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Distribution of omega-3 fatty acids in tissues of rabbits fed a flaxseed-supplemented diet

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Abstract

Diets rich in omega-3 polyunsaturated fatty acids are associated with decreased incidences of cardiovascular disease. The extent of incorporation and distribution of these beneficial fats into body tissues is uncertain. Rabbits were fed regular rabbit chow or a diet containing 10% ground flaxseed that is highly enriched with the omega-3 polyunsaturated fatty acid α-linolenic acid (ALA). The high-flaxseed diet resulted in an incorporation of ALA in all tissues, but mostly in the heart and liver with little in the brain. Docosahexaenoic and eicosapentaenoic acid levels were also selectively increased in some tissues, and the effects were not as large as ALA. Arachidonic acid and the ratio of ω-6/ω-3 fatty acids were decreased in all tissues obtained from the flax-supplemented group. Consumption of dietary flaxseed appears to be an effective means to increase ALA content in body tissues, but the degree will depend upon the tissues examined.

1. Introduction

Increasing evidence points to the efficacy of nutritional interventions in the treatment and prevention of disease. Although the intent of many studies is to focus on one specific target, investigators need to be aware of the overall tissue changes that arise from their intervention, which may have an important impact in all of these tissues or even have direct consequences for any functional effects observed. In particular, ω-3 polyunsaturated fatty acids (PUFAs) can have profound physiologic effects [1]. The ω-3 fatty acids are essential fatty acids that cannot be made by the human body and must, therefore, be ingested in the diet [2]. Once in the body, these fatty acids can be metabolized through a series of elongations and desaturations into longer-chain fatty acids in the same family. α-Linolenic acid (ALA) is the parent ω-3 PUFA; and it can be metabolized into the longer-chain PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are commonly found in fish oils. The majority of research on ω-3 PUFA has been conducted on fish oils, and relatively little is known about the biological effects of ALA. Flaxseed is one of the richest sources of ALA. α-Linolenic acid may be beneficial on its own, or the benefits may arise upon its metabolism to the longer-chain PUFAs. The ω-3 fatty acids compete directly with the ω-6 family of PUFAs for metabolic enzymes [3], so the ratio of the 2 families of PUFAs is important in determining the type of metabolites produced. Typically, the Western diet contains very high amounts of ω-6 PUFAs [4], limiting the metabolism of ω-3 fatty

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Table 1
Nutritional compositions of the control and flaxseed-containing diets (percentage of dry diet)

	Control	Flaxseed
Crude protein	21.3	20.5
Carbohydrates	51.4	51.7
Crude fat	5.4	8.1
Crude fiber	13.5	11.7
Ash	8.4	8.1
Digestible energy (cal/g)	3.38	3.54

Table 2
Fatty acid compositions of the control and flaxseed-containing diets (percentage of total fatty acids)

	Control	Flaxseed
14:0	0.8	0.6
16:0	18.3	14.2
18:0	6.2	5.9
16:1 ω-9	1.1	0.8
18:1 ω-9 OA	30.0	25.4
18:1 ω-7	4.8	4.1
18:2 ω-6 LA	31.4	17.5
18:3 ω-3 ALA	5.6	29.9

acids. Lowering the ratio of ω-6/ω-3 fatty acids can have beneficial health implications.

It is reasonable to hypothesize that the consumption of flaxseed in the diet will augment tissue ω-3 PUFA levels. However, the overall and relative distribution of ALA in the body after dietary flaxseed intake is not well known. It is not clear if there will be a differential accumulation of the ω-3 PUFA levels in different tissues of the body. This is important because the physiologic effects of ALA may be tissue specific. Alternatively, it is possible that the physiologic action of ALA may be limited because of poor incorporation into some tissues of the body. The purpose of this study, therefore, was to determine if ALA is preferentially incorporated into different tissues of the body in the rabbit after supplementation of the diet with flaxseed. The rabbit is a well-used model for the study of ophthalmic diseases [5,6], stroke [7], atherosclerosis [8,9], and other cardiovascular diseases [10,11].

