Decreased linoleic acid concentration in the bile-diverted rat is not due to decreased uptake of dietary linoleic acid

Deanna M. Minich, Mini Kalivianakis, Rick Havinga, Harry van Goor, Frans Stellaard, Roel J. Vonk, Folkert Kuipers and Henkjan J. Verkade

Biochimica et Biophysica Acta, 1999;1438 (1):111-119
Abstract

Decreased bile secretion into the intestine has been associated with low plasma concentrations of essential fatty acids (EFA) in humans. We studied the mechanism behind this relationship by determining the status and absorption of the major dietary EFA, linoleic acid (LA), in control and 1 wk bile-diverted rats. The absorption of LA was quantified by a balance method and by measuring plasma concentrations of $^{13}$C-LA after its intraduodenal administration. Absolute and relative concentrations of LA in plasma were decreased in bile-diverted rats ($P<0.01$ and $P<0.001$, respectively). Fecal excretion of LA was increased at least 20-fold in bile-diverted rats ($0.72 \pm 0.11$ mmol/d vs $0.03 \pm 0.00$ mmol/d; $P<0.0001$). Due to increased chow ingestion by bile-diverted rats, net intestinal absorption of LA was similar between bile-diverted and control rats ($1.96 \pm 0.14$ mmol/d vs $1.91 \pm 0.07$ mmol/d, respectively; $P>0.05$). After intraduodenal administration of $^{13}$C-LA, plasma concentrations were approximately 3-4 fold lower in bile-diverted rats for at least 6 h ($P<0.001$). Plasma concentrations of both $^{12}$C- and $^{13}$C-arachidonic acid were increased in bile-diverted rats ($P<0.05$). We conclude that decreased plasma concentrations of LA in 1 wk bile-diverted rats are not due to decreased net intestinal absorption of LA, but may be related to increased metabolism of LA.
Introduction

Since essential fatty acids (EFA) cannot be synthesized de novo by mammals, their concentration in the body depends on adequate ingestion and efficient intestinal absorption of EFA from exogenous sources. In case of insufficient dietary supply, decreased absorption and/or increased metabolism of EFA, a deficiency may develop, eventually leading to serious clinical symptoms [1-3]. A decreased absorption of EFA has been implied to cause impaired EFA status when bile secretion into the intestine is decreased or absent, such as in cholestatic conditions [4-6]. To determine if indeed the lack of bile flow to the intestine can be per se responsible for changes in EFA status, we studied the status and absorption of linoleic acid (LA), the major dietary EFA, in a rat model of bile diversion, in the absence of cholestatic liver injury. This rat model allows absorption to be studied under physiological conditions (normal bile secretion into the duodenum) and under conditions of permanent biliary drainage (lack of bile secretion into the duodenum) [7]. The absorption of LA was quantified by a balance method and by measuring plasma concentrations of $[^{13}\text{C}]$-LA after its intraduodenal administration. Using this approach, we were able to demonstrate that the diversion of bile from the intestine in the absence of cholestatic liver injury does affect the plasma concentration of LA, but does not influence the net absorption of LA from the intestine.

Materials and methods

Animals and diets

Male Wistar rats (Harlan Laboratories, Zeist, The Netherlands), weighing 300-350 g (mean ± SD: 327 ± 19 g), were kept in an environmentally controlled facility with diurnal light cycling and free access to chow, tap water, and, in the case of bile-diverted rats, saline. Experimental protocols were approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen.

Experimental procedures

Rats were individually-housed in metabolic cages and fed a high-fat chow (35 en% lipid; major long-chain fatty acid composition as measured by gas chromatography: 16:0, 31.9%; 18:0, 5.2%; 18:1n-9, 32.7%; 18:2n-6, 30.2%) (Hope Farms BV, Woerden, The Netherlands). After 1 week, rats were equipped with permanent catheters in the right jugular vein, bile duct and duodenum, as described by Kuipers et al. [7]. This experimental model allows for physiological studies in unanesthetized rats with long-term bile diversion without the interference of stress or restraint. After surgery, catheters in bile duct and duodenum were either connected, to restore the enterohepatic circulation (control rats) (n=13), or were chronically interrupted (bile-diverted rats) (n=9). Animals were allowed to recover from surgery for 6 days.

