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Uptake and interconversion of plasma unesterified ^{14}C linoleic acid by gastrointestinal tract and blood forming tissues: An experimental study in the rat

Ning Xu and Åke Nilsson*

*Cell Biology Department 1, University Hospital, Lund, Sweden; *Gastroenterology Division, Department of Medicine, Lund, Sweden*

The origin of the arachidonic acid (20:4, n-6) in the gastrointestinal tract and blood forming tissues is unknown. This study examines the rate of uptake and interconversion of unesterified ^{14}C linoleic acid (18:2, n-6) by the liver, gastrointestinal tract, bone marrow, and spleen. Albumin-bound unesterified ^{14}C-18:2 was injected intravenously into young male rats. The clearance rate of the ^{14}C-18:2 and the mass concentration of unesterified 18:2 in plasma were measured. After 5 and 10 min, the radioactivity of the tissue lipids and the degree of interconversion of ^{14}C-18:2 were determined. The rate of retention and interconversion of plasma unesterified 18:2 in different tissues could then be calculated. The retention of ^{14}C in liver lipids (3.6 to 4.5%/g of tissue) was several-fold higher than in the stomach (0.4%/g), small intestine (0.3%/g), colon (0.2%/g), and spleen (0.2%/g). Higher proportions of the ^{14}C were in phospholipids (PL) in the extrahepatic tissues (50 to 80%) than in the liver (42 to 44%). The main part of the PL radioactivity was in phosphatidylcholine. The percent of labeling in cardiolipin was 4 to 10 fold higher in the gastrointestinal tract and 8 to 20 fold higher in the spleen and bone marrow than in the liver. The percent of interconversion of retained ^{14}C-18:2 to δ6 desaturase products was higher in colon (16%), spleen (18%), and bone marrow (15 to 16%) than in liver (10 to 11%). The rate of 20:4 formation from plasma-free 18:2 was 14,200 pmol/min in liver, 106 pmol/min in the stomach, 751 pmol/min in the small intestine, and 159 pmol/min in the colon. This study indicates that a large part of the 20:4 pools in the gastrointestinal tract and blood-forming tissues can be formed by local interconversion reactions. (J. Nutr. Biochem. 7:16–22, 1996.)

Keywords: arachidonic acid; bone marrow; linoleic acid; small intestine; spleen; stomach

Introduction

Albumin-bound free fatty acids (FFA) are rapidly cleared from blood to undergo oxidation, acylation, or interconversion reactions in the tissues.[1–6] Although oxidation of plasma FFA is an important source of energy, fatty acids may also be targeted to specific tissues and used for synthesis of membrane phospholipids (PLs).[5–11]

The origin of the polyunsaturated fatty acids (PUFAs) of the mucosal PLs in the stomach, small intestine, and colon is not known. δ6 desaturase, which catalyzes the first rate-limiting step in the desaturation-elongation of 18-carbon PUFA to eicosanoid precursors, is present both in absorptive villus cells and regenerating crypt cells of rat small intestine.[12,13] Furthermore, part of the absorbed 20:4 is retained in mucosal PLs of the small intestine.[14,15] Recently it was shown that linolenic acid (18:3, n-3), which is the preferred substrate of the δ6 desaturase, when given intravenously as unesterified fatty acid was actively taken up and desaturated-elongated by the stomach, small intestine, and colon.[18] The quantitative role of the local desaturation-

Address reprint requests to Dr. Åke Nilsson at Gastroenterology Division, Department of Medicine, University Hospital of Lund, S-221 85 Lund, Sweden.
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655 Avenue of the Americas, New York, NY 10010

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elongation reaction in these tissues is, however, unknown since other pathways such as the uptake of lipoproteins or of 2-lyso-phosphatidylcholine[23] might also supply eicosanoid precursors. No evidence for a significant production of eicosanoid precursors in circulating blood cells has been reported. In platelets there was no measurable interconversion of radioactive 18:2.[16] It is therefore likely that the formation of the 20:4 containing PL pools is an integral part of the blood cell formation.[17]

In this study we injected unesterified ^{14}C-18:2 intravenously and studied the retention and interconversion in different tissues and found that a considerable 20:4 formation occurs not only in the liver but also in the gastrointestinal tract, bone marrow, and spleen.

