Intestinal conversion of linoleic acid to arachidonic acid in the rat

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The arachidonic acid (C20:4, n-6) appearing in intestinal lymph during linoleic acid (C18:2, n-6) absorption may originate from enterocyte synthesis or from the liver either after secretion in biliary phospholipids at the same time dietary linoleic acid absorption occurs or via plasma. The radioactivity measured in the total bile collected during the 6 hours of linoleic acid absorption is too small to explain hepatic origin of the C20:4 detected by high performance liquid chromatography analysis of labeled fatty acids recovered in the lymph, in the intestinal mucosa, and the intestinal wall at the peak of linoleic acid intestinal absorption. This study confirms the probability that under in vivo conditions, during the absorption processes, rat intestine is able to convert dietary linoleic acid independent of liver desaturases and elongase activities. (J. Nutr. Biochem. 4:655–658, 1993.)

Keywords: linoleic acid; intestine; conversion; rat

Introduction

Previous authors have demonstrated in vitro the capacity of small intestinal microsomes to elongate[1] and to desaturate[2,3] the long chain fatty acids. Information on the ability of enterocytes to desaturate long chain fatty acids in vivo is lacking or sparse, although these enzymatic activities are well known in the liver. Garg et al.[2] showed the presence of some desaturase enzymes in the rat enterocyte and suggested that a significant amount of arachidonic acid may originate from de novo synthesis within the enterocyte via desaturation and elongation of linoleic acid. In a recent study we observed the presence of significant labeled arachidonic acid in intestinal lymph during [1-^{14}C] linoleic acid absorption in the rat.[4]

This observation suggests that the arachidonic acid recovered in lymph may originate from synthesis in enterocytes, without excluding a liver origin, through both blood and biliary phospholipids.

The goal of this work was to investigate the site of synthesis of labeled arachidonic acid recovered in lymph after intestinal labeled-linoleic administration and to estimate the intestinal conversion of this essential fatty acid.

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Methods and materials

Animals

Official French regulation (No 87848 and No 03056) for the care and use of laboratory animals were followed. Male Wistar rats weighing 200–220 g (Centre Elevage Dépré, St Doulchard, France) were housed in the animal room for 5 days before the experiments.

The animals were fed a balanced diet (4% wt:wt lipid, UAR Villemoisson sur Orge, France) and had free access to tap water.

Surgical procedures

In one group of rats, the main mesenteric lymph duct was cannulated with a Biotrol No 1 catheter (Biotrol, BP 33, Louvres, France) for lymph collection.

In two other groups of rats, the bile was collected by a catheter (Biotrol No 2) introduced in the upper part of the common bile duct.

In all groups of rats, a catheter (Biotrol No 4) was sutured in the duodenal lumen to introduce the lipids. The lipid emulsion was infused at 37° C, 16 hours after the surgery.

After surgery, the rats were placed in restraining cages in a warm room (25° C). They had free access to a saline solution containing 0.7% sodium chloride and 0.2% potassium chloride.

From the first group the mesenteric lymph was collected hourly during the 6 hours following the lipid administration. From a second group, the bile was collected hourly during the 6 hours following the lipid administration. From the parallel group with bile fistula the intestinal mucosa was removed by scraping and the intestinal wall recovered 2 hours after the lipid administration.

The surgical procedures were performed under slight ethyl ether anesthesia as used in a previous work.[5] The duration of anesthesia during the main mesenteric lymph duct cannulation did not exceed 45 minutes; for the other surgical preparations, anesthesia did not exceed 15 minutes. Thus the collections of both bile and lymph were performed on conscious animals that had been placed in the restraining cages for 16 hours.

Infusate

Ninety μmol of an equimolar mixture (30/30/30) of linoleic acid, oleic acid, and monopalmitin were prepared containing 5–6 mCi of [1-^{14}C] linoleic acid (specific activity: 58 mCi · mmol^{-1}) (CEA, Saclay, France). The radioactivity purity was checked by high performance liquid chromatography (HPLC). The lipids were emulsified with sodium taurocholate (60 μmol) in 1.5 mL of 0.9% NaCl solution. The lipids were obtained from NU Check Prep Company (Elysian, MN USA).

Lipid analysis

The lipids were extracted from the lymph and bile samples from the intestinal mucosa and intestinal wall using Delsal's method,[6] and the fatty acid methyl esters were prepared.[7] The methyl esters of fatty acids were analyzed by reverse phase HPLC; all fatty acid peaks were recovered and radioactivity measured.

Reverse phase HPLC

The distribution of radioactivity between substrate (18:2 n-6) and desaturation-elongation products was determined by reverse phase HPLC as previously described[8] using commercial instrumentation and a lichrocart column (Superspher RP 18, 250 mm × 4 mm i.d., Waters, Milford, MA USA). Each HPLC fraction, corresponding to a known fatty acid methyl ester, was collected at the outlet of the detector (differential refractometer) and the radioactivity was measured directly in the solvent by liquid scintillator counting. The elution conditions (acetonitrile/water 93/7 vol/vol, 30° C) permitted an effective separation of the different n-6 unsaturated fatty acid methyl esters in the order: 18:3 < 20:4 < 18:2 < 20:3 n-6. Results were expressed as percent of conversion.