On day 7, 1.67 mL lipid/kg body weight was slowly administered as a bolus via the duodenal catheter. Medium chain triglyceride oil was included in the bolus, as a carrier oil in order to
have a sufficient amount to allow a reliable, reproducible delivery of the label. Specifically, the lipid bolus was composed of olive oil (25% v/v; fatty acid composition: 16:0, 14%; 18:1n-9, 79%; 18:2n-6, 8%) and medium chain triglyceride oil (75% v/v; composed of extracted coconut oil and synthetic triglycerides; fatty acid composition: 6:0, 2%; 8:0, 50-65% max.; 10:0, 30-45%; 12:0, 3% max.) and contained 6.7 mg [U-\(^{13}\)C]-LA/kg body weight (Martek Biosciences Corporation, Columbia, MD, USA; [U-\(^{13}\)C]-LA was 99% enriched with a chemical purity exceeding 97%). The lipid bolus represented <10% of the daily lipid intake for control and bile-diverted 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 \([^{13}\text{C}]\)-LA and \([^{13}\text{C}]\)-arachidonic acid was taken at 24 h after label administration. 24 h was selected as a time point since absorption of the label was demonstrated to exceed 90% of the amount absorbed in 48 h. Plasma was separated by centrifugation (10 min, 2000 rpm, 4°C) and stored at -20°C until further analysis. Feces was collected in 24 h fractions starting 24 h before label administration and ending 48 h afterwards (72 h total). Feces samples were stored at -20°C prior to analysis. During bile diversion, bile was collected in 6 h increments for 24 h using a fraction collector. Chow ingestion was documented for 72 h by daily weighing of the chow container. At the end of the experiment, rats were sacrificed and small intestines were removed (n=3 per group, at random) and stored at -80°C for further histological analysis.

In order to determine whether the observed effects could be attributed to the charged character of the lipid molecules, such as in fatty acids, a set of control experiments was performed with an uncharged lipid, retinol. Water-soluble retinol (50,000 IU) in an olive oil bolus was administered via the duodenal catheter to control and bile-diverted rats (n=4 per group), after which blood samples (see above) were taken hourly up to 6 h.

**Analytical techniques**

*Plasma and biliary lipids.* Total plasma lipids (triacylglycerols, phospholipids, etc.) and biliary phospholipids were extracted, hydrolyzed and methylated according to Lepage and Roy [8]. In order to account for losses during lipid extraction, heptadecanoic acid (17:0) was added to all samples as an internal standard before the start of the extraction and methylation procedure. Resulting fatty acid methyl esters were analyzed by gas chromatography to measure total and individual amounts of major fatty acids. Cumulative fatty acid concentrations were calculated using the sum of the area of major fatty acid (>90%) peaks (16:0 + 18:0 + 18:1n-9 + 18:2n-6 + 20:4n-6). For plasma and bile, relative fatty acid concentrations (molar percentages) were calculated by expressing the area of each individual fatty acid as a percentage of the sum of the area of major fatty acids (16:0 + 18:0 + 18:1n-9 + 18:2n-6 + 20:4n-6).

Methylated plasma samples were analyzed by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) to measure the \([^{13}\text{C}]\)-enrichment of LA and arachidonic acid. The concentration of \([^{13}\text{C}]\)-fatty acid in plasma at each time point was determined from the fatty acid concentration and \([^{13}\text{C}]\) enrichment and expressed as a percentage of the dose administered per milliliter plasma (% dose/mL). Metabolism of \([^{13}\text{C}]\)-LA to \([^{13}\text{C}]\)-arachidonic acid...
acid was expressed as a ratio between the % dose $[^{13}\text{C}]-\text{arachidonic acid/mL plasma}$ and the % dose $[^{13}\text{C}]-\text{LA/mL plasma}$ at 24 h after $[^{13}\text{C}]$ bolus administration.