Methods and materials

Intravenous injection of ^{14}C 18:2

1-^{14}C-18:2 (specific activity 53.0 mCi/mmol) was obtained from New England Nuclear). The labeled fatty acid was bound to albumin in serum from a nonfasted rat kept on the standard pellet diet as described earlier.[18]

Male Sprague-Dawley rats were obtained from Möllegaard (Denmark). The rats were kept under a controlled dark–light schedule and had free access to a standard commercial pellet diet until the time of the experiment. The rats weighed 137 to 152 g. Labeled serum, 0.5 mL (10 μCi ^{14}C-18:2), was injected into the jugular vein under a light ether anesthesia. After 5 or 10 min, the rats (5 at each time point) were anesthetized with diethyl ether and killed by aortic puncture. The liver, stomach, upper half of the small intestine, lower half of the small intestine, colon, the bones including bone marrow of two femurs, tibias and humerus, spleen, heart, and lungs were removed and lipids were extracted with chloroform:methanol (1:1) containing 0.005% butylated hydroxytoluene as an antioxidant. For determining the clearance of ^{14}C-18:2 in plasma, 4 rats weighing 139 to 142 g were intravenously injected with 0.5 mL of labeled serum containing 2.4 × 10^6 dpm ^{14}C-18:2 into the jugular vein under a light ether anesthesia. Blood samples (0.3 to 0.4 mL) were obtained by cutting the rat's tail at 15, 30, 45, 60, 90, 120, and 180 sec. One hundred microliters of plasma was counted in a Packard Tri Carb 460 CD liquid scintillation system using the automatic external standard for quench correction.

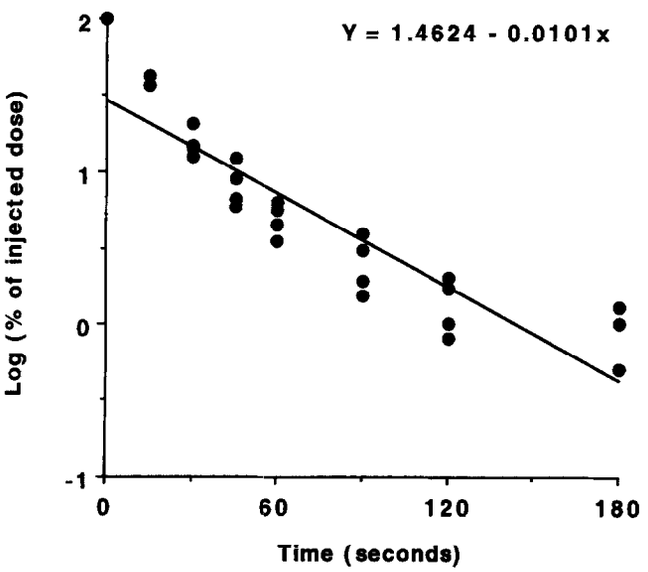

Figure 1 Linear regression of clearance of ^{14}C-18:2 in plasma. Rats were injected intravenously with 0.5 mL of labeled serum containing 2.4 × 10^6 dpm ^{14}C-18:2. Blood was collected (0.3 to 0.4 mL) by cutting the rat's tail at the time intervals indicated. The correlation coefficient is 0.85, $P = 0.0001$. Data are from four individual observations.

Determination of radioactivity in different lipid classes

After lipid extraction and two-phase distribution as described earlier,[19] aliquots of the lower phase were taken to dryness with nitrogen and redissolved in a small volume of chloroform. Aliquots were taken for thin-layer chromatography (TLC). Nonpolar lipids were separated by TLC on silica gel G plates which were developed in petroleum ether:diethyl ether:acetic acid 80:20:2. PLs were separated on Merck silica gel 60 plates developed in chloroform:methanol:acetic acid:water 100:80:12:1.2 (vol/vol). Spots were identified by staining with iodine vapor and scraped into counting vials. One milliliter of methanol:water 1:1 (vol/vol) and 10 mL of Instagel:toluene 1:1 (vol/vol) were added, and the radioactivity of the samples was determined in a Packard Tri Carb 460 CD liquid scintillation system using the automatic external standard for quench correction.