2. Materials and methods

2.1. Diet and feeding

Male New Zealand white rabbits (2.8 ± 0.1 kg) were randomly assigned to receive a standard rabbit diet or a diet containing 10% ground high-ALA flaxseed (Promega Flax; Polar Foods, Fisher Branch, Manitoba, Canada). This flaxseed contains 71% ALA compared with 57% in regular flaxseed. Diets were prepared by grinding the required amount of standard ration and then mixing in the appropriate amount of ground flaxseed [12]. Only milled flaxseed was given to the animals in this study to avoid the varying ALA bioavailability when diets contain whole flaxseed, milled flaxseed, or flaxseed oil [13]. Once mixed, the diets were moistened, repelleted, and then fan-dried. All experimental diets were kept refrigerated and protected from light. Nutritional and fatty acid compositions of the diets are shown in Tables 1 and 2. Each rabbit was fed 125 g/d of diet for 8 weeks.

2.2. Tissue sampling and analysis

Blood was drawn from the left marginal ear vein of fasted rabbits at 8 weeks and collected into Vacutainer tubes (BD, Mississauga, ON, Canada) containing EDTA. Blood samples were centrifuged at 4500*g* for 10 minutes at 4°C, and plasma was stored at −80°C until analyzed.

Fatty acids were extracted from plasma and derivatized using the method of Lepage and Roy [14]. Briefly, 100 μL of plasma was added to 2 mL of methanol-benzene (4:1) in a test tube. While vortexing, 200 μL of acetyl chloride was added. The tubes were sealed and heated to 100°C for 1 hour. Five milliliters of 6% K_2CO_3 was then added to neutralize the solution, and the upper benzene layer was removed for analysis.

Brains, kidneys, livers, and muscle from heart, aorta, carotid arteries, and gastrocnemius were isolated from the rabbits, flushed with phosphate-buffered saline, and quick frozen in liquid nitrogen before storage at −80°C. Lipids were extracted from the tissues with chloroform-methanol and derivatized with boron trifluoride using the method of Folch et al [15]. A Varian (Palo Alto, CA) CP-3800 gas chromatograph equipped with a flame ionization detector and Varian CP-Sil 88 capillary column (60 m × 0.25 mm × 0.20 μm) were used to analyze 1.0 μL of each extract, which was injected with a CP-8400 autosampler at a split ratio of 1:100 [12]. Flow rate of the helium carrier gas was 1 mL/min. The initial oven temperature was held at 80°C for 1 minute, raised to 140°C at 30°C/min, and then raised to 225°C at 5°C/min and held for 10 minutes. The total run time for each sample was 30 minutes. Components were identified by comparison with authentic standards (NuCheck Prep, Elysian, MN).

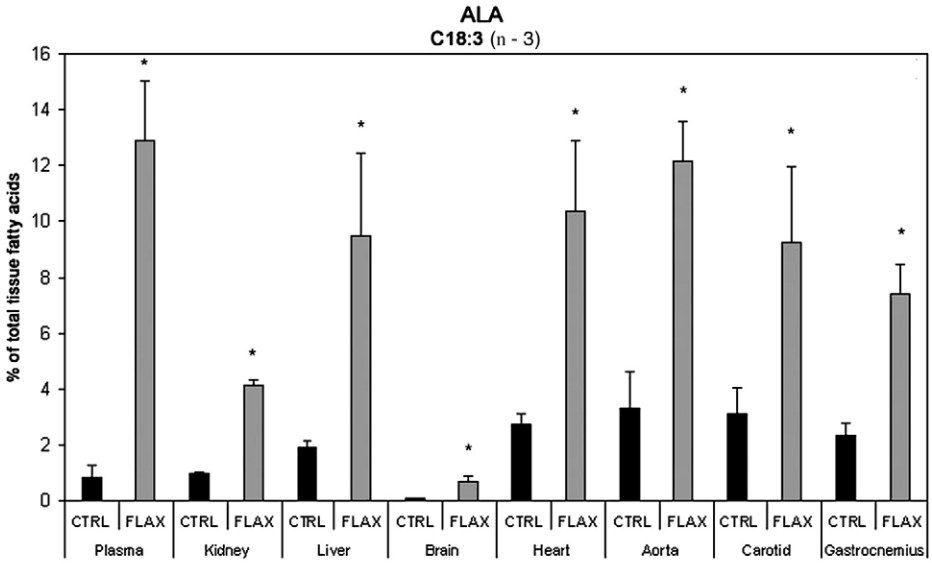
2.3. Statistical analysis

Comparison between the fatty acids in the tissues of the control or flaxseed fed rabbits was performed using a 1-way analysis of variance followed by a Student-Newman-Keuls post hoc test. $P < .05$ was considered significant. All results are expressed as mean ± SEM.