Quantification of radioactivity

The radioactivity was measured with a liquid scintillation spectrophotometer (Prias model, Packard Instruments, Downer's Grove, IL USA).

Calculations

Results were expressed in nanomole equivalents of linoleic or arachidonic acids using the specific activity of the [1-^{14}C] linoleic acid of the infusate. Values are means ± SEM.

Results

Table 1 shows the recoveries of radiolabeled fatty acids in the lymph at different times of sampling. The radioactivity was found mainly in the linoleic acid (11,341 nmoles) during the 6-hour experiment with only a low amount of radioactivity in the arachidonic acid (399 nmoles).

Table 1 Absorption profile of linoleic and arachidonic acids in the mesenteric lymph

Time period (hours)	C18:2 (nmol)	C20:4 (nmol)
0–1 hr	155 ± 18.5	0
1–2 hr	1,448 ± 145	0
2–3 hr	5,453 ± 170	225 ± 4.4
3–4 hr	2,237 ± 210	140 ± 16
4–5 hr	1,131 ± 123	34 ± 5.6
5–6 hr	917 ± 144	4 ± 1
0–6 hr	11,341 ± 131	399 ± 6

The values are expressed as nanomoles of C 18:2 and C 20:4 recovered in lymph during the 6 hours following the intraduodenal lipid infusion. The rats had intact bile ducts and cannulated lymph ducts.
Values are means ± SEM for n = 6 animals.

The appearance of the labeled linoleic acid increased from the 1st to the 3rd hour following the duodenal lipid infusion. The peak of radioactivity in the lymph during labeled linoleic acid absorption appeared between the 2nd and the 3rd hour. The radioactivity decreased gradually from the 3rd to the 6th hour. During the period of absorption, labeled arachidonic acid appeared in the lymph from the 3rd hour with a maximum occurring during the 2–3 hr period (225 nanomoles). Prior to peak linoleic acid absorption, no significant arachidonic acid was detected in lymph. The total recovered radioactive arachidonic acid was around 400 nanomoles, corresponding to 3.5% of the total radiolabeled fatty acid in the lymph.

The total radioactivity recovered in 6 hours on the biliary lipids (*Figure 1*) of the bile fistula animals represented only 90.7 equivalent nanomoles of linoleic acid.

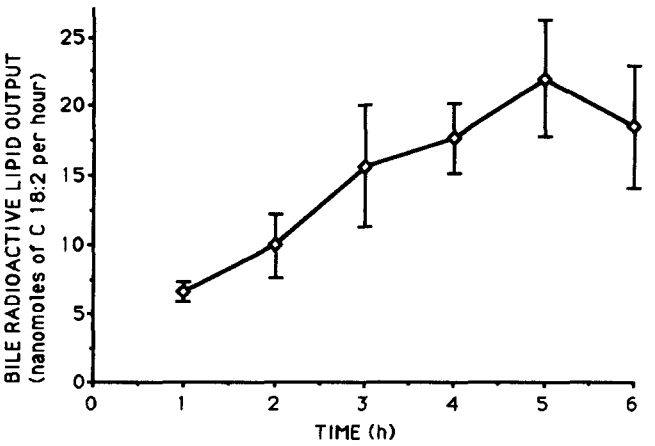

Figure 1 Hourly recoveries of radioactivity in the biliary lipids during the 6 hours following intraduodenal lipid infusion. The animals had intact lymph ducts and canulated bile ducts. Values are expressed in nanomole equivalents of C 18:2 ± SEM (bars) for n = 6 animals.

This low quantity did not permit the analysis of specific radiolabeled molecules nor an estimation of whether the radioactivity was associated with arachidonic acid. Moreover, whereas the lymph linoleic acid recovery showed a significant peak of absorption, the bile flow rate (*Figure 2*) measured during the period of absorption showed a steady state. The peak of radioactive lipids recovered in the bile appeared after the 4th hour, much later than for lymph.

The analysis of the labeled fatty acids in intestinal mucosa and intestinal wall recovered 2 hours after the duodenal lipid infusion (*Table 2*) showed an accumulation of C18:2 (14,030 nanomoles and 4,840 nanomoles, respectively) and simultaneously the presence of low amounts of C20:4 (161 and 53 nanomoles, respectively).

Discussion

During long chain fatty acid absorption phospholipids are continuously used for chylomicron formation and turn-over of enterocyte membranes. The rapid phospholipid turn-over requires polyunsaturated fatty acids such as arachidonic acid (C20:4, n-6) or eicosapentaenoic acid (C20:5, n-3). In a recent review, Thomson et al.[9] suggested that C20:4, n-6 might be the rate-limiting factor for phospholipid synthesis in the intestine. It would be interesting to know the metabolic origin of this essential fatty acid.