Determination of radioactivity in different n-6 fatty acids

Aliquots of the lipid extracts were transmethylated in toluene: methanol 1:1 (vol/vol) containing 2% concentrated sulfuric acid at 65°C for 4 hr. One half the volume of water was then added, and the methyl esters were extracted in 1.5 vol of petroleum ether. The upper phase was taken to dryness with nitrogen and was redissolved in acetonitrile:water 90:10. Fatty acid methyl esters were separated by high performance liquid chromatography (HPLC) using a Shimadzu SPD 6A high pressure liquid chromatograph equipped with a Nucleosil C_{18} column (4.6 × 250 mm) and a UV detector set at 205 nm. The mobile phase was acetonitrile:water 90:10 (vol/vol),[20] and the flow rate was 0.5 mL/min. Radioactivity of the different fractions was estimated by continuous radioactivity detection with a Radiomatic Flow One continuous flow detector.

Separation of plasma unesterified 18:2 by gas chromatography

Plasma unesterified fatty acids were separated by TLC on silica gel 60 plates as described above. The unesterified fatty acid spots were collected, re-extracted, and transmethylated as already described. The residue was redissolved in 0.5 mL of chloroform. Sample, 1.5 μL, was analyzed in a Perkin Elmer Model 30 apparatus, equipped with a flame ionization detector on a 200 cm glass column (3 mm in inner diameter), packed with 15% diethylene glycol succinate (DEGS), and coated on Chromosorb W DMCS, 80 to 100 mesh. Nitrogen was used as carrier gas, and the column was operated at 193°C. Individual fatty acids were identified by comparison with the relative retention time of standards. Heptadecanioc acid (17:0) was used as internal standard.

Statistical analysis

Values are reported as the mean ± standard error (SE). Data were analyzed by using a statistical software package in an IBM personal computer. One-way analysis of variance (ANOVA) followed

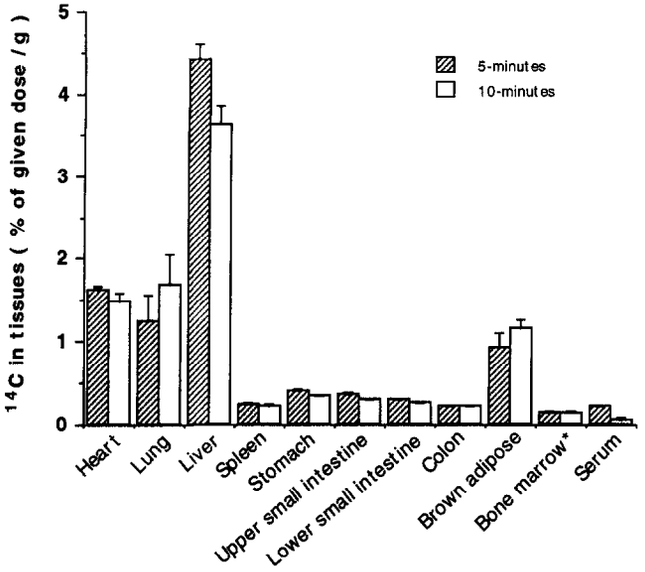

Figure 2 Lipid radioactivity per gram of tissue in different organs. Rats were injected intravenously with 10 μCi of ^{14}C-18:2 as described in the Methods and materials section. Values are means ± SE of five observations. *Bone marrow radioactivity per gram was calculated as radioactivity per total weight of both femurs, tibias, and humerus.

by unpaired Student's *t*-test was employed for statistical analysis. A P value <0.05 in a two-tailed test was considered significant.

