Effect of dietary docosahexaenoic acid on biosynthesis of docosahexaenoic acid from alpha-linolenic acid in young rats[S]

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Abstract Docosahexaenoic acid (DHA), a crucial nervous system n-3 PUFA, may be obtained in the diet or synthesized in vivo from dietary α-linolenic acid (LNA). We addressed whether DHA synthesis is regulated by the availability of dietary DHA in artificially reared rat pups, during p8 to p28 development. Over 20 days, one group of rat pups was continuously fed deuterium-labeled LNA (d5-LNA) and no other n-3 PUFA (d5-LNA diet), and a second group of rat pups was fed a d5-LNA diet with unlabeled DHA (d5-LNA + DHA diet). The rat pups were then euthanized, and the total amount of deuterium-labeled docosahexaenoic acid (d5-DHA) (synthesized DHA) as well as other n-3 fatty acids present in various body tissues, was quantified. In the d5-LNA + DHA group, the presence of dietary DHA led to a marked decrease (3- to 5-fold) in the total amount of d5-DHA that accumulated in all tissues that we examined, except in adipose. Overall, DHA accretion from d5-DHA was generally diminished by availability of dietary preformed DHA, inasmuch as this was found to be the predominant source of tissue DHA. When preformed DHA was unavailable, d5-DHA and unlabeled DHA were preferentially accreted in some tissues along with a net loss of unlabeled DHA from other organs.—DeMar, Jr., J. C., C. DiMartino, A. W. Baca, W. Lefkowitz, and N. Salem, Jr. **Effect of dietary docosahexaenoic acid on biosynthesis of docosahexaenoic acid from alpha-linolenic acid in young rats. *J. Lipid Res.* 2008.** 49: **1963–1980.**

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Docosahexaenoic acid (DHA; 22:6n-3) is an n-3 PUFA that is particularly enriched in the phospholipids of cells constituting the mammalian nervous system (1, 2). Functionally, DHA enhances membrane elasticity and molecular motion and thus promotes signal transduction via enhanced protein/receptor interactions (3–6). DHA is also the activating ligand for multiple transcriptional factors that control the expression of enzymes involved in fatty acid synthesis and β-oxidation (7). DHA may be directly obtained in the diet, as preformed DHA, or synthesized in vivo from other common dietary n-3 PUFAs such as α-linolenic acid (LNA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), or docosapentaenoic acid (DPA, 22:5n-3) (8). All of these n-3 PUFAs may be converted in vivo to DHA through sequential steps of elongation, desaturation, and peroxisomal β-oxidation (9). Prolonged dietary deprivation of all n-3 PUFAs in rat pups, initiated prior to weaning, depletes up to 80% of their brain DHA (10–12). Such depletion of brain DHA in rodents leads to distinct impairments in brain function (11, 13–17). Piglets and monkeys also show impaired neural function when deprived of n-3 PUFA for an extended period during infancy (18, 19). The essentiality of DHA for human infant nutrition in support of neuronal function has been shown by DHA supplementation, enhancing visual acuity and cognition-related test scores in human infants (20–28). In adult humans, low DHA blood levels have been correlated with psychological disturbances such as alcoholism, major depression (nonpsychotic), postpartum depression, and senile dementia (29–34).

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Abbreviations: AA, arachidonic acid (20:4n-6); BHT, butylated hydroxytoluene; DHA, docosahexaenoic acid (22:6n-3); d5-DHA, deuterium-labeled docosahexaenoic acid; DPA, docosapentaenoic acid (22:5n-3); d5-DPA, deuterium-labeled docosapentaenoic acid; EPA, eicosapentaenoic acid (20:5n-3); d5-EPA, deuterium-labeled eicosapentaenoic acid; FAME, fatty acid methyl ester; LA, linoleic acid (18:2n-6); LNA, α-linolenic acid (18:3n-3); d5-LNA, deuterium-labeled α-linolenic acid; RBC, red blood cell.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of nine tables.

Although dietary sources of LNA are often more readily available than that of DHA, feeding animals high levels of LNA does not produce identical tissue levels of DHA compared with animals that are provided lower levels of dietary DHA (35–37). Whereas dietary LNA can be converted to DHA in vivo, the efficiency of conversion is limited (38). This has recently been investigated through kinetic intravenous infusion experiments in young rats using radiolabeled LNA to obtain the in vivo conversion rates in brain and liver (39–42). It was shown that the mature rat brain has little if any capacity to synthesize its own DHA, even when stimulated to do so under dietary conditions of feeding LNA alone or during n-3 PUFA dietary deprivation (39, 41). In contrast, the adult rat liver does convert LNA to DHA, but at a rate sufficient to convert <2% of the total LNA that enters the liver per unit time, with the majority (>70%) of the entering LNA being lost to β-oxidation; and similar results were obtained in both the presence and the absence of dietary DHA (40, 42). In these studies, adult rat liver production of DHA is elevated 3-fold in the absence of dietary DHA and the rate of liver-to-plasma secretion of synthesized DHA exceeds the replacement rate for brain DHA losses by 10-fold, suggesting that LNA metabolism is able to approach the DHA demands of the adult brain (42, 43).

Although the above intravenous infusion studies provide kinetically accurate rates for DHA biosynthesis/accretion in adult rat tissues at a fixed time period and physiological state, they do not take into account the large changes over time in DHA degradative losses, biosynthesis, and the resulting net accretion found in growing neonatal animals (43–47). This can only be truly examined using experiments that can follow the total accumulation of DHA over a prolonged period. We recently reported a study in which rat pups were continuously fed all of their dietary LNA as deuterium-labeled LNA (d5-LNA), over a 20 day postgestational time period (days p8–p28), to quantify the accumulation of newly biosynthesized deuterium-labeled docosahexaenoic acid (d5-DHA) in the developing brain and liver (10). These rat pups were provided a diet containing only d5-LNA (d5-LNA diet) or a diet containing d5-LNA plus unlabeled DHA at twice the level of the d5-LNA (d5-LNA + DHA diet), in order to test whether dietary preformed DHA decreases the net accretion of biosynthesized d5-DHA. In the rat pups receiving a d5-LNA + DHA versus d5-LNA diet, we found that brain and liver accretion of biosynthesized d5-DHA over 20 days were both decreased by 4-fold. This is consistent with findings by others that an absence of dietary DHA significantly upregulates the liver biosynthesis of DHA from LNA (35, 42, 48).

