CO$_2$ were affected to a similar extent (~80% and ~54%, respectively), indicating that $\beta$-oxidation of absorbed linoleic acid was not greatly altered. In long-term BDL rats, a decreased capacity in hepatic $\beta$-oxidation has been reported (15, 16).

In summary, we conclude that a cholestatic condition in rats (short-term bile duct ligation) is associated with decreased net uptake of linoleic acid but with quantitatively rather unaffected metabolism of absorbed linoleic acid. Although differences between our experimental model and humans with cholestatic liver disease may apply, the present observations are in agreement with a decreased net uptake of linoleic acid in patients with chronic cholestatic disease, not as much due to a greatly decreased efficacy of its absorption per se, but to the associated decrease in food and linoleic acid ingestion (7, 21). A protracted decrease in the net uptake of dietary linoleic acid can be expected to eventually result in decreased linoleic acid levels. An implication of the present findings may be that the occurrence of EFA deficiency in patients with cholestatic liver disease can be counteracted by nutritional means, given the relatively preserved absorption efficiency of EFA. It remains to be demonstrated in patient studies, however, whether these implications can indeed be extrapolated from our present results in the experimental animal model used.

H. J. Verkade is a Fellow of the Royal Netherlands Institute for Arts and Sciences.

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