Results

Turnover of plasma unesterified linoleic acid

In the first series of experiments, the kinetics for the disappearance of ^{14}C-18:2 from plasma was determined. After 15 to 60 sec, 92 to 96% of the plasma radioactivity was in FFA, and after 120 to 180 sec 80 to 90%. The remaining part was in polar compounds that were not further identified. The rate of clearance was estimated by a linear regression analysis of the amounts of ^{14}C-FFA radioactivity remaining in serum at different time intervals. The initial half-life was 24 sec (*Figure 1*), and the labeled fatty acid was thus almost completely cleared from plasma within 3 min. In two other series of animals (4 rats in each series), we tested whether replenishing the amount of blood drown with 0.9% NaCl influenced the data. We found no difference between the groups, and values for the clearance were very similar to those in *Figure 1*. Neither did we see any difference when analysis was performed on serum or ethylendiamine-tetraacetic acid (EDTA)–plasma (data not shown). The average concentration of FFA was 0.89 μmol/mL estimated from the sum of the major fatty acids (palmitic, oleic, stearic, linoleic, and arachidonic acids) at gas chromatographic (GC) analysis of the FFA fraction. The concentration of unesterified 18:2 was 0.16 ± 0.05 μmol/mL of plasma. With the assumption that the plasma volume is 4% of the body weight, the average plasma pool of unesterified 18:2 was calculated to be 0.88 μmol. From the half-life of 24 sec it could be calculated that 1.07 μmol/min is cleared from plasma. Since 1% of the plasma pool corresponds to 8.8 nmol of 18:2, about 16 nmol of unesterified 18:2 is eliminated per second.

Tissue retention of ^{14}C-18:2

Tissue uptake and the degree of interconversion was studied in two series of animals in which the rats were killed after 5 and 10 min (5 rats for each time point). At these time intervals, almost all of the ^{14}C-18:2 had been cleared from plasma but not yet recirculated in lipoproteins. Moreover, since the data at 5 and 10 min did not differ, the values for retention and the interconversion must reflect the initial fate after the uptake of 18:2 as FFA. The distribution of ^{14}C-18:2 in serum in these animals was determined by TLC indicating that more than 60% of ^{14}C-18:2 was in unesterified fatty acid fractions, and less than 2% was found in the TG fraction after either 5 or 10 min, demonstrating that there was no recirculation of ^{14}C-18:2 as very low density lipoprotein (VLDL). No ^{14}C-labeled desaturation-elongation productions were found in serum during the designed experimental period. The retention of ^{14}C in lipids of different tissues is shown in *Figure 2*. In agreement with the earlier study with ^{14}C-18:3 and ^{3}H-20:5, the retention of radioactivity was several-fold higher in liver than in the gastrointestinal tract.[18] The uptake in heart, lung, and brown adipose tissue was higher than in the gastrointestinal tract but lower than in the liver (*Figure 2*). The total uptake in the gastrointestinal tract including stomach, upper small intestine, lower small intestine, and colon was 2.49 ± 0.13% of the injected dose. The 5 and 10 min values did not differ significantly. Accordingly it is concluded that the acylation and interconversion of ^{14}C-18:2 occurred soon after the uptake when all the labeled fatty acid is converted to acyl-CoA derivatives. We therefore assume that the degree of retention of ^{14}C-18:2 is the same as that of unlabeled 18:2 continuously taken up from the plasma FFA fraction. Based on this assumption, it is possible to calculate the rate of retention of plasma unesterified 18:2 in the different tissues since we know the mass of 18:2 that disappears from blood per time unit and the

Table 1 Retention of plasma unesterified 18:2 in different tissues (n = 9)*

Organ	Amount of (nmol/min/g of tissue)†
Heart	14.7 ± 1.2
Lung	16.7 ± 3.6
Liver	35.9 ± 2.6
Spleen	2.3 ± 0.2
Stomach	3.4 ± 0.2
Upper small intestine	3.0 ± 0.1
Lower small intestine	2.5 ± 0.1
Colon	2.1 ± 0.2
Brown adipose	11.5 ± 1.2
Bone marrow‡	1.5 ± 0.2

*Calculations are based on the finding that the mass of plasma unesterified 18:2 that disappeared from blood was 1.07 μmol/min and on the assumption that the degree of retention of ^{14}C-18:2 is the same as that of unlabeled 18:2 delivered to the tissues. The retention of plasma unesterified 18:2 in different tissues was thus calculated by multiplying the percent retention of ^{14}C-18:2 in different tissues with 1.07 μmol/min.
†Values are means ± SE.
‡The weight of bone marrow based on the total weight of both femurs, tibias, and humerus including both bone and bone marrow.