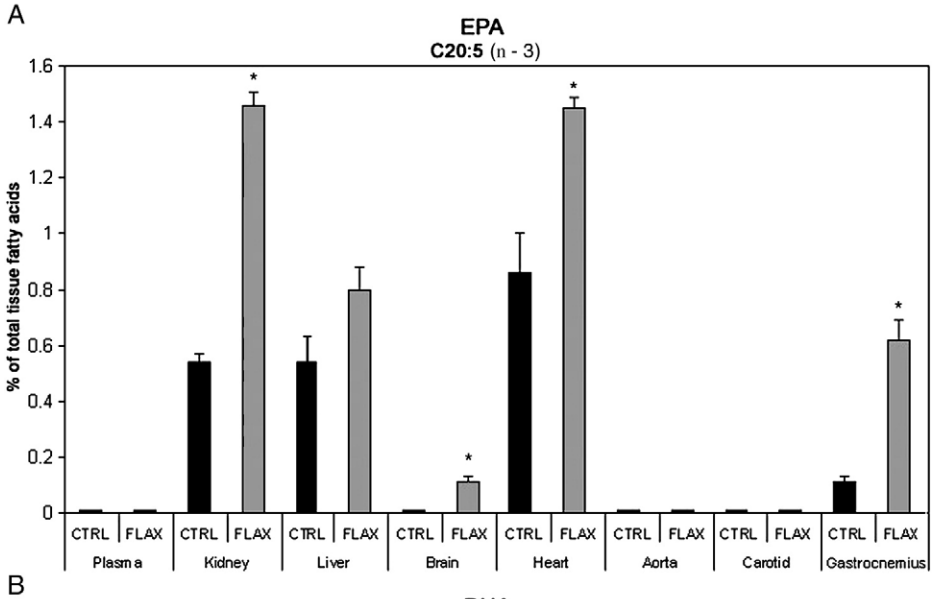
3. Results

3.1. Dietary supplementation of flaxseed increases ω-3 PUFA concentration in tissues

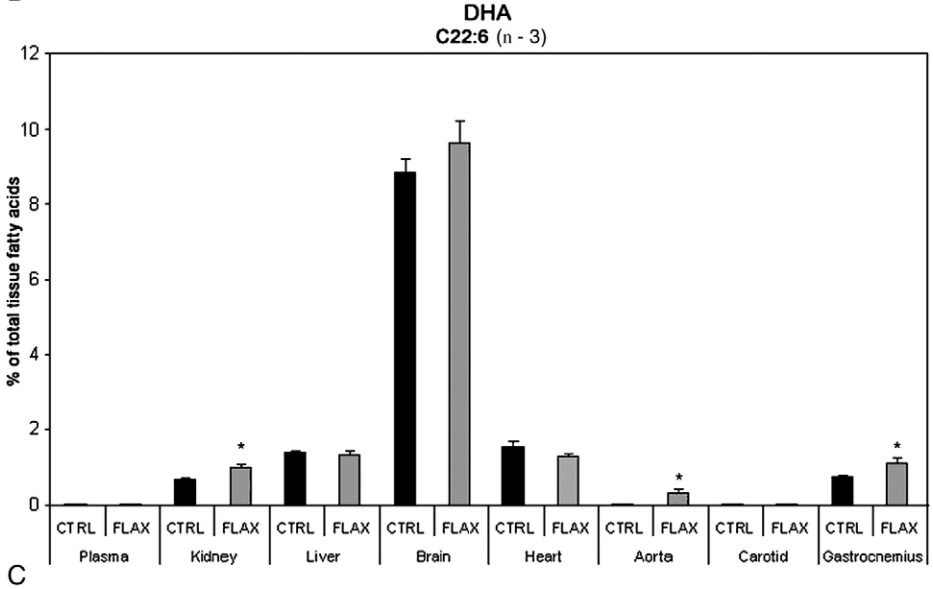
After 8 weeks of dietary supplementation with 10% ground flaxseed, ALA levels were significantly elevated in the plasma, kidney, liver, and brain, as well as in heart, aorta,


ALA
C18:3 (n - 3)
% of total tissue fatty acids
0
2
4
6
8
10
12
14
16
CTRL FLAX
Plasma
Kidney
Liver
Brain
Heart
Aorta
Carotid
Gastrocnemius

A


EPA
C20:5 (n - 3)
% of total tissue fatty acids
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
CTRL FLAX
Plasma
Kidney
Liver
Brain
Heart
Aorta
Carotid
Gastrocnemius