**Chow and fecal lipids.** After freeze-drying and mechanically homogenizing, aliquots of high-fat chow and feces were extracted, hydrolyzed and methylated [8]. Heptadecanoic acid (17:0) was added to all samples as an internal standard before the extraction and methylation procedure. Resulting fatty acid methyl esters were analyzed by gas chromatography to calculate ingestion and fecal excretion of major fatty acids, including LA. Fatty acid methyl esters were analyzed by GC-C-IRMS to calculate the enrichment of $[^{13}\text{C}]-\text{LA}$. Total fecal lipid excretion was calculated as the sum of excretion rates of the major long-chain fatty acids ($16:0 + 18:0 + 18:1\text{n-9} + 18:2\text{n-6}$). Total fecal lipid (LA) excretion was expressed as mmol/day. The percentage of lipid (LA) absorption was calculated from daily lipid (LA) ingestion and daily lipid (LA) excretion and expressed as a percentage of the daily lipid (LA) ingestion.

$$\text{% total dietary lipid (LA) absorbed} = \frac{\text{Daily lipid (LA) ingestion (mol)} - \text{Daily fecal lipid (LA) output (mol)}}{\text{Daily lipid (LA) ingestion (mol)}} \times 100\%$$

Net lipid (LA) absorption was calculated as the molar ingestion minus the molar excretion of lipid (LA). Using the ingestion and fecal excretion of $[\text{U-}^{13}\text{C}]-\text{LA}$, identical calculations were performed to measure the percentage and the net amount of $[\text{U-}^{13}\text{C}]-\text{LA}$ absorbed by the intestine.

**Gas liquid chromatography.** Fatty acid methyl esters were separated and quantified by gas liquid chromatography on a Hewlett Packard gas chromatograph Model 6890 with a 50 m x 0.32 mm Ultra 1 capillary column (Hewlett Packard, Palo Alto, CA, USA). The oven temperature was programmed from an initial temperature of 160°C to a final temperature of 290°C in 3 temperature steps (160°C held 2 min; 160-240°C, ramp 2°C/min, held 1 min; 240-290°C, ramp 10°C/min, held 10 min). Fatty acids were quantified using heptadecanoic acid (17:0) as internal standard.

$[^{13}\text{C}]-\text{enrichments of fatty acid methyl esters were determined by GC-C-IRMS (Delta S/GC Finnigan MAT, Bremen, Germany). Separation of the methyl esters was achieved on a 50 m x 0.32 mm CP-SIL 88 capillary column (Chrompack, Middelburg, The Netherlands). The gas chromatograph oven temperature was programmed from an initial temperature of 80°C to a final temperature of 225°C in three temperature steps (80°C held 1 min; 80-150°C, ramp 30°C min$^{-1}$; 150-190°C, ramp 5°C min$^{-1}$; 190-225°C, ramp 10°C min$^{-1}$, held 5 min).}$

$^{12}\text{CO}_2^+$ and $^{13}\text{CO}_2^+$ ions were measured at m/z 44 and 45. Correction for $^{17}\text{O}$ was achieved through measurement of $^{18}\text{O}$ abundance at m/z 46 [14]. $[^{13}\text{C}]$ abundance was expressed as the $^{13}\text{C}_{\text{PDB}}$ value, i.e., the difference between the sample value and the reference compared to the
Pee Dee Belemnite limestone. $^{13}\text{C}_{\text{PDB}}$ values were converted to atom % $[^{13}\text{C}]$ values. Enrichment was expressed by subtracting the baseline $[^{13}\text{C}]$ abundance from all enriched values. The enrichment (atom % excess) was converted to mol % $[^{13}\text{C}]$ fatty acid. The $[^{13}\text{C}]$ fatty acid concentration was calculated from the fatty acid concentration and mol % $[^{13}\text{C}]$ fatty acid. The concentration of $[^{13}\text{C}]$ fatty acid in plasma was then expressed as the percentage of the dose administered per milliliter plasma (% dose/mL).

Retinyl palmitate. Retinyl palmitate concentration in 50 µL plasma samples was determined after two extractions with hexane [9]. Retinyl acetate was added to plasma samples before lipid extraction as an internal standard. Samples were resuspended in ethanol (15 µL) and analyzed by reverse-phase HPLC using a 150 x 4.6 mm Symmetry RP18 column (Waters Corp., Milford, MA, USA) [10]. Peak area of retinyl palmitate was normalized to that of retinyl acetate. At each time point, concentrations were expressed as µmol retinyl palmitate/mL plasma.

Intestinal lipids and histology. Intestinal mucosa fractions were obtained by scraping proximal and distal sections of the small intestine. Triglyceride concentrations in mucosal tissue were determined after lipid extraction [11] as described previously [12]. Specimens (~0.5 cm) of duodenum, jejunum, and ileum of control and bile-diverted rats were fixated in 4% paraformaldehyde (v:v). Cross-sections of the tissues were embedded in paraffin, microsectioned and processed in a routine manner for histologic examination with hematoxylin and eosin stain.