Table 2 Percentage distribution of ^{14}C-18:2 in lipid classes in different tissues*

Organ	Time (min)	DG	FFA	TG	CE	PL + MG
Heart	5	3.6 (0.3)	1.4 (0.1)	72.7 (3.9)	0.8 (0.1)	21.4 (3.6)
	10	3.3 (0.4)	1.4 (0.2)	71.2 (4.1)	0.9 (0.1)	23.2 (3.5)
Lung	5	3.8 (0.5)	28.9 (11.3)	11.6 (2.3)	0.7 (0.1)	55.0 (8.5)
	10	2.4 (0.4)	49.7 (10.7)	7.9 (2.1)	0.4 (0.1)	39.6 (8.3)
Liver	5	5.1 (0.3)	0.9 (0.0)	48.6 (2.2)	1.3 (0.1)	44.2 (1.9)
	10	3.9 (0.3)	0.8 (0.1)	54.8 (4.0)	0.4 (0.1)	42.1 (2.6)
Spleen	5	3.7 (0.1)	3.5 (0.3)	7.5 (0.5)	3.2 (0.2)	82.1 (0.8)
	10	2.8 (0.1)	2.7 (0.3)	7.4 (0.4)	1.9 (0.1)	85.3 (0.3)
Stomach	5	4.1 (0.1)	5.6 (1.0)	21.6 (1.9)	0.7 (0.1)	68.0 (1.2)
	10	3.2 (0.1)	5.5 (0.6)	21.0 (1.1)	0.6 (0.0)	69.7 (1.1)
Bone marrow	5	4.5 (0.2)	4.1 (0.2)	19.0 (1.6)	3.1 (0.3)	69.2 (1.5)
	10	3.6 (1.0)	3.1 (0.3)	17.6 (1.2)	2.0 (0.1)	73.7 (1.2)
Upper small intestine	5	4.0 (0.2)	3.9 (0.6)	32.1 (2.1)	2.2 (0.3)	57.9 (1.7)
	10	4.1 (0.1)	5.8 (0.3)	31.7 (0.5)	2.7 (0.2)	55.8 (0.4)
Lower small intestine	5	4.3 (0.1)	4.3 (0.3)	27.0 (0.9)	1.8 (0.2)	62.6 (0.8)
	10	4.1 (0.1)	6.4 (0.7)	26.9 (0.4)	2.0 (0.2)	60.6 (1.2)
Colon	5	4.1 (0.1)	2.5 (0.2)	11.6 (0.9)	1.1 (0.1)	80.7 (1.0)
	10	3.7 (0.1)	2.5 (0.1)	11.0 (0.7)	0.8 (0.1)	82.1 (0.7)

*Values are mean ± SE.

percentage of this amount that is retained in the tissues over the time span studied (*Table 1*).

Distribution of ^{14}C between lipid classes

The distribution of ^{14}C between individual nonpolar lipids and total phospholipids is given in *Table 2*. As a general feature, the distribution was similar after 5 and 10 min. Furthermore, the proportion in triacylglycerol (TG) was higher in the liver than in the tissues of the gastrointestinal tract, bone marrow, and spleen. The TG radioactivity in the heart was, however, most predominant. Among the individual PLs 72 to 81% was in phosphatidylcholine (PC), the values being similar after 5 and 10 min (*Table 3*). Phosphatidylethanolamine (PE) contained more of the PL radioactivity in the liver than in other tissues. In contrast, cardiolipin (CL) contained 4 to 20 fold more of the PL ^{14}C in the gastrointestinal tract, spleen, and bone marrow. ^{14}C in phosphatidylinositol (PI) was similar for most tissues and varied between 3.7 and 7.8% of the PL-^{14}C.

Interconversion of ^{14}C-18:2 in different tissues

The percent distribution of ^{14}C between different fatty acids is shown in *Table 4*. The spleen, bone marrow, and colon exhibited the highest degree of interconversion after 5 and 10 min. Significant amounts of both 20:4 and 20:3 were found in all these tissues, and in the case of the stomach, spleen, and bone marrow there was also some radioactivity in 18:3. About 6% of the liver radioactivity was in 20:4, 3% in 20:3, and 2% in 18:3. In lung, the degree of interconversion was lower than in these tissues, and in the heart the degree of interconversion was just at the detection limit of the method. No interconversion at all was observed in brown adipose tissue.