B


DHA
C22:6 (n - 3)
% of total tissue fatty acids
0
2
4
6
8
10
12
CTRL FLAX
Plasma
Kidney
Liver
Brain
Heart
Aorta
Carotid
Gastrocnemius

C

Table 3
Fatty acid content (percentage of total fatty acids) of plasma, kidney, liver, and brain tissues in rabbits after 8 weeks of feeding of a regular or 10% flaxseed diet

Fatty acid	Tissue							
	Plasma		Kidney		Liver		Brain	
	Control	Flaxseed	Control	Flaxseed	Control	Flaxseed	Control	Flaxseed
C14:0	1.14 ± 0.23	0.36 ± 0.13*	0.28 ± 0.04	0.28 ± 0.05	1.23 ± 0.11	1.4 ± 0.31	0.23 ± 0.01	0.27 ± 0.02
C14:1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.17 ± 0.11	0.17 ± 0.11	0 ± 0	0 ± 0
C16:0	23.59 ± 2.55	20.18 ± 2.21	16.29 ± 1.33	15.64 ± 1.22	19.56 ± 2.7	22.7 ± 7.85	19.63 ± 0.39	19.91 ± 0.41
C16:1	2.96 ± 0.47	1.71 ± 0.3	0.99 ± 0.15	0.83 ± 0.11	2.18 ± 0.44	2.09 ± 0.63	0.38 ± 0.02	0.51 ± 0.05
C18:0	23.46 ± 1.65	22.94 ± 1.62	14.9 ± 0.39	15.07 ± 0.41	17.27 ± 0.97	14.09 ± 1.77	22.26 ± 0.52	21.52 ± 0.57
C18:1 (n-9) Ol	20.48 ± 3.15	18.75 ± 2.11	16.31 ± 0.75	15.38 ± 0.69	18.68 ± 2.49	17.85 ± 5.29	23.67 ± 1.66	23.92 ± 1.47
C18:1 Vac	4.62 ± 3.88	1.99 ± 0	2.42 ± 0.19	2.16 ± 0.08	2.95 ± 0.39	2.58 ± 0.53	4.61 ± 0.19	4.41 ± 0.2
C18:2 (n-6) LA	19.84 ± 3.57	18.5 ± 2.46	26.12 ± 1.13	26.64 ± 0.97	24.87 ± 2.05	21.97 ± 4.15	1.66 ± 0.04	2.43 ± 0.22*
C20:0	0 ± 0	0 ± 0	0.37 ± 0.01	0.39 ± 0.02	0 ± 0	0.08 ± 0.06	0.43 ± 0.06	0.42 ± 0.05
C18:3 (n-6) GLA	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C20:1 (n-9)	0 ± 0	0 ± 0	0.32 ± 0.03	0.27 ± 0.01	0.57 ± 0.15	0 ± 0*	1.82 ± 0.27	1.8 ± 0.23
C20:2 (n-6)	0 ± 0	0 ± 0	0.46 ± 0.02	0.39 ± 0.03	1.05 ± 0.08	0.76 ± 0.03*	0.35 ± 0.04	0.41 ± 0.05
C22:0	0 ± 0	0 ± 0	0.7 ± 0.03	0.66 ± 0.04	0.93 ± 0.02	0.7 ± 0.01*	1.42 ± 0.08	1.1 ± 0.08*
C20:3 (n-6) 8-11-14	0 ± 0	0 ± 0	1.01 ± 0.03	0.66 ± 0.05*	0 ± 0	0 ± 0	0.57 ± 0.03	0.66 ± 0.05
C20:3 (n-3) 11-14-17	0 ± 0	0 ± 0	0.08 ± 0.02	0.48 ± 0.05*	0 ± 0	0 ± 0	0 ± 0	0.05 ± 0.02*
C20:4 (n-6)	3.09 ± 0.11	2.66 ± 0.71	16.59 ± 0.53	13.6 ± 0.58*	6.39 ± 0.27	3.93 ± 0.47*	11.43 ± 0.42	9.72 ± 0.43*
C24:0	0 ± 0	0 ± 0	0.4 ± 0.03	0.42 ± 0.04	0 ± 0	0 ± 0	1.02 ± 0.12	0.94 ± 0.15
C24:1 (n-9)	0 ± 0	0 ± 0	0.55 ± 0.06	0.51 ± 0.04	0.37 ± 0.19	0.1 ± 0.1	1.7 ± 0.28	1.5 ± 0.25
Total FA (mg/g)	0.63 ± 0.04	0.51 ± 0.02	22.00 ± 0.45	21.01 ± 0.39	41.56 ± 1.82	50.62 ± 5.54	25.40 ± 0.49	24.83 ± 0.43

n = 4.
* $P < .05$ compared with control group.