Statistics
The experimental data are reported as means ± S.E.M. for the indicated number of animals per group. Differences between control and bile-diverted groups were calculated using the two-tailed Student’s t-test for unpaired data. Significance was considered at $P<0.05$.

Results

Body weight and food ingestion
During the bolus and fat balance experiments, there was no significant difference in body weight between bile-diverted and control rats (321.7 ± 7.8 g vs 335.8 ± 4.6 g; $P>0.05$). Mean values revealed that bile-diverted rats ingested considerably more chow than control rats (20.3 ± 1.2 g vs 14.7 ± 0.6 g; $P<0.001$; equal to 4283 kcal/kg chow).

Absolute and relative linoleic acid (LA) concentrations in plasma
Already after 1 week of bile diversion, the total concentration of major fatty acids was decreased compared to control rats (5.43 ± 0.30 mM vs 6.77 ± 0.47 mM; $P<0.05$). LA concentration was significantly lower in bile-diverted rats compared with control rats (0.90 ± 0.08 mM vs 1.45 ± 0.12 mM; $P<0.01$). Also, the molar percentage (Fig. 1) was decreased in bile-diverted rats compared with control rats (16.37 ± 0.79% vs 21.18 ± 0.44%; $P<0.001$).
Absolute concentrations of arachidonic acid were not significantly different between bile-diverted and control rats (1.57 ± 0.09 mM vs. 1.47 ± 0.12 mM, \(P>0.05\)), but its relative concentration was increased in bile-diverted rats (29.21 ± 1.40% vs 21.91 ± 1.40%; \(P<0.01\)) (Fig. 1). The triene:tetraene ratio (20:3n-9/20:4n-6, biochemical indicator of EFA deficiency) was significantly higher in the bile-diverted rats (0.020 ± 0.002 vs 0.011 ± 0.001; \(P<0.01\)), although values remained considerably below 0.2, the accepted threshold cutoff value for EFA deficiency in humans [13].

**Calculation of daily linoleic acid (LA) supply to the intestine via bile**

For accurate fat balance measurements, we quantified LA in chow and in biliary phospholipid. The high-fat chow contained 0.15 \(\mu\)mol LA/mg chow, therefore providing on average (mean chow intake for control rats: 16.3 ± 0.5 g) about 2.4 millimoles LA/day. The LA content of bile from male Wistar rats fed high-fat chow was 21.8 ± 1.0 mol% \((n=4)\). Using the biliary phospholipid secretion rate in rats under physiological conditions as determined by us previously [14] (569 nmol/min/kg), total daily LA input into the intestine via bile for a 300 g rat was calculated to be 0.11 millimoles. Using these values, the amount of LA in the intestine delivered via bile is <5% of that provided by the high-fat, LA-rich chow. Therefore, the contribution of biliary LA to the LA balance was relatively minor.

**Lipid balance**

In Table 1, dietary lipid absorption data of control and bile-diverted rats are shown. Bile-diverted rats excreted significantly increased amounts of lipid and of LA in feces compared to control rats \((P<0.0001)\). Although the percentages of lipid and LA absorption were decreased in bile-diverted rats by 38 and 22%, respectively, when compared to control rats \((P<0.0001)\), net lipid and net LA absorption values remained similar \((P>0.05)\). In Table 2, the lipid balance data for \(^{13}\)C-LA are presented for control and bile-diverted rats. No quantitative differences in excretion or net absorption of the labeled substrate by the intestine were found between control and bile-diverted rats under the conditions studied.
Table 1. Dietary lipid and linoleic acid (LA) balance data for control and bile-diverted rats