From the data on the concentration of unesterified

Table 3 Percentage distribution of ^{14}C-18:2 in phospholipid subclasses in different tissues*

Organ	Time (min)	LPC	PC	PS	PI	PE	PA	CL
Liver	5	1.8 (0.4)	72.7 (0.4)	1.7 (0.0)	3.7 (0.2)	17.9 (0.3)	1.3 (0.1)	0.6 (0.1)
	10	1.0 (0.1)	75.3 (0.7)	1.1 (0.0)	4.2 (0.2)	17.0 (0.6)	0.9 (0.0)	0.4 (0.1)
Spleen	5	1.4 (0.1)	72.0 (0.8)	3.5 (0.2)	4.7 (0.1)	8.2 (0.2)	1.7 (0.1)	7.9 (0.5)
	10	0.5 (0.0)	73.0 (0.6)	2.1 (0.1)	5.8 (0.2)	8.8 (0.1)	1.2 (0.0)	8.6 (0.3)
Stomach	5	1.6 (0.3)	79.0 (0.5)	2.4 (0.2)	5.7 (0.3)	7.6 (0.2)	1.3 (0.1)	1.9 (0.1)
	10	1.3 (0.2)	79.5 (0.8)	1.9 (0.1)	6.2 (0.3)	7.2 (0.2)	1.2 (0.0)	2.1 (0.1)
Bone marrow	5	1.6 (0.1)	75.5 (0.4)	2.6 (0.1)	5.4 (0.1)	9.3 (0.2)	0.9 (0.1)	3.9 (0.1)
	10	0.7 (0.1)	77.5 (0.3)	2.1 (0.1)	5.4 (0.1)	9.4 (0.1)	0.6 (0.0)	4.3 (0.2)
Upper small intestine	5	1.4 (0.1)	78.1 (0.5)	3.1 (0.3)	6.1 (0.8)	6.7 (0.3)	1.8 (0.1)	2.7 (0.3)
	10	1.4 (0.1)	78.0 (0.3)	2.1 (0.2)	7.0 (0.3)	6.5 (0.1)	1.5 (0.1)	3.7 (0.2)
Low small intestine	5	1.2 (0.1)	78.1 (0.8)	2.3 (0.2)	6.2 (0.2)	6.6 (0.2)	2.0 (0.1)	3.2 (0.1)
	10	1.8 (0.6)	75.4 (0.5)	1.9 (0.1)	7.8 (0.4)	7.4 (0.3)	1.7 (0.1)	3.8 (0.3)
Colon	5	1.2 (0.1)	78.2 (0.6)	1.9 (0.2)	6.8 (0.4)	6.5 (0.2)	1.8 (0.1)	3.2 (0.1)
	10	0.6 (0.0)	81.1 (0.4)	1.5 (0.1)	5.6 (0.1)	6.5 (0.1)	1.5 (0.1)	3.2 (0.2)

*Values are mean ± SE. $n = 5$.

Table 4 Percentage interconversion of ^{14}C-18:2 in different tissues*

Organ	Time (min)	18:2	18:3	20:2	20:3	20:4
Heart	10	97.7 (0.3)	0.7 (0.1)	1.2 (0.2)	1.0 (0.2)	0.8 (0.0)
Lung	10	89.9 (1.8)	0.7 (0.1)	4.8 (0.8)	2.0 (0.4)	2.6 (0.6)
Liver	5	87.4 (1.6)	2.2 (0.3)	2.3 (0.4)	2.7 (0.6)	5.8 (0.7)
	10	85.8 (0.7)	2.0 (0.2)	4.0 (0.7)	3.1 (0.1)	5.8 (0.4)
Spleen	5	79.3 (0.6)	2.0 (0.5)	5.1 (0.2)	4.3 (0.5)	11.7 (0.8)
	10	78.3 (1.1)	2.1	7.4 (0.4)	5.0 (0.3)	10.9 (0.9)
Stomach	5	90.6 (0.9)	2.1 (0.3)	2.6 (0.6)	2.2 (0.1)	2.9 (0.3)
	10	87.1 (0.8)	2.6 (0.3)	4.0 (0.3)	2.8 (0.2)	3.5 (0.5)
Upper small intestine	5	83.8 (1.1)	3.7 (0.3)	3.9 (0.5)	3.7 (0.4)	5.1 (0.9)
	10	86.2 (0.9)	1.9 (0.2)	4.1 (0.4)	4.9 (0.4)	3.3 (0.5)
Lower small intestine	5	83.3 (0.9)	1.8 (0.8)	4.4 (0.2)	5.2 (0.5)	6.0 (0.4)
	10	81.3 (1.1)	2.8 (0.2)	5.4 (0.7)	5.3 (0.7)	6.9 (0.4)
Colon	5	77.0 (0.9)	—	6.7 (0.8)	8.1 (0.7)	8.3 (0.7)
	10	77.6 (0.5)	—	6.8 (0.5)	7.7 (0.7)	7.9 (0.7)
Bone marrow	5	80.4 (0.8)	2.0 (0.3)	4.1 (0.4)	4.1 (0.4)	10.3 (0.3)
	10	78.7 (1.2)	2.2 (0.5)	6.5 (0.6)	4.2 (0.4)	8.9 (0.7)