The present study is an extension of our previous analysis (10) of LNA metabolism in growing rat pups and its regulation by dietary DHA to a consideration of all the tissues in the body. Our hypothesis was that, as we had previously observed for brain and liver, preformed dietary DHA would decrease the amount of biosynthesized d5-DHA accretion in the major organs of the rat pups during development. We also hypothesized that the total body accumulation of preformed dietary DHA would exceed that of biosynthesized d5-DHA in the d5-LNA + DHA versus d5-LNA diet group, and we would demonstrate that dietary LNA is not nutritionally equivalent to dietary preformed DHA in developing animals. Overall, the underlying goal of this study is to generate an organ-based representation of whole-body n-3 PUFA metabolism to determine how it is regulated by input of dietary DHA. An abstract of this work has been presented (49).

MATERIALS AND METHODS

Materials

$^{2}H_{5}$-17, 17, 18, 18, 18-18:3n-3 ethyl ester (d5-LNA) was purchased from Cambridge Isotope Labs (Andover, MA), and was previously shown by GC-MS to have a purity of >98% (10). 13, 16, 19-Docosatrienoic acid (22:3n-3), for use as an internal standard during fatty acid composition analyses by GC, was purchased from NuChek Prep (Elysian, MN). A standard mixture of fatty acid methyl esters (FAMEs) used for peak identifications during GC analyses was purchased from NuChek Prep (GLC reference mixture 462). PBS (pH 7.4) was from Invitrogen Corp. (Gibco™ brand; Grand Island, NY). Butylated hydroxytoluene (BHT; 2,6-di-tertbutyl-4-methylphenol) was purchased from Acros Organics, Inc. (Morris Plains, NJ). All solvents were high-purity HPLC/GC grade and were purchased from Burdick and Jackson (B and J brand®; VWR International, West Chester, PA) and EMD Chemicals (Gibbstown, NJ).

Animals

All procedures for rearing animals, formulation of their respective experimental diets, and associated feeding regimes have been previously described in detail by Lefkowitz et al. (10). All animal experiments were performed under protocols approved by the Animal Care and Use Committee of the National Institute of Alcohol Abuse and Alcoholism, and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication 80-23). Animals were maintained in a conventional animal facility with controlled temperature, humidity, and illumination.

In brief, Long Evans male rat pups were delivered and reared until 8 days of age (p8) by dams fed an AIN-93 diet modified to contain 3.1% of fatty acids as unlabeled LNA as the only source of n-3 fatty acids. Dams were provided the food and water on an ad libitum basis. Starting at p8 and continuing to p28, the rat pups were removed from the dams and placed on one of two artificial milk formulations. The first artificial milk formulation contained 1.0 ± 0.03% (n = 14) of its fatty acids as deuterium-labeled LNA (d5-LNA diet). Likewise, the second artificial milk formulation contained 1.1 ± 0.02% (n = 25) d5-LNA plus the addition of 2.0% of its fatty acids as unlabeled DHA (d5-LNA + DHA diet). The d5-LNA content of both diets, however, was not statistically different. Both diets contained essentially no unlabeled LNA (<0.01% of total fatty acids), as well as no other n-3 PUFA intermediates. In both diets, n-6 PUFA was entirely composed of unlabeled linoleic acid (LA; 18:2n-6) at 10.3–10.4% of total fatty acids, to give an n-6/n-3 PUFA ratio of 10.3:1 and 3.3:1 in the d5-LNA and d5-LNA + DHA diets, respectively.

The rat pups were hand fed their respective d5-LNA- and d5-LNA + DHA-containing milk diets every 3–4 h for a total of seven feedings each day. Feeding volumes ranged from 0.07 ml/g body weight at p15 to 0.2 ml/g body weight by p28. In this manner, from p8 to p28, the rat pups in both dietary groups were provided a continuous oral input of dietary LNA that was solely in the form

of d5-LNA. During this 21 day period of growth, any DHA that was derived from biosynthesis using the dietary d5-LNA as substrate and accreted in the rat pup tissues would also be labeled with five deuterium atoms, maintained at their original site in the fatty acyl chain, but now as incorporated into ^{2}H-21, 21, 22, 22, 22-22:6n-3 (d5-DHA). Rat pups receiving the d5-LNA + DHA diet, unlike those on the d5-LNA diet, would accumulate new unlabeled DHA in their tissues, as derived from the preformed dietary DHA they were ingesting.

At p28, rat pups taken from the d5-LNA and d5-LNA + DHA diet groups (n = 7 and n = 6, respectively) were fasted overnight, weighed, and euthanized by decapitation. Animals were fully eviscerated and skinned, with the whole carcass, skin, and subdivided internal organs wrapped separately in aluminum foil and immediately frozen on dry ice. During decapitation, a sample of blood was collected from the severed neck and centrifuged at 3,000 rpm for 10 min to collect plasma. In the same fashion, fully dam-reared reference groups of p8 and p28 rat pups (n = 11 and n = 4, respectively), with p8 representative of a starting baseline group, were euthanized, and whole tissues and plasma were collected for analysis of percent body composition as described below. All tissues were weighed and stored frozen at −80°C until utilized for analysis.

Sampling of various animal tissues

Individual organs taken for fatty acid compositional analysis were the brain, heart, kidneys, liver, lungs, retinas, spleen, and testes. The digestive tract was left intact and consisted of the esophagus, stomach, pancreas, and small and large intestines. Before use, the heart and kidneys were thawed on ice and cut laterally in half, and internal cavities were washed clean of blood and urine, respectively, using excess ice-cold PBS; they were then reweighed. All other organs, except the digestive tract, were thawed, and their external surfaces were picked clean of extraneous tissues then washed with PBS. The skin was thawed, the outside surface was shaved of hair with an electric razor, and the underside was cleaned of excess subcutaneous white adipose. Sections of skin, 1 cm^{2} in size, located at the mid-back region of the cleaned pelt, were cut out for analysis.

The carcass was partially thawed, and samples of skeletal muscle, bone, and brown, white, and visceral adipose were taken. General skeletal muscle was sectioned from the right thigh region and included tissue from the gluteus maximus, vastus lateralis, and caput vertebralis muscle bundles. After collection of the thigh muscle, the right hip was further dissected to remove the intact femur bone (including posterior cartilage end cap), which was cleaned of adhering muscle, tendons, and fascia, then washed with PBS.