<table>
<thead>
<tr>
<th></th>
<th>Ingestion (mmol/d)</th>
<th>Fecal excretion (mmol/d)</th>
<th>Absorption (%)</th>
<th>Absorption (mmol/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Total lipid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.59 ± 0.23</td>
<td>0.41 ± 0.04</td>
<td>93.8 ± 0.8</td>
<td>6.18 ± 0.25</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>9.05 ± 0.47*</td>
<td>3.72 ± 0.37**</td>
<td>58.6 ± 4.1**</td>
<td>5.33 ± 0.50</td>
</tr>
<tr>
<td><strong>II. LA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.94 ± 0.06</td>
<td>0.03 ± 0.00</td>
<td>98.5 ± 0.3</td>
<td>1.91 ± 0.07</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>2.68 ± 0.14*</td>
<td>0.72 ± 0.11**</td>
<td>73.3 ± 4.1</td>
<td>1.96 ± 0.14</td>
</tr>
</tbody>
</table>

Data are means ± S.E.M. of 13 control and 9 bile-diverted rats. Mean values represent the average of 3 days per rat. Included in these 3 days is the experimental day in which the lipid bolus was administered. Total lipid includes 16:0, 18:0, 18:1-n-9 and 18:2-n-6. * P<0.001, ** P<0.0001.

Table 2. 13C-linoleic acid (LA) balance data for control and bile-diverted rats

<table>
<thead>
<tr>
<th></th>
<th>Amount administered (µmol)</th>
<th>Fecal excretion (µmol/48 h)</th>
<th>Absorption (%)</th>
<th>Absorption (µmol/48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.44 ± 0.12</td>
<td>0.19 ± 0.05</td>
<td>97.3 ± 0.7</td>
<td>7.25 ± 0.15</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>0.57 ± 0.20</td>
<td>0.57 ± 0.20</td>
<td>92.5 ± 2.6</td>
<td>6.83 ± 0.26</td>
</tr>
</tbody>
</table>

Data are means ± S.E.M. of 13 control and 9 bile-diverted rats. No statistical significance noted in any category between groups.

Plasma [13C]-linoleic acid (LA) concentrations

We studied absorption kinetics by determining the appearance of [13C]-LA in plasma after its duodenal administration. Figure 2 shows the time course of [13C]-LA appearance in plasma after intraduodenal administration of [13C]-LA to control and bile-diverted rats, respectively. In control rats, plasma [13C]-LA concentrations increased within 1 h, reaching a maximum value of 0.12 ± 0.02% dose/mL plasma at 6 h after bolus administration. Upon bile diversion, plasma [13C]-LA concentrations were significantly lower than in controls (P<0.001). A maximum value of 0.05 ± 0.01% dose/mL plasma was obtained at 5 h.

![Figure 2. Time course of [13C]-linoleic acid concentration in plasma of control (circles) and bile-diverted (squares) rats after intraduodenal administration of [13C]-linoleic acid (6.7 mg/kg body weight) at time zero. Data are means ± S.E.M of 14 control and 10 bile-diverted rats. Statistical significance was reached at all individual time points (t=1, 2, 3 h, P<0.001; t=5, 6 h, P<0.01; t=4, P<0.05) and at the area under the curve (0-6 h) (P<0.001).](image)
**Plasma retinyl palmitate concentrations**

To compare the plasma appearance of $[^{13}C]$-LA with that of an uncharged lipid molecule, we investigated the appearance of retinyl palmitate in plasma after intraduodenal retinol (vitamin A) administration. The metabolism of postprandial lipoproteins is commonly studied after the ingestion of a fat load supplemented with retinol. Figure 3 shows the time course of retinyl palmitate appearance in plasma after intraduodenal administration of retinol to control and bile-diverted rats. In control rats, plasma retinyl palmitate concentration reached a maximum of 21.1 ± 4.3 µmol retinyl palmitate/L plasma at 2 h after administration. Upon bile diversion, plasma concentrations of retinyl palmitate were significantly lower than in controls ($P<0.001$). Maximum values of 1.99 ± 0.62 µmol retinyl palmitate/L plasma were obtained at 4 h.

**Intestinal morphology**

To investigate whether bile-diversion induced changes in intestinal morphology, we examined the morphology of the small intestine of control and bile-diverted rats. Villus length of duodenal mucosa was increased in bile-diverted rats compared with control rats (Fig. 4).

**Figure 3.** Time course of retinyl palmitate concentration in plasma of control (circles) and bile-diverted (squares) rats after intraduodenal administration of 50,000 IU retinol at time zero. Data are means ± S.E.M of 4 rats per group. Statistical significance was reached at all individual time points (t=1, 4, 5, 6 h, P<0.001; t=3 h, P<0.01; t=2 h, P<0.05 ) and at the area under the curve (0-6 h) (P<0.001).