carotid artery, and gastrocnemius muscle (Fig. 1). This increase was accompanied by a rise in the tissue concentrations of the EPA in heart and gastrocnemius muscle, kidney, and brain tissue in the rabbits. In the kidney and aortic and gastrocnemius muscle, tissue concentrations of DHA also significantly rose under flaxseed supplementation. All *cis*-11,14,17-eicosatrienoic acid was nondetectable in most tissues, but made a marginal but significant increase in concentration in brain and kidney tissue (Table 3).

3.2. Effects of dietary supplementation of flaxseed on ω-6 PUFA accumulation in tissues

Arachidonic acid was greatly reduced after flaxseed supplementation in all tissues but carotid arterial tissue, gastrocnemius muscle, and plasma showed no statistically significant change. Linoleic acid (C18:2 n-6) concentration significantly increased in the brain, but showed no significant changes in other tissues. C20:2 (n-6) was significantly reduced in the liver, the only tissue in which it initially comprised more than 1% of the total fatty acid content, and did not make any significant changes in the other tissues. C20:3 (n-6) 8-11-14 decreased in kidney, did not change in brain tissue or gastrocnemius muscle, and was nondetectable in other tissues. γ-Linoleic acid (C18:3 n-6) was nondetectable in all tissues examined.

3.3. Effects of dietary supplementation of flaxseed on the bioaccumulation of other fatty acids

C14:0 was significantly reduced in the plasma of flaxseed-fed animals, but did not change after 8 weeks of feeding in any other tissues. There was no difference in C14:1, C16:0, and C16:1 concentration in any samples.

Flaxseed did not induce a change in the accumulation of C18:0 in any tissues investigated. There was also no difference in oleic acid (C18:1 n-9) or vaccenic acid (C18:1 n-6) concentrations in any tissues. Low-level baseline C20:0 concentrations were increased in both heart and carotid muscle, but remained at near-undetectable amounts in most tissues.

C22:0 concentrations were lowered by flaxseed supplementation in liver and brain tissue, whereas they were nondetectable in plasma, gastrocnemius, and carotid muscle and experienced no change in the other tissues observed. C24:0 and C24:1 (n-9) remained in nondetectable amounts in most tissues and showed no change in the rest of the samples after flaxseed supplementation.

3.4. Total FA concentrations changed only in the heart and plasma

Total fatty acids decreased significantly in the carotid artery and gastrocnemius muscle, but was unchanged in

Fig. 1. α-Linolenic acid content of various rabbit tissues (A), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (C) content and in muscle (B) after 8 weeks of dietary supplementation with flaxseed. Values shown are mean ± standard error of the mean (n = 4-5). Values represent percentage of total fatty acids in each tissue. Absolute values (in milligrams fatty acid per gram wet tissue weight) for each tissue are reported in Tables 3 and 4. *$P < .05$ compared with control group of respective tissue.

Table 4
Fatty acid content (percentage of total fatty acids) of rabbit muscle tissues after 8 weeks of feeding of a regular or 10% flaxseed diet