To obtain muscle fibers representing fast-oxidative (type IIa-fast twitch), fast-glycolytic (type IIb-fast twitch), and slow-oxidative (type I-slow twitch) types, the red gastrocnemius, white gastrocnemius, and soleus muscles were dissected from the lower shank of the right-side rear leg, as described by Stark, Lim, and Salem (50). Whereas the soleus is found as a single muscle bundle, red and white gastrocnemius are actually zones of tissue located within the margin and center portions, respectively, of the medial and lateral heads of the gastrocnemius. Strips of red and white gastrocnemius were carefully cut from the middle of these regions to avoid any junctions of their intermixing. Samples of general thigh muscle and the three muscle fiber subtypes from the lower leg were cleaned of extraneous fascia and adipose and then rinsed with PBS before use.

Brown adipose tissue was taken from the shoulder region of the carcass, inside the pocket formed between the scapula bones and attached surpraspinatus muscles. Significant deposits of brown adipose were also sampled from the body wall underneath the supraspinatus, infraspinatus, teres major, and serratus ventralis shoulder muscles. White adipose was gathered from large patches found to be loosely attached to the outer surface of the mid-back region and combined with fat that was attached to the underside of the opposing skin. Visceral fat was collected from deposits of whitish adipose lining the back wall of the abdominal cavity adjacent to both sides of the lumbar spinal column. At the end of the tissue collections, the remaining carcass, consisting of leftover muscle, bones, and adipose, was also saved for fatty acid composition analysis.

Gross dissection of animals for percent body composition analysis

Dam-reared reference animals 8 days and 28 days old (n = 4, each) were subjected to gross dissection to determine the total weights of hair, skin, skeletal muscle, bones, internal organs, and brown, white, and visceral adipose. The weights for each tissue were then converted to a percentage of the original body weight of the animal at time of euthanization (see supplementary Table I). All major internal organs were removed (brain, digestive tract, heart, kidneys, liver, lungs, retinas, spleen, and testes) and lumped together for weighing. Total hair, skin, and brown and visceral adipose present within the animals were collected for weighing as described above. Significant hair was not yet present on the 8 day-old animals, and thus their hair was not removed and measured. Total skeletal muscle was stripped from all bones then weighed. In the process of collecting total skeletal muscle, all substantial deposits of white adipose were dissected out and combined for weighing, along with loose white adipose removed from the underside of the skin. All bones, including tail and spinal vertebra, were carefully cleaned of adhering muscle, tendons, and fascia then polished with a soft tissue paper and weighed. Summed weights of all of the above tissues were subtracted from the original body weight of the animals and the remainder taken to represent blood and other body fluids such as urine. This arbitrary value for whole-body fluid content, however, was not applied to estimating the whole-body volumes for plasma and red blood cells (RBCs), as found in supplementary Table II.

Homogenization of tissues

Samples of all tissues collected for fatty acid composition analysis from the 8 day-old dam-reared reference, d5-LNA diet, and d5-LNA + DHA diet groups were first homogenized, using a motor-driven homogenizer equipped with a 5 or 10 mm sheer generator probe (Model TH115; Omni International, Marietta, GA), into 3 ml of ice-cold methanol containing 0.05 mg/ml BHT. Femur bone was first crushed, before homogenization into methanol-BHT, using a hammer-driven cryopluverization device (BioPulverizer, Model 59013N; Biospec Products Inc., Bartlesville, OK). Typically, a piece of each tissue ≤1.0 g in size was utilized per homogenate, except for the remaining carcass and intact digestive tract, as to be described below. Plasma was not subjected to homogenization; a 50–100 μl aliquot was added directly to the methanol-BHT for analysis. Prior to homogenization, docosatrienoic acid (22:3n-3), as the free fatty acid, in chloroform solution (<100 μl), was added to the samples as an internal standard for fatty acid composition analysis, such that the 22:3n-3 would constitute 5–10% of the total fatty acid concentration.

To homogenize the carcasses and digestive tracts, they were frozen on dry ice, fragmented with surgical bone clippers, and crushed using the previously mentioned cryopluverization device. The crushed carcasses and digestive tracts were then homogenized in 10–100 ml of ice-cold methanol-BHT using a motor-driven homogenizer equipped with a 19 mm sheer generator

probe (Ultra-Turrax, Model T18; VWR International). For both the carcass and digestive tract homogenates, an even dispersion of very fine particles in methanol was achieved. The tissue densities of the digestive tract and carcass homogenates were 0.24–0.39 g/ml and 0.07–0.14 g/ml, respectively. Out of each homogenate, duplicate aliquots for analysis were taken immediately after vortexing. For the carcass homogenates, this represented 2–8% of the total volume, whereas 5–25% of each digestive tract homogenate was sampled. This gave a final tissue sample size of 0.2–0.8 g per analysis. If required, homogenate samples were brought to 3 ml with methanol-BHT, then the appropriate amount of 22:3n-3 internal standard was added to all homogenates.

Fatty acid compositional analysis

Total lipids were extracted from tissue homogenates in 3 ml methanol-BHT (as described above), by the method of Folch, Lees, and Sloane-Stanley (51), using appropriate portions of chloroform and an aqueous solution of 0.5 M KCl. All extraction procedures were done under nitrogen. At the end of the extraction procedure, the crude total lipid extracts in chloroform were washed using a theoretical aqueous phase of methanol-BHT and 0.5 M KCl (1:1; v/v) as described by Folch, Lees, and Sloane-Stanley (51), dried under nitrogen, reconstituted in 1–3 ml of chloroform, and stored at −80°C. FAMEs were prepared from a portion of each total lipid extract using 14% wt/vol BF_3-methanol at 100°C for 60 min (52), followed by extraction into hexane using the method of Salem et al. (38) as modified by Lefkowitz et al. (10). The extracts were subjected to fast-GC analysis as previously described by Masood, Stark, and Salem (53). Fast-GC analyses were carried out on an Agilent 6980 gas chromatograph in the split mode (200:1) and equipped with a DB-FFAP (J and W Scientific; LaPalma, CA) capillary column (15 m × 0.1 mm ID × 0.1 μm film thickness), a flame ionization detector, and hydrogen as the carrier gas at a linear velocity of 56 cm/s and constant head pressure of 344.7 kPa. The injector and detector temperatures were set to 250°C, and the temperature program was as follows: initial, 150°C with a 0.25 min hold; 150–200°C at 35°C/min; 200–225°C at 8°C/min with a 3.2 min hold; then 225–245°C at 80°C/min with a 2.75 min hold. Peaks were identified by comparison with a standard mixture (#462; NuChek Prep) and against chromatograms for tissues from the 8 day-old dam-reared reference group. Using the fast-GC method, complete identification and separation was achieved for the unlabeled (endogenous) and deuterium-labeled n-3 PUFA species of LNA, EPA, DPA, and DHA, as previously described for conventional capillary GC (10). Fatty acid concentrations in each tissue (mg fatty acid/g tissue) were calculated by comparison of the fatty acid peak areas in the GC chromatograms to that of the 22:3n-3 internal standard.