**Figure 4.** Morphology of duodenal mucosa from control (A) and bile-diverted (B) rats as determined by light microscopy (10x) after fixation by paraformaldehyde and hematoxylin eosin staining.

**Metabolism of $[^{13}C]$-linoleic acid (LA) to $[^{13}C]$-arachidonic acid**

The decreased plasma concentration of LA during bile diversion could be due to increased metabolic conversion to, for example, arachidonic acid (20:4n-6). At 24 h after $[^{13}C]$-LA
administration, bile-diverted rats had an increased ratio of plasma $[^{13}\text{C}]$-arachidonic acid to plasma $[^{13}\text{C}]$-LA concentration compared to controls (1.38 ± 0.31 vs. 0.47 ± 0.08; $P<0.05$).

**Discussion**

The relationship between lack of bile secretion into the intestine and lipid malabsorption is well known. Many investigators have suggested that the decreased LA status in cholestatic patients (~30% lower than control patients) is due to lipid malabsorption [15-18]. However, actual balance studies have not been performed. In addition, it has not been determined whether decreased levels in these patients are associated with the lack of bile secretion into the intestine or with other phenomena related to cholestasis, for example, cholestatic hepatocellular injury. In our present study, we found that plasma LA concentration was decreased in rats already 1 week after bile diversion. Yet, although the percentage dietary LA absorption was lower, the net absorption of dietary LA by the intestine was not altered by bile diversion. The results were compatible with an increased metabolism of LA to arachidonic acid, as a possible cause for the decreased LA concentration in plasma of bile-diverted rats.

We investigated lipid malabsorption as a possible cause of impaired LA status in bile-diverted rats. In control rats, the percentage of dietary lipid absorbed, as determined from lipid balance calculations, was ~94%, which is within the normal range of lipid absorption (92-95%) [19]. The percentage of dietary LA absorbed was even more efficient, averaging about 99%. In contrast, the percentage of dietary lipid and LA absorption in bile-diverted rats was decreased to 58% and 73%, respectively. Similarly, Demarne et al. demonstrated that bile-ligated rats fed chow containing 10 wt% lipid (22 en%) experienced a reduced percentage of dietary fatty acid absorption (56%) [20]. Percentage of LA absorbed as a free fatty acid ($[^{13}\text{C}]$-LA), as determined by the lipid balance, was more efficient than when LA was given in the diet as triacylglycerols. The higher percentage of $[^{13}\text{C}]$-LA absorbed may be due to the fact that the amount of $[^{13}\text{C}]$-LA administered to the rats was <1% of the daily LA intake (tracer effect) and that it was administered in a specific, relatively soluble form. Compared with the diet, the lipid bolus contained a majority of MCT along with the tracer, which could have potentially facilitated enteral and even portal transport of $[^{13}\text{C}]$-LA.

In spite of the decrease in the percentage of dietary lipid and LA absorption in bile-diverted rats, the rats managed to maintain a quantitatively similar lipid and LA absorption. This adaptive response can be due to a number of changes. An important compensatory effect for bile diversion seemed to be an increased food ingestion (~40%). It has been observed previously that, during bile diversion, rats ingest more chow in order to maintain an adequate energy balance [7]. Mechanisms underlying this adaptation of food intake are unclear, although it is tempting to speculate that a decreased concentration of apoprotein (apo) A-IV is involved. Apo A-IV, which is only synthesized in the intestine, has been identified as having characteristics of a ‘satiety factor’ [21,22]. Intestinal synthesis of apo A-IV is stimulated upon transport of absorbed lipid via chylomicrons into lymph. Impaired chylomicron assembly in
bile-diverted rats would decrease concentrations of apo A-IV in plasma, which theoretically could result in enhanced chow ingestion [23].