During exogenous labeled linoleic acid absorption, labeled arachidonic acid is detected in lymph radioactive lipids. It is not known whether this arachidonate is derived from linoleate in the intestine, from blood origin, or from luminal hydrolysis and absorption of bile phospholipids. Evidence appears to be controversial. GARG et al.[2] suggested that a significant amount of C20:4 originates from enterocyte de novo synthesis; Christiansen et al.[1] and Hjelte et al.,[10,11] on the contrary, feel that the arachidonic acid recovered in intestine of essential fatty acid-deficient rats after administration of linoleic acid originates only from a conversion in the liver.

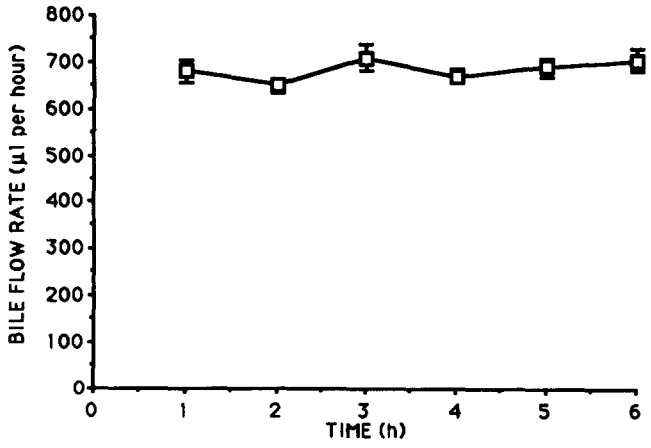

Figure 2 Bile flow rate during the 6 hours following intraduodenal lipid infusion. Values are expressed in mL ± SEM (bars) for $n = 6$ animals

Table 2 Nanomole equivalents of ^{14}C C18:2 and ^{14}C C20:4 recovered in intestinal mucosa and intestinal wall 2 hours after the intraduodenal lipid infusion

	Mucosa (nmol)	Wall (nmol)	Mucosa + wall (nmol)
C 18:2	14,030 ± 2,000	4,840 ± 1,300	18,870 ± 2,262
C 20:4	161 ± 55	53 ± 21	215 ± 38

Values are means ± SEM for $n = 6$ animals. The rats had intact lymph ducts and cannulated bile ducts.

Our experiments carried out on the bile fistula rats (with intact lymph duct) and those involving the quantification of arachidonic acid recovered in lymph after linoleic acid absorption on rats with intact bile ducts demonstrate that hepatic conversion would not appear to be the sole origin of arachidonic acid appearance in mesenteric lymph. Indeed, the total radioactivity recovered in biliary lipids during the 6 hours was greatly inferior to that of arachidonic acid in lymph radioactive lipids. Likewise, the quantity of labeled arachidonic acid present in the mucosa and in the intestinal wall after a 2-hr infusion in the group of rats with intact lymph ducts and cannulated bile ducts was clearly greater than the total radioactivity found in 6 hours in the labeled biliary lipids of diverted bile rats. Consequently, the arachidonic acid quantified in mucosa cannot have originated from the biliary phospholipids. Indeed, differences in the time of liver synthesis followed by its appearance in bile could suggest that only arachidonic acid of intestinal origin was present in lymph at the early time period of absorption. Furthermore, the low quantity of radioactivity recovered in bile cannot be due to deficient bile collection. The bile flow in our experiment was very reproducible and similar to the bile flow generally observed during intestinal fat absorption, particularly as shown by Boquillon and Clement.[12]

Scraping of the intestinal mucosa only 2 hr after infusion was sufficient to demonstrate the conversion of linoleic to arachidonic acid during the initial stage of absorption and before the delivery of any arachidonic acid synthetized in the liver because in the same time period no arachidonate was recovered on the biliary lipids. However, further investigations might be carried out to estimate parallel eventual blood supply of arachidonic acid from liver origin at the intestine. But, as shown by Gangl and Ockner,[13] endogenous plasmatic free fatty acids are preferentially integrated into enterocyte phospholipids instead of contributing to chylomicron triacylglycerol synthesis.

The in vivo studies confirm the probability that some bioconversion of linoleic acid to arachidonic acid appears during the initial passage of metabolism in the intestine. These results are consistent with the presence of Δ6 desaturase activity in intestine observed in vitro by Garg et al.,[2] and with an elongase activity reported from in vitro experiments by Christiansen et al.[1] Recently, Chen and Nilsson[14] demonstrated the presence of Δ6 and Δ5 desaturase activities in human CaCo2 cells,

which further supports existence of enzymes required for the conversion of linoleate to arachidonate in the intestinal epithelial cells. More recently, Garg et al.[15] demonstrated that in vitro the level of arachidonic acid and the biosynthesis of this fatty acid by desaturation-chain elongation of linoleic acid in intestinal microsomes respond rapidly to changes in physiological conditions such as fasting and dietary fat composition.

In our experimental conditions, taking into account radioactive assays conducted on bile, hepatic synthesis of arachidonate seemed to be delayed compared with lymph radioactive arachidonic acid output. It would seem reasonable to assume that hepatic synthesis might be followed by simultaneous appearance of arachidonic acid both in blood and bile.

Thus, in conclusion, for the time period of absorption studied, we found that in vivo the rat intestine seems able to convert linoleic into arachidonic acid independent of liver desaturase and elongase activities.

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