Calculations and statistics

Tissue fatty acid concentrations (mg fatty acid/g tissue) were converted to the total amount of fatty acid per organ by multiplying these values by the whole-organ weight, as reported in supplementary Table II. Whole-body weights for skin, adipose, skeletal muscle, and bones were based on their determined percent body composition values (see above) multiplied by the total body weights of the animals, as found in supplementary Tables I and II, respectively. Total body plasma and RBC weights (1 g ≈ 1 ml) were estimated using the equations of Lee and Blaufox (54), where whole-body blood volume = 0.06 × body weight (g) + 0.77 and RBC/plasma = 0.45/0.55 (v/v). Retina, spleen, and testes fatty acid data are reported as micrograms of fatty acid per whole organ, whereas all other whole organs were reported in milligrams of fatty acid. Red gastrocnemius, white gastrocnemius, and soleus leg muscle types were expressed as fatty acid concentration values and reported as milligrams of fatty acid per gram tissue.

All data were expressed as the mean ± SD. Statistical comparisons between means were carried out using the Student's *t*-test. Because this study involves independent and repetitive measurements of various fatty acids in multiple tissues from each diet group, to help reduce the likelihood of making type-2 statistical errors, a significant difference was taken as $P < 0.010$; however, differences near significance, where $P = 0.010–0.050$, were also separately noted in the tables and, where warranted, in the Results section. For the tissue percent body composition data (see supplementary Table I), comparisons were carried out between the means of the 8 versus the 28 day-old dam-reared reference groups (n = 4, each). For the organ weight (see supplementary Table II), tissue fatty acid composition (**Tables 1–13** and

TABLE 1. Plasma fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/whole-body plasma	
Saturates			
10:0	0.006 ± 0.006	ND	ND
12:0	0.08 ± 0.09	0.06 ± 0.02	0.07 ± 0.03
14:0	0.13 ± 0.11	0.10 ± 0.03	0.13 ± 0.05
16:0	0.59 ± 0.28	1.4 ± 0.3	2.0 ± 0.8
18:0	0.33 ± 0.06	2.6 ± 0.7	2.7 ± 1.0
20:0	0.004 ± 0.001	0.01 ± 0.004	0.01 ± 0.01
22:0	0.004 ± 0.001	0.02 ± 0.01	0.02 ± 0.01
24:0	0.01 ± 0.001	0.05 ± 0.02	0.03 ± 0.01
Total saturates	1.2 ± 0.5	4.2 ± 1.1	4.9 ± 1.8
Monounsaturates			
16:1n-7	0.03 ± 0.01	0.05 ± 0.07	0.18 ± 0.06[b]
18:1n-7	0.04 ± 0.01	0.09 ± 0.07	0.07 ± 0.03
18:1n-9	0.21 ± 0.07	1.7 ± 0.4	2.4 ± 1.0
20:1n-9	0.004 ± 0.001	0.05 ± 0.01	0.05 ± 0.02
22:1n-9	0.001 ± 0.0003	0.008 ± 0.002	0.005 ± 0.006
24:1n-9	0.005 ± 0.004	0.21 ± 0.10	0.27 ± 0.25
Total monounsaturates	0.28 ± 0.09	2.2 ± 0.5	2.9 ± 1.3
n-6 PUFA			
18:2n-6	0.38 ± 0.08	0.96 ± 0.20	2.1 ± 0.9[a]
18:3n-6	0.006 ± 0.002	0.02 ± 0.005	0.03 ± 0.01
20:2n-6	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
20:3n-6	0.03 ± 0.01	0.07 ± 0.03	0.20 ± 0.09[a]
20:4n-6	0.37 ± 0.06	3.1 ± 0.9	4.2 ± 1.3
22:2n-6	0.02 ± 0.005	0.007 ± 0.006	ND[a]
22:4n-6	0.01 ± 0.003	0.07 ± 0.04	0.03 ± 0.01[a]
22:5n-6	0.02 ± 0.003	0.59 ± 0.17	0.03 ± 0.01[b]
Total n-6 PUFA	0.85 ± 0.12	4.9 ± 1.3	6.6 ± 2.3
n-3 PUFA			
d5-18:3n-3	–	0.02 ± 0.004	0.03 ± 0.01
18:3n-3	0.01 ± 0.01	ND	ND
d5-20:5n-3	–	0.01 ± 0.003	0.04 ± 0.02
20:5n-3	0.02 ± 0.002	ND	0.16 ± 0.07[b]
d5-22:5n-3	–	0.04 ± 0.01	0.02 ± 0.01[b]
22:5n-3	0.03 ± 0.01	0.005 ± 0.003	0.06 ± 0.02[b]
d5-22:6n-3	–	0.69 ± 0.15	0.24 ± 0.09[b]
22:6n-3	0.11 ± 0.02	0.10 ± 0.03	2.1 ± 0.9[b]
Total n-3 PUFA	0.16 ± 0.02	0.85 ± 0.19	2.6 ± 1.1
Total fatty acids	2.5 ± 0.7	12.1 ± 3.0	17.1 ± 6.5

ND, not detected (i.e., < 0.0001 mg/whole-body plasma). Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