*Values are mean ± SE. $n = 5$.

plasma 18:2, the elimination kinetics for ^{14}C-18:2, the percent retention in tissues, and the degree of interconversion, the molar rate of interconversion of 18:2 to 20:4 originating from the plasma unesterified 18:2 pool in different organs could be calculated (*Table 5*). The total amount of 20:4 formed in the liver was 16 fold higher than that formed in the gastrointestinal tract. Somewhat more 20:3 accumulated in tissues of the gastrointestinal tract (*Table 4*) with the total formation of δ6 desaturase products being 13 fold lower than in liver (*Table 5*). The total amount of the 20:4 from the bone marrow cannot be calculated exactly since we did not know the weight of the bone marrow. Based on the assumptions that bone marrow accounts for 3% of the body weight and for about 25% of the weight of femurs, tibias, and humerus, it can be calculated that the total 20:4 formation in bone marrow was 2,074 pmol/min, i.e., about one seventh of the formation in the liver (*Table 5*).

Table 5 20:4 formation from plasma unesterified 18:2 pool in different organs*

Organ	Amount (pmol/min/whole organ)†
Heart	60.5 ± 1.8
Lung	360.6 ± 59.3
Liver	14182.6 ± 2587.5
Spleen	125.2 ± 17.6
Stomach	106.1 ± 20.3
Upper small intestine	360.0 ± 66.4
Lower small intestine	391.3 ± 47.0
Colon	158.9 ± 30.2
Bone marrow‡	2073.8 ± 68.0

*Values are calculated by multiplying the values for retention of plasma unesterified 18:2 given in *Table 1* with the proportion of the retained ^{14}C-radioactivity that was in 20:4. $n = 9$.
†Values are mean ± SE.
‡Based on the assumptions that bone marrow accounts for 3% of body weight and for 25% of the weight of femurs, tibias, and humerus.

Discussion

This study shows that significant proportions of 18:2 taken up from blood as FFA is interconverted to δ6 desaturase products not only in the liver but also in the gastrointestinal tract and blood-forming tissues. The total formation of δ6 desaturase products in the intestine amounted to about 8% of that in the liver. The quantitative contribution of the interconversion of 18:2 taken up as FFA to the 20:4 pools of the gastrointestinal tract cannot be fully evaluated at present since 20:4 may also be acquired by the pathways listed in *Table 6*. For the following reasons we believe, however, that local interconversion is an important source.

First, the large differences in the molar rate of 20:4 production from 18:2 FFA between liver and the other tissues is mainly due to the large difference in the rate of 18:2 uptake. The high percentage interconversion of 18:2 FFA in the blood-forming and gastrointestinal tissues implies that any 18:2 CoA formed during the deacylation reactions in the tissues might undergo interconversion to the same extent. The total differences in the rates of formation of 20:4 from 18:2 between liver and the gastrointestinal and blood-forming tissues may thus be much smaller than those observed (*Table 5*).