Fatty acid	Muscle							
	Heart		Aorta		Carotid		Gastrocnemius	
	Control	Flaxseed	Control	Flaxseed	Control	Flaxseed	Control	Flaxseed
C14:0	1.61 ± 0.15	2.37 ± 0.52	1.54 ± 0.61	1.69 ± 0.32	2.26 ± 0.42	1.55 ± 0.5	1.55 ± 0.29	1.33 ± 0.1
C14:1	0.18 ± 0.07	0.33 ± 0.12	0 ± 0	0 ± 0	0.06 ± 0.04	0 ± 0	0.32 ± 0.05	0.26 ± 0.01
C16:0	17.75 ± 1.97	21.47 ± 5.14	26.32 ± 7.48	24.63 ± 3.84	30.54 ± 5.63	24.23 ± 5.75	26.46 ± 3.53	24.79 ± 1.63
C16:1	2.37 ± 0.25	3.11 ± 0.71	2.86 ± 1.16	2.5 ± 0.57	4.61 ± 1.02	2.59 ± 0.64	3.66 ± 0.64	2.53 ± 0.38
C18:0	10.96 ± 0.49	7.75 ± 0.66*	12.28 ± 1.95	11.11 ± 0.77	11.44 ± 1.16	13.81 ± 3.05	8.81 ± 0.92	9.05 ± 0.57
C18:1 (n-9) Ol	18.48 ± 2.17	21.24 ± 4.73	26.68 ± 8.2	24.4 ± 2.48	25.76 ± 5.68	23.95 ± 6.37	24.04 ± 3.56	21.84 ± 1.61
C18:1 Vac	3.65 ± 0.22	3.23 ± 0.46	1.91 ± 0.49	1.62 ± 0.14	2.05 ± 0.39	2.51 ± 0.14	2.73 ± 0.38	2.25 ± 0.17
C18:2 (n-6) LA	26.01 ± 1.37	19.85 ± 2.45	14.82 ± 4.52	13.76 ± 1.02	13.45 ± 3.35	13.4 ± 3.71	18.77 ± 2.44	19.23 ± 1.61
C20:0	0.24 ± 0.06	0.47 ± 0.07*	0 ± 0	0.07 ± 0.05	0.1 ± 0.07	0.91 ± 0.19*	0.01 ± 0.01	0.03 ± 0.03
C18:3 (n-6) GLA	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.01 ± 0.01
C20:1 (n-9)	0 ± 0	0 ± 0	0.23 ± 0.15	0.13 ± 0.14	0.16 ± 0.1	0 ± 0	0.18 ± 0.06	0.17 ± 0.05
C20:2 (n-6)	0.57 ± 0.1	0.76 ± 0.04	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.17 ± 0.04	0.12 ± 0.04
C22:0	0.58 ± 0.12	0.84 ± 0.03	0.4 ± 0.15	0.46 ± 0.09	0 ± 0	0 ± 0	0 ± 0	0.03 ± 0.03
C20:3 (n-6) 8-11-14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.05 ± 0.03	0 ± 0	0.57 ± 0.03	0.53 ± 0.07
C20:3 (n-3) 11-14-17	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.01 ± 0.01	0.14 ± 0.08
C20:4 (n-6)	12.5 ± 0.12	5.49 ± 0.23*	9.66 ± 0.51	7.15 ± 0.28*	6.35 ± 0.66	7.81 ± 1.48	9.51 ± 0.78	8.54 ± 0.41
C24:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C24:1 (n-9)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Total FA (mg/g)	28.14 ± 0.94	46.80 ± 3.70*	9.32 ± 1.15	10.46 ± 0.52	7.60 ± 0.68	4.03 ± 0.42*	7.20 ± 0.42	6.44 ± 0.20*

n = 4.
* $P < .05$ compared with control group.

plasma, aorta, kidney, liver, and brain tissue (Tables 3 and 4). Dietary flaxseed induced a significant increase in total fatty acids in the heart.

3.5. ALA accumulates primarily in liver and cardiac tissue

α-Linolenic acid, the principle fatty acid in flaxseed, accumulated to the greatest extent in heart and liver tissue, each amassing 39% of the total increase in ALA measured (Fig. 2). The next highest ALA increase was detected in the aorta, followed by the kidney, then gastrocnemius, brain, and carotid arteries; and the lowest proportion was in the plasma.

3.6. ω-6/ω-3 PUFA ratio improves in all tissue

In all tissues examined, the ratio of ω-6/ω-3 PUFA was significantly decreased through dietary supplementation with flaxseed (Fig. 3). The ratios were significantly lowered (63%-77%) in all the tissues examined except the brain that was decreased by a much smaller amount (20%), but this remained a statistically significant decrease in the ratio compared with control ($P < .05$).

4. Discussion

This study represents the first detailed analysis of the distribution of fatty acids in 8 different tissue sites within the body, with a particular focus on ALA, in response to a diet enriched in milled flaxseed. Past information on this topic has been limited because of a smaller number of tissues studied (usually 2-4) [16-20] and the use of oils like canola, mixed vegetable, or flaxseed [16-20] instead of milled flaxseed. Previous data have also been limited by the use of special lipid fractions (ie, plasma phospholipids, triglycerides, cholesteryl fractions, etc) [17] instead of a complete lipid extraction profile as was done in the present investigation and by the study of animal populations in unstable physiologic conditions (ie, growing neonatal animals) [20] instead of healthy adult populations. Of course, the present data are limited by the same 3 considerations: it is applicable only to the diet administered here; it applies only to healthy, adult rabbits; and it provides information about total lipid fractions and not specialized species of lipids. However, the best way to fulfill our primary objective (to provide an overall identification of the storage sites for ALA and other fatty acids in response to the flaxseed-supplemented diet) was through the use of total lipid extractions from as many of the major organs as possible, including arterial tissues that may be involved in pathologic conditions like atherosclerosis.