TABLE 2. Heart fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/whole heart	
Saturates			
10:0	0.001 ± 0.001	0.003 ± 0.003	0.006 ± 0.005
12:0	0.01 ± 0.01	0.07 ± 0.05	0.05 ± 0.03
14:0	0.03 ± 0.02	0.08 ± 0.04	0.06 ± 0.02
16:0	0.30 ± 0.07	0.80 ± 0.10	0.75 ± 0.034
18:0	0.41 ± 0.06	1.8 ± 0.1	1.7 ± 0.1
20:0	0.008 ± 0.001	0.03 ± 0.004	0.03 ± 0.002
22:0	0.005 ± 0.001	0.02 ± 0.003	0.02 ± 0.002
24:0	0.007 ± 0.001	0.02 ± 0.003	0.02 ± 0.005
Total saturates	0.8 ± 0.2	2.8 ± 0.3	2.6 ± 0.2
Monounsaturates			
16:1n-7	0.007 ± 0.002	0.01 ± 0.005	0.01 ± 0.002
18:1n-7	0.07 ± 0.01	0.15 ± 0.03	0.11 ± 0.01[a]
18:1n-9	0.12 ± 0.03	1.4 ± 0.4	1.1 ± 0.3
20:1n-9	0.004 ± 0.001	0.03 ± 0.01	0.02 ± 0.003
22:1n-9	0.002 ± 0.0004	0.008 ± 0.001	0.007 ± 0.0005
24:1n-9	0.007 ± 0.001	0.04 ± 0.01	0.05 ± 0.01[b]
Total monounsaturates	0.21 ± 0.05	1.6 ± 0.5	1.3 ± 0.3
n-6 PUFA			
18:2n-6	0.11 ± 0.04	1.0 ± 0.1	0.87 ± 0.10[a]
18:3n-6	0.001 ± 0.0001	0.006 ± 0.001	0.005 ± 0.002
20:2n-6	0.01 ± 0.002	0.04 ± 0.004	0.04 ± 0.002
20:3n-6	0.02 ± 0.01	0.06 ± 0.01	0.07 ± 0.004
20:4n-6	0.48 ± 0.07	1.6 ± 0.2	1.1 ± 0.04[b]
22:2n-6	0.001 ± 0.0003	0.003 ± 0.001	0.004 ± 0.002[b]
22:4n-6	0.05 ± 0.01	0.13 ± 0.02	0.04 ± 0.004[b]
22:5n-6	0.02 ± 0.004	0.54 ± 0.04	0.04 ± 0.002[b]
Total n-6 PUFA	0.70 ± 0.12	3.4 ± 0.3	2.1 ± 0.1[b]
n-3 PUFA			
d5-18:3n-3	–	0.01 ± 0.005	0.01 ± 0.003
18:3n-3	0.002 ± 0.001	0.001 ± 0.0005	0.001 ± 0.001
d5-20:5n-3	–	0.004 ± 0.001	0.005 ± 0.001[a]
20:5n-3	0.005 ± 0.002	0.001 ± 0.0007	0.01 ± 0.002[b]
d5-22:5n-3	–	0.06 ± 0.01	0.02 ± 0.001[b]
22:5n-3	0.06 ± 0.01	0.02 ± 0.005	0.04 ± 0.01[b]
d5-22:6n-3	–	0.38 ± 0.02	0.15 ± 0.02[b]
22:6n-3	0.16 ± 0.03	0.12 ± 0.02	1.5 ± 0.2[b]
Total n-3 PUFA	0.23 ± 0.04	0.61 ± 0.04	1.8 ± 0.2[b]
Total fatty acids	1.9 ± 0.4	8.4 ± 1.0	7.8 ± 0.6

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).
[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.
[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

supplementary Tables III–IX), and amount of d5-DHA accumulated in tissues data (**Table 14**), comparisons were only carried out between the d5-LNA versus d5-LNA + DHA diet groups (28 day-old animals; n = 7 and n = 6, respectively), and not against the 8 day-old dam-reared reference group (n = 7). For the amount of unlabeled DHA accumulated in tissues data (**Table 15**), however, comparisons were carried out between the 8 day-old dam-reared reference group versus the d5-LNA and d5-LNA + DHA diet groups.

RESULTS

Percent body composition analysis

Supplementary Table I shows the total body weights and the weight percentages of the whole body represented by the combined major internal organs (brain, digestive tract, heart, kidneys, liver, lungs, retinas, spleen, and testes) and various extraneous tissues for the 8 and 28 day-old dam-reared reference group animals (n = 4, each). For the two dam-reared reference groups, significant differences were detected between the mean total body weights and the related mean weight percentages of hair, skin, skeletal muscle, brown adipose, visceral adipose, and blood/other fluids. The total body weight of the 28 day-old dam-reared reference group was found to have increased 6-fold over that of the 8 day-old group. In the 28 day-old rat pups, the weight percentages representing the skeletal muscle and visceral fat were increased compared with the 8 day-old animals, at 1.9- and 2.4-fold, respectively; whereas for brown adipose and blood/other fluids, the weight percentages were decreased by 2.2- and 1.6-fold, respectively. Significant hair was not present on the skin of the 8 day-old rat pups, but it was found on the 28 day-old animals. There were no significant differences detected between the two age groups in the mean weight percentages of total bones, white adipose, and combined internal organs, but the skin approached a significantly lower percentage of the body