Apart from increased ingestion of chow, present data indicate the presence of adaptive responses to bile diversion. Similar net absorption of dietary lipid by bile-diverted rats may be explained in part by altered morphological and/or functional characteristics of the bile-diverted rat intestine. We demonstrated that the villus length of enterocytes of bile-diverted rats was longer than those of the control rats. This observation is in accordance with those of Bloch et al. [24], who found that 12-day bile-diverted rats have increased villus and crypt height (40%) compared to control rats. The mechanism responsible for this adaptation has not been elucidated, but may be related to the absence of detergent bile salts in the intestinal lumen [25,26]. The increased surface area available for nutrient absorption, provided by the longer villi, may partially compensate for the lack of bile secretion into the intestine. Additionally, the functional area of absorption may also be increased by using the distal section of the intestine in conditions which interfere with the efficiency of lipid absorption, such as in bile diversion [27]. In support of this possibility, our analysis of the proximal and distal small intestine of bile-diverted rats revealed greater amounts of triglyceride in the distal compared to the proximal small intestine (0.16 ± 0.02 µmol triglyceride/mg protein vs. 0.08 ± 0.02 µmol triglyceride/mg protein for distal and proximal sections, respectively). In contrast, control rats had similar amounts of triglyceride in proximal and distal sections of the small intestine (0.17 ± 0.02 µmol triglyceride/mg protein vs. 0.17 ± 0.03 µmol triglyceride/mg protein, respectively).

Theoretically, the mechanism behind these morphological and/or functional changes may not be related (only) to the intestinal absence of bile salts, but rather to the lack of biliary phospholipid EFA input to the intestine. Depending on the quantity of EFA in the diet, the biliary supply of EFA can be a substantial source of EFA for structural (i.e., lipid membrane constituents) and/or functional (i.e., prostaglandin formation, energy) needs of the intestine. Since the small intestinal cells have a relatively rapid turnover (5-6 days in humans, 2-3 days in rodents), a constant supply of EFA are needed for continual cell renewal [28,29]. Using control and bile-diverted rats, Melin et al. [28] have estimated that absorbed bile arachidonic acid contributes significantly (30%) to the arachidonic acid pools of the absorptive villus cells. Yet, in the present study, biliary supply of LA contributed <5% to the total dietary LA intake, suggesting that the potential effects of the lack of biliary LA on either small intestinal function, or on plasma LA concentrations, were minimal.

Although net intestinal absorption was similar between control and bile-diverted rats, plasma concentrations of [13C]-LA and retinyl palmitate were significantly lower in bile-diverted rats for 6 h after administration compared to control rats. This finding is compatible with the hypothesis that the intestine can compensate for the net amount of lipid absorbed, but is unable to meet the physiological rate of absorption, due to the lack of bile. The absorption of dietary lipids from more distal parts of the intestine compared with the physiological condition can certainly play a role. In addition, the bile-diverted intestine may be able to compensate for the
lack of biliary phospholipids by using alternate routes of transport. The concept that relatively hydrophilic, long-chain fatty acids such as LA are partially transported via the portal vein cannot be excluded. Since the intestinal lipoprotein production is partially regulated by biliary lipids [30], it would be interesting to study if bile-diversion leads to a different distribution of LA over lipid classes (phospholipids, triacylglycerols) or lipoproteins.

Based on our data, a decreased net absorption could be ruled out as a possible cause for the decreased LA concentration in bile-diverted rats. Yet, the present results suggest an alternative explanation, namely an increased conversion of LA to its long-chain polyunsaturated fatty acid metabolites, such as arachidonic acid. Relative concentrations of arachidonic acid were increased by ~25% in bile-diverted rats compared to control rats. Also, an increased conversion of $[^{13}\text{C}]$-LA to $[^{13}\text{C}]$-arachidonic acid was observed in bile-diverted rats at 24 h after $[^{13}\text{C}]$ label administration. These two independent observations indicate that in bile diversion without cholestasis, there is an increased conversion of LA to its major metabolite, arachidonic acid. Although there may be other contributors to the decreased LA concentration in plasma of bile-diverted rats, it seems likely to suggest that an accelerated metabolism plays a role.

In summary, we conclude that absolute and relative concentrations of LA in plasma are decreased in bile-diverted rats. However, decreased levels are not due to decreased net absorption of dietary LA by the intestine. Our data are compatible with an accelerated metabolism of LA to arachidonic acid upon bile diversion, as indicated by increased relative concentrations of arachidonic acid in plasma and by increased conversion of $[^{13}\text{C}]$-LA to $[^{13}\text{C}]$-arachidonic acid after $[^{13}\text{C}]$ label administration. It presently remains to be determined whether cholestasis, apart from its effects on bile secretion into the intestine, influences LA status and metabolism.

References