Our results demonstrate for the first time that dietary supplementation with milled flaxseed can significantly enrich ALA levels in all 8 of the tissues examined. This enrichment was not uniform. Most of the fatty acids present in the flaxseed-containing diet were deposited in the heart and liver. The relatively large size of the observed changes in the liver is not surprising given that the liver is the primary site of fatty acid metabolism [21]. It was somewhat unexpected, however, to detect the substantial changes in the heart relative to other muscles that were examined.

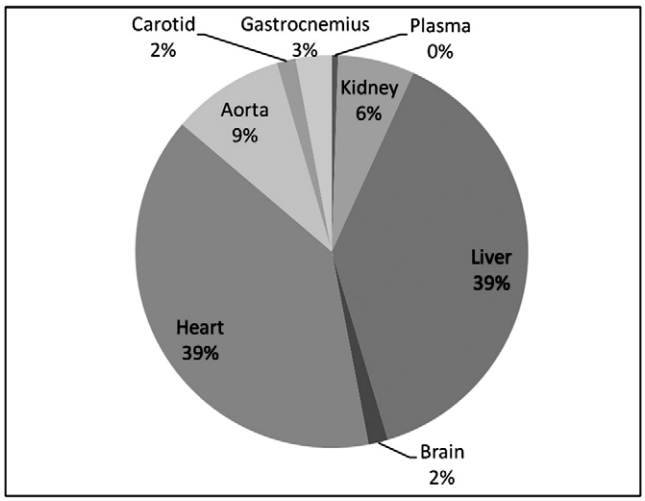


Fig. 2. Relative distribution of ALA in rabbit tissues after consumption of a diet rich in flaxseed for 8 weeks. Values shown represent the percentage of the combined total ALA augmented in the selected tissues compared with that in the tissues of animals fed a control diet.

Again, this may be due to its unusually heavy reliance on fatty acid metabolism as an energy source. It is important to note, however, that the accumulation of ALA by the heart is comparable to other tissues when expressed relative to total fatty acid content (Fig. 1). Others [20] have observed that about 85% of a PUFA-blended oil that was ingested "disappeared" presumably through β-oxidation. If the contracting cardiac muscle is a constant source of this oxidative metabolic activity, it would appear natural that a major storage site would be the heart. In view of the documented need for PUFAs in brain function, it was surprising that brain tissue did not react with large increases in ALA or PUFA levels in response to the flaxseed-supplemented diet. It is possible that transport of the fatty acid into the brain or that storage within the tissue is limited in some undefined way. Alternatively, it is also possible that this tissue uses the fatty acid so efficiently that it does not accumulate with the dosages of flaxseed that were used.

It was also possible for us to determine if there were any tissue-dependent differences in the capacity to convert ALA into longer-chain PUFAs like EPA and DHA as a function of flaxseed consumption. Eicosapentaenoic acid and DHA were undetectable in the plasma from regular diet or flaxseed-supplemented rabbits. However, a significant increase in the levels of these metabolites was found in other tissues, suggesting that the long-chain ω-3 PUFAs were being metabolized in the periphery, although levels remained relatively low. No striking patterns could be detected, but the kidney and the gastrocnemius muscle were the only 2 tissues that showed a consistent elevation in both DHA and EPA after dietary supplementation with flaxseed. The lack of an increase in DHA in the heart after flaxseed feeding may be due to a deficiency of elongase-2 expression, as has previously been reported in rat heart [22]. The extent of ALA metabolism to the longer-chain PUFAs in humans is controversial. Reports indicate ranges of metabolism from as little as 0.2% to as high as 21% [22-25]. Our results here in rabbits would be consistent with a limited capacity to metabolize ALA into longer-chain PUFAs.