TABLE 3. Lung fatty acid compositions of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/both whole lungs	
Saturates			
10:0	0.004 ± 0.002	0.03 ± 0.02	0.02 ± 0.01
12:0	0.06 ± 0.02	0.28 ± 0.12	0.20 ± 0.14
14:0	0.22 ± 0.06	0.42 ± 0.09	0.33 ± 0.13
16:0	1.4 ± 0.2	2.8 ± 0.4	2.5 ± 0.5
18:0	0.69 ± 0.08	1.6 ± 0.2	1.5 ± 0.1
20:0	0.02 ± 0.002	0.05 ± 0.01	0.05 ± 0.01
22:0	0.02 ± 0.004	0.05 ± 0.01	0.06 ± 0.01
24:0	0.03 ± 0.006	0.08 ± 0.01	0.10 ± 0.02[a]
Total saturates	2.5 ± 0.4	5.3 ± 0.8	4.7 ± 0.7
Monounsaturates			
16:1n-7	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.04
18:1n-7	0.14 ± 0.02	0.08 ± 0.02	0.07 ± 0.01[b]
18:1n-9	0.79 ± 0.08	6.2 ± 1.6	4.9 ± 1.3
20:1n-9	0.02 ± 0.001	0.13 ± 0.02	0.13 ± 0.01[b]
22:1n-9	0.007 ± 0.001	0.05 ± 0.01	0.06 ± 0.01
24:1n-9	0.03 ± 0.004	0.24 ± 0.03	0.28 ± 0.04
Total monounsaturates	1.0 ± 0.1	6.8 ± 1.6	5.5 ± 1.3
n-6 PUFA			
18:2n-6	0.41 ± 0.06	1.2 ± 0.3	1.1 ± 0.3
18:3n-6	0.008 ± 0.001	0.01 ± 0.003	0.01 ± 0.001
20:2n-6	0.04 ± 0.004	0.05 ± 0.01	0.05 ± 0.004[b]
20:3n-6	0.07 ± 0.03	0.08 ± 0.01	0.10 ± 0.02[b]
20:4n-6	0.64 ± 0.08	1.2 ± 0.1	1.0 ± 0.1[b]
22:2n-6	0.009 ± 0.006	0.007 ± 0.001	0.009 ± 0.002
22:4n-6	0.18 ± 0.01	0.25 ± 0.03	0.13 ± 0.02[b]
22:5n-6	0.05 ± 0.006	0.26 ± 0.03	0.03 ± 0.01[b]
Total n-6 PUFA	1.4 ± 0.1	3.1 ± 0.5	2.4 ± 0.2[b]
n-3 PUFA			
d5-18:3n-3	–	0.05 ± 0.01	0.04 ± 0.02
18:3n-3	0.02 ± 0.004	0.007 ± 0.003	0.004 ± 0.002
d5-20:5n-3	–	0.009 ± 0.001	0.01 ± 0.003[b]
20:5n-3	0.03 ± 0.01	0.002 ± 0.001	0.04 ± 0.01[b]
d5-22:5n-3	–	0.05 ± 0.01	0.03 ± 0.004[b]
22:5n-3	0.14 ± 0.02	0.01 ± 0.002	0.05 ± 0.01[b]
d5-22:6n-3	–	0.15 ± 0.02	0.06 ± 0.01[b]
22:6n-3	0.27 ± 0.03	0.04 ± 0.01	0.70 ± 0.08[b]
Total n-3 PUFA	0.46 ± 0.06	0.31 ± 0.04	0.94 ± 0.10[b]
Total fatty acids	5.4 ± 0.6	15.5 ± 2.9	13.5 ± 2.1

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010$–0.050.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

weight in the 28 day-old animals (1.3-fold; $P = 0.011$). The percent body composition data (see supplementary Table I) for skin, skeletal muscle, bones, and brown, white, and visceral adipose were utilized in supplementary Table II to derive the whole-body masses of these corresponding tissues using the body weights of the d5-LNA and d5-LNA + DHA diet groups (28 day-old animals) and the 8 day-old dam-reared reference group.

Body and organ/tissue weights

Supplementary Table II shows the baseline body and whole-organ/tissue weights for the 8 day-old dam-reared reference, d5-LNA diet, and d5-LNA + DHA groups (n = 7, 7, and 6, respectively). After 21 days (p8–p28) on their respective diets, the final mean body weights of the rat pups in the d5-LNA and d5-LNA + DHA diet groups did not differ significantly from each other, but they both had increased ~6-fold in size over the 8 day-old group. Growth curves showing the weight gain per day for these animals were previously reported by Lefkowitz et al. (10). Likewise, the weights for the whole organs/tissues did not significantly differ between the two diet groups, but the retinas approached a significantly lower weight in the d5-LNA + DHA diet group (1.2-fold; $P = 0.011$). As anticipated, the mean organ/tissue weights recorded for 8 day-old dam-reared reference group all underwent a dramatic increase in size in the 28 day-old animals in both dietary groups.

Fatty acid compositional analyses

Tables 1–13 present the fatty acid compositional data, expressed as micrograms (testes only) or milligrams fatty acid/whole organ or tissue, for the plasma (Table 1), heart (Table 2), lungs (Table 3), kidneys (Table 4), testes (Table 5), skin (Table 6), skeletal muscle (Table 7), bones (Table 8), brown adipose (Table 9), white adipose (Table 10), visceral adipose (Table 11), brain (Table 12), and liver (Table 13) of the two 28 day dietary (d5-LNA and d5-LNA + DHA) groups and the 8 day-old dam-reared reference group (n = 7, 6, and 7, respectively). Likewise, located in supplementary Tables III–IX are the corresponding data for retina (see supplementary Table III), spleen (see supplementary Table IV), digestive tract (see supplementary Table V), carcass (see supplementary Table VI), and the lower rear leg muscle subtypes, red gastrocnemius (see supplementary Table VII), white gastrocnemius (see supplementary Table VIII), and soleus (see supplementary Table IX), which are also expressed as milligrams (digestive tract and carcass), micrograms (retina and spleen only) or milligrams fatty acid/gram tissue (muscle subtypes). The fatty acid composition data shown here for brain and liver (Tables 12 and 13, respectively) has been previously reported by Lefkowitz et al. (10). In this paper, the fatty acid compositional data for liver has undergone mathematical correction to account for a mistaken repeat in multiplication by the liver total weights in the previous paper (10). Brain values did not merit any changes. In order to condense the presentation of this enormous volume of data, the whole-organ/tissue fatty acid composition results will be generalized under saturated, monounsaturated, n-6 PUFA, unlabeled LNA and d5-LNA, unlabeled EPA and deuterium-labeled EPA (d5-EPA), unlabeled DPA and deuterium-labeled DPA (d5-DPA), and unlabeled DHA and d5-DHA fatty acid sections to follow below. Data only for significant ($P < 0.010$) and near-significant ($P = 0.010$–0.050) differences between the d5-LNA and d5-LNA + DHA diet groups will be described in these Result sections, and all comparisons of these two diet groups to the 8 day-old dam-reared reference group will be reserved for the Discussion section. The units of data expression for red gastrocnemius, white gastrocnemius, and soleus leg muscle (see supplementary Tables VII–IX) are on a per gram tissue basis and their data will be presented separately at the end of the Results section.