Second, there is evidence that the uptake of 20:4 from plasma lipoproteins by the gastrointestinal tract is slow. When ^{14}C-20:4 as FFA and ^{3}H-20:4 labeled chyle was injected intravenously, less than 1% of the ^{3}H-20:4 was recovered in the tissues of the gastrointestinal tract after 0.5 to 4 hr. Furthermore, the ^{3}H/^{14}C ratio of the gastrointestinal tract was 3 fold lower than in the liver and blood. Even after 24 hr most of these differences persisted, except that the total ^{3}H and ^{14}C radioactivity and the ^{3}H/^{14}C ratio of the upper half of the small intestine had increased (T. Melin, Q. Chen, and Å. Nilsson, unpublished data). In bile-drained rats these changes with time were eliminated. The conclusion was that 20:4 pools of the liver and of the gastrointestinal tract mix very slowly except in the upper part where 20:4 released from bile PLs is reabsorbed. At the present

Table 6 Possible pathways by which the tissues of the gastrointestinal tract may acquire arachidonic acid

Pathways	References	Hypothetical evaluation of size of contribution
Uptake and interconversion of 18:2 as FFA	18, this paper	Probably large
Interconversion of tissue pools of 18:2 after deacylation		Unknown, possibly large
Uptake of 20:4 as chylomicron or very low density lipoprotein remnants	Unpublished*	Probably small
Uptake of 20:4 as low density lipoproteins	22	Probably small
Uptake of 20:4 of high density lipoproteins	Unpublished*	Unknown, probably small
Uptake of 2-20:4 lysophospholipids	23	Unknown, possibly large
Uptake of 20:4 as FFA	Unpublished*	Small
Retention of reabsorbed 20:4 from bile phospholipids	14, 15	Large in small intestine

*T. Melin, Q. Chen, and Å. Nilsson, unpublished data.

stage we thus believe that local interconversion may be a more important pathway than has generally been believed in view of the high capacity of the liver to desaturate-elongate 18-carbon PUFAs.

Platelets do not interconvert 18:2 to 20:4.[15] In other studies we found a very slow uptake of 20:4 by platelets after injection of chylomicrons labeled with ^{3}H-20:4 within 30 min (N. Xu and Å. Nilsson, unpublished data). The present data indicate that bone marrow and spleen, i.e., the blood forming tissues of young rats form considerable amounts of 20:4 from unesterified blood 18:2. This may mean that 20:4 formation is an integral part of the formation of cell membranes during cell proliferation in the bone marrow,[16] although at the present stage we cannot tell which cell types account for most of the interconversion reactions.

Large proportions of the ^{14}C-18:2 in the extrahepatic tissues was in PLs. Since the amount of PUFAs available is an important determinant of their partitioning between TGs and PLs, the higher proportion of ^{14}C in TGs in the liver may reflect the larger uptake of FFA by the liver than by the other tissues. In an earlier study, the incorporation of dietary ^{14}C-18:2 into PLs, particularly CL, in essential fatty acid–deficient rats was increased.[24] The several-fold higher incorporation of ^{14}C-18:2 in CL in the gastrointestinal tract, spleen, and bone marrow than in the liver suggests that the supply of essential fatty acids to these tissues is not in excess in relation to the need for phospholipid synthesis in growing rats on a normal pellet diet. One may speculate that a large part of this supply occurs by the uptake of 18:2 as FFA. If this is so, the PL and eicosanoid formation in the gastrointestinal tract and blood-forming tissues may be directly influenced by variation in the fatty acid composition of the FFA fraction caused by variation in the dietary level of essential fatty acids.

In summary, our studies show that substantial amounts of 20:4 may be formed by interconversion of 18:2 at the acyl CoA stage soon after the uptake by gastrointestinal and blood-forming tissues. It is likely that 18:2 appearing as acyl CoA derivatives during the continuous deacylation reacylation reactions in these tissues undergoes desaturation-elongation to 20:4 to a similar extent as 18:2 taken up as FFA. Considering this, and the fact that only a limited proportion of the 20:4 synthesized in the liver is likely to be transferred to these tissues, the best hypothesis at the present stage is that considerable parts of the 20:4 pools of both bone marrow and gastrointestinal tract are formed in situ. An accurate quantitation of the role of in situ 20:4 formation requires, however, that the difficult problem to measure the net transport of 20:4 from the liver to extrahepatic tissues via lipoproteins,[22] or lyso-PC[23] is solved. Furthermore, it would be of interest to test the hypothesis that the dietary influence on the composition of the plasma FFA fraction may mediate the increased mucosal resistance seen on linoleate-rich diets.[25–27]

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