Consumption of flaxseed also significantly altered the non–omega-3 fatty acid composition of the same body tissues. All tissues showed a significant reduction in the ω-6/ω-3 ratio after consumption with a flaxseed-rich diet, even the brain tissue, which had the lowest ratio of all of the

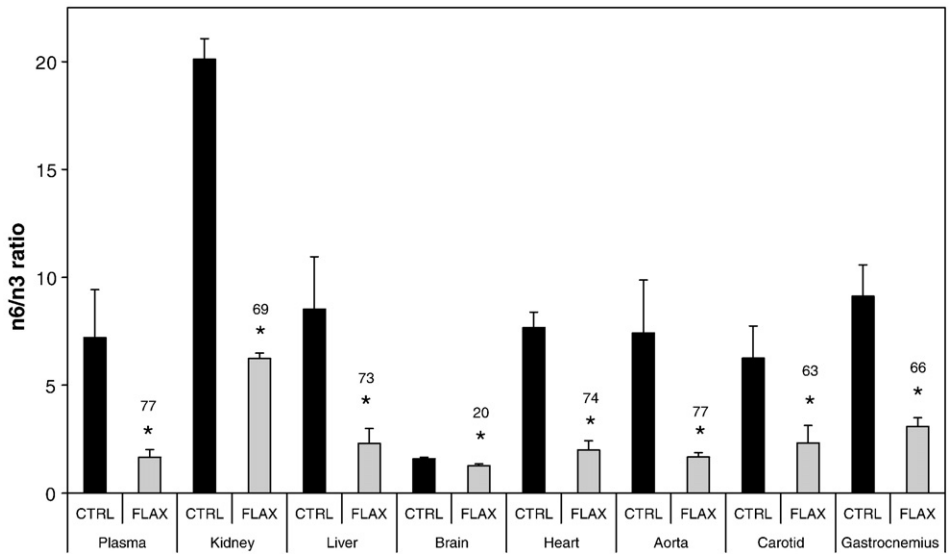


Fig. 3. Ratio of ω-6/ω-3 fatty acids in tissues of rabbits after 8 weeks of feeding with a control diet (black) or a diet containing 10% ground flaxseed (gray). Values shown are mean ± standard error of the mean (n = 4-5). Text values on figure represent percentage decrease in ratio compared with control group of respective tissue. *P < .05 compared with control group of respective tissue.

tissues examined. The smallest change in the ratio of ω-6/ω-3 PUFA was observed in the brain. This was likely because the brain already exhibited a relatively large amount of DHA, which is known to be important for normal brain function, including learning, vision, and mental health [26-30]. The overall reduction in the ω-6/ω-3 ratio was not just due to an increase in the ω-3 content. In nearly all of the tissues, the reduction in the ω-6/ω-3 ratio was due to both an increase in the ω-3 PUFA content and a decrease in the content of the ω-6 PUFAs linoleic acid and particularly arachidonic acid. These observations are consistent with results in both guinea pig and hamster models, and suggest that the increased presence of ω-3 PUFA favors a balanced use of the Δ-6 desaturase for metabolism of the ω-6 and ω-3 PUFA [31,32]. Our findings highlight the relative importance of a lower ω-6/ω-3 ratio to the endogenous production of long chain ω-3 PUFA.

The increase in ALA content in the tissues of the body may have important physiologic and pathophysiologic effects. For example, endogenous stores of ALA in the heart have been shown to provide antiarrhythmic action during ischemic/reperfusion challenge [12]. Hepatic fatty acid composition may influence steatosis development in patients with chronic hepatitis C [33]. Hepatic ALA levels are higher in patients with steatosis [33]. Higher ALA concentrations have been shown to induce apoptosis in vascular smooth muscle cells in culture [34]. This has been suggested to play a role in plaque destabilization and the progression of atherosclerosis [34]. Conversely, ALA deficiencies in the diet have been proposed to render the brain more vulnerable to neuropathologic insults [35]. Clearly then, changes in ALA content within any of the tissues examined in this study could not only have important functional implications but alter cell/tissue viability as well.

In conclusion, dietary flaxseed produced a significant increase in ALA with a preferential distribution to the heart and liver. Flaxseed is an effective dietary supplement for lowering the ω-6/ω-3 ratio in numerous pathologically relevant tissues in the rabbit. We also observed an increase in tissue levels of its downstream ω-3 products, although the degree of ALA metabolism to longer-chain PUFAs was limited and tissue specific. Flaxseed may be a useful dietary supplement for any condition in which an elevation of ω-3 PUFA would be expected to produce positive health effects.

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