TABLE 4. Kidney fatty acid compositions of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/both whole kidneys	
Saturates			
10:0	0.001 ± 0.0005	0.003 ± 0.003	0.001 ± 0.001
12:0	0.01 ± 0.01	0.04 ± 0.03	0.04 ± 0.01
14:0	0.05 ± 0.02	0.14 ± 0.03	0.13 ± 0.02
16:0	0.50 ± 0.11	2.4 ± 0.3	2.6 ± 0.4
18:0	0.40 ± 0.08	2.9 ± 0.2	3.0 ± 0.4
20:0	0.01 ± 0.002	0.06 ± 0.01	0.07 ± 0.01
22:0	0.02 ± 0.004	0.08 ± 0.01	0.08 ± 0.01
24:0	0.02 ± 0.004	0.29 ± 0.03	0.33 ± 0.06
Total saturates	1.0 ± 0.2	5.9 ± 0.6	6.3 ± 0.8
Monounsaturates			
16:1n-7	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
18:1n-7	0.08 ± 0.02	0.16 ± 0.04	0.14 ± 0.03[b]
18:1n-9	0.24 ± 0.05	2.8 ± 0.4	3.0 ± 0.3
20:1n-9	0.005 ± 0.002	0.10 ± 0.01	0.10 ± 0.01
22:1n-9	0.001 ± 0.0002	0.02 ± 0.00	0.02 ± 0.003
24:1n-9	0.02 ± 0.005	0.43 ± 0.04	0.48 ± 0.07[b]
Total monounsaturates	0.37 ± 0.07	3.6 ± 0.5	3.8 ± 0.4
n-6 PUFA			
18:2n-6	0.19 ± 0.04	1.4 ± 0.1	2.1 ± 0.3[b]
18:3n-6	0.001 ± 0.0003	0.008 ± 0.001	0.01 ± 0.001[a]
20:2n-6	0.009 ± 0.001	0.10 ± 0.01	0.09 ± 0.010
20:3n-6	0.04 ± 0.01	0.19 ± 0.04	0.25 ± 0.05[a]
20:4n-6	0.58 ± 0.12	4.2 ± 0.5	3.4 ± 0.4[b]
22:2n-6	0.0006 ± 0.0001	0.011 ± 0.003	0.009 ± 0.002[b]
22:4n-6	0.04 ± 0.01	0.12 ± 0.03	0.06 ± 0.01[b]
22:5n-6	0.01 ± 0.003	0.26 ± 0.04	0.02 ± 0.01[b]
Total n-6 PUFA	0.87 ± 0.18	6.3 ± 0.6	5.9 ± 0.7
n-3 PUFA			
d5-18:3n-3	–	0.02 ± 0.005	0.03 ± 0.00
18:3n-3	0.003 ± 0.001	0.001 ± 0.001	0.001 ± 0.001
d5-20:5n-3	–	0.02 ± 0.002	0.05 ± 0.011[b]
20:5n-3	0.01 ± 0.005	0.003 ± 0.001	0.27 ± 0.07[b]
d5-22:5n-3	–	0.04 ± 0.01	0.02 ± 0.003[b]
22:5n-3	0.03 ± 0.01	0.01 ± 0.001	0.05 ± 0.01[b]
d5-22:6n-3	–	0.38 ± 0.05	0.10 ± 0.02[b]
22:6n-3	0.13 ± 0.03	0.07 ± 0.01	1.0 ± 0.2[b]
Total n-3 PUFA	0.18 ± 0.05	0.54 ± 0.06	1.5 ± 0.3[b]
Total fatty acids	2.4 ± 0.5	16.3 ± 1.6	17.5 ± 2.2

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).
[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.
[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

Saturated fatty acid composition of rat pup organs

The most abundant and physiologically relevant saturated fatty acids typically found in tissues are 16:0 and 18:0 (palmitic and stearic acids), which also represent the main products of the saturated fatty acid biosynthetic pathway (55). Only the retina showed significant differences in these saturated fatty acids, where there was a 1.3- and 1.2-fold increase in 16:0 and 18:0 content, respectively, for the d5-LNA + DHA compared with the d5-LNA diet group. For the retina, total saturated fatty acid content was also significantly increased by 1.2-fold in the d5-LNA + DHA diet group.

Monounsaturated fatty acid composition of rat pup organs

The most abundant and physiologically relevant monounsaturated fatty acid typically found in tissues is 18:1n-9 (oleic acid), which can be biosynthesized by the Δ-9 desaturation of 18:0 (56). Only retina was found to significantly differ between the two dietary groups in oleic acid content, with a decrease of 1.3-fold in the d5-LNA + DHA compared with d5-LNA diet group. Correspondingly, retina was also decreased by 1.3-fold in total monounsaturated fatty acid content in the d5-LNA + DHA diet group.

n-6 PUFA composition of rat pup organs

The most abundant and physiologically relevant n-6 PUFAs typically found in tissues are 18:2n-6 (LA), a dietary essential PUFA, and its biosynthetic end product, 20:4n-6 (arachidonic acid, AA) (9). During times of n-3 PUFA deprivation, it is apparent that the accretion of AA from LA is increased and AA is also further metabolized to produce 22:5n-6 (DPAn-6), which accumulates in tissues so as to reciprocally replace lost DHA (57–60). Of all tissues examined, only kidney and brain were found to be changed in LA content, with an increase of 1.5- and 1.3-fold, respectively, in the d5-LNA + DHA compared with d5-LNA diet group. Despite an overall lack in effect of the diets on LA in the tissues, the AA content of the tissues was significantly

TABLE 5. Testes fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		μg/both whole testes	
Saturates			
10:0	0.49 ± 0.15	16 ± 27	1.3 ± 1.3
12:0	0.16 ± 0.04	129 ± 240	18 ± 11
14:0	1.9 ± 0.5	124 ± 176	59 ± 20
16:0	48 ± 11	1,626 ± 513	1,414 ± 181
18:0	34 ± 6	657 ± 231	547 ± 93
20:0	0.06 ± 0.03	14 ± 4	14 ± 2
22:0	0.90 ± 0.17	10 ± 2	11 ± 1
24:0	1.1 ± 0.2	9.5 ± 2.4	9.3 ± 1.0
Total saturates	86 ± 17	2,586 ± 1155	2,073 ± 296
Monounsaturates			
16:1n-7	3.1 ± 0.7	27 ± 26	18 ± 5
18:1n-7	5.4 ± 1.0	79 ± 12	85 ± 14
18:1n-9	32 ± 6	2,326 ± 2,763	1,055 ± 267
20:1n-9	0.44 ± 0.09	22 ± 13	16 ± 3
22:1n-9	0.24 ± 0.01	3.6 ± 2.0	3.0 ± 1.3
24:1n-9	1.5 ± 0.2	19 ± 7	21 ± 4
Total monounsaturates	43 ± 8	2,477 ± 2,812	1,198 ± 291
n-6 PUFA			
18:2n-6	9.8 ± 2.4	469 ± 526	280 ± 70
18:3n-6	0.12 ± 0.01	4.5 ± 3.4	3.0 ± 0.8
20:2n-6	0.46 ± 0.13	19 ± 7	18 ± 2
20:3n-6	2.0 ± 0.4	48 ± 14	72 ± 10[b]
20:4n-6	39 ± 6	1,068 ± 228	925 ± 129[b]
22:2n-6	0.08 ± 0.02	4.4 ± 0.9	4.9 ± 2.3[b]
22:4n-6	8.5 ± 1.8	118 ± 26	106 ± 13
22:5n-6	1.6 ± 0.6	613 ± 97	350 ± 28[b]
Total n-6 PUFA	62 ± 11	2,344 ± 780	1,757 ± 220
n-3 PUFA			
d5-18:3n-3	–	7 ± 10	4.5 ± 3.2
18:3n-3	0.05 ± 0.02	0.67 ± 0.37	0.88 ± 0.22
d5-20:5n-3	–	3.1 ± 1.1	2.7 ± 0.5
20:5n-3	0.95 ± 0.21	0.83 ± 0.39	7.0 ± 1.5[b]
d5-22:5n-3	–	6.9 ± 4.5	2.4 ± 1.0[a]
22:5n-3	2.2 ± 0.4	1.2 ± 1.1	6.2 ± 1.9[b]
d5-22:6n-3	–	115 ± 24	34 ± 5[b]
22:6n-3	9.0 ± 1.6	24 ± 7	287 ± 38[b]
Total n-3 PUFA	12 ± 2	171 ± 64	345 ± 48[b]
Total fatty acids	203 ± 37	7,564 ± 4,698	5,374 ± 809

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010$–0.050.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

TABLE 6. Skin fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total skin	
Saturates			
10:0	6.4 ± 3.9	7.5 ± 3.8	8.0 ± 6.7
12:0	45 ± 24	99 ± 17	95 ± 22
14:0	37 ± 18	64 ± 9	62 ± 12
16:0	66 ± 26	110 ± 18	115 ± 26
18:0	16 ± 5	45 ± 4	45 ± 3.7
20:0	0.33 ± 0.08	1.2 ± 0.2	1.1 ± 0.1
22:0	0.43 ± 0.07	1.3 ± 0.3	1.2 ± 0.2
24:0	1.4 ± 0.3	3.3 ± 0.6	3.5 ± 0.3
Total saturates	173 ± 77	330 ± 49	331 ± 70
Monounsaturates			
16:1n-7	8.3 ± 4.5	9.4 ± 2.9	10 ± 4
18:1n-7	6.6 ± 2.1	6.6 ± 1.1	5.8 ± 0.9
18:1n-9	52 ± 17	774 ± 102	761 ± 135
20:1n-9	0.86 ± 0.20	3.8 ± 0.5	3.6 ± 0.4
22:1n-9	0.13 ± 0.02	0.68 ± 0.07	0.67 ± 0.06
24:1n-9	0.21 ± 0.04	1.6 ± 0.1	1.6 ± 0.2
Total monounsaturates	68 ± 23	796 ± 104	783 ± 138
n-6 PUFA			
18:2n-6	30 ± 8	147 ± 18	156 ± 26
18:3n-6	0.52 ± 0.17	0.76 ± 0.14	0.63 ± 0.13
20:2n-6	1.6 ± 0.4	1.6 ± 0.3	1.7 ± 0.2
20:3n-6	1.7 ± 0.4	1.7 ± 0.5	2.1 ± 0.5
20:4n-6	6.7 ± 1.4	17 ± 2	13 ± 1[b]
22:2n-6	0.12 ± 0.04	0.41 ± 0.14	0.43 ± 0.15
22:4n-6	1.4 ± 0.2	1.9 ± 0.4	1.0 ± 0.1[b]
22:5n-6	0.45 ± 0.07	3.3 ± 0.5	0.97 ± 0.23[b]
Total n-6 PUFA	42 ± 11	174 ± 21	176 ± 26
n-3 PUFA			
d5-18:3n-3	–	8.8 ± 1.5	9.5 ± 2.1
18:3n-3	2.6 ± 1.2	0.72 ± 0.29	0.68 ± 0.14
d5-20:5n-3	–	0.15 ± 0.03	0.23 ± 0.05[a]
20:5n-3	0.48 ± 0.20	0.05 ± 0.01	0.36 ± 0.08[b]
d5-22:5n-3	–	0.49 ± 0.13	0.28 ± 0.06[b]
22:5n-3	1.2 ± 0.3	0.14 ± 0.04	0.49 ± 0.09[b]
d5-22:6n-3	–	1.6 ± 0.2	0.94 ± 0.15[b]
22:6n-3	2.4 ± 0.5	0.50 ± 0.10	12 ± 3[b]
Total n-3 PUFA	6.8 ± 2.2	12 ± 2	24 ± 5[b]
Total fatty acids	291 ± 112	1,313 ± 171	1,314 ± 234

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010$–0.050.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

decreased in the d5-LNA + DHA compared with d5-LNA diet group, except in plasma, brown adipose, white adipose, visceral adipose ($P = 0.025$), brain ($P = 0.042$), and liver ($P = 0.019$). The largest decrease in AA content for the internal organs and other various tissues was found in heart and skeletal muscle, at 1.5- and 1.7-fold, respectively, of the d5-LNA diet group. As anticipated, all tissues examined in this study also had significantly lower amounts of DPAn-6, a marker of DHA deficiency, in the d5-LNA + DHA compared with the d5-LNA diet group, ranging from a 2- to a 36-fold decrease. For the internal organs and various other tissues, the liver and skeletal muscle displayed the largest decreases in DPAn-6 content at 36- and 10-fold, respectively, of the d5-LNA diet group. Plasma also had a large decrease in DPAn-6 at 19-fold of the d5-LNA diet group. Despite decreased accumulation of LA, AA, and/or DPAn-6 in the d5-LNA + DHA diet group for all tissues, only the heart, lungs, retina, skeletal muscle, brain, and liver showed a significantly decreased content of total n-6 PUFA (1.6-, 1.3-, 1.7-, 1.5-, 1.2-, and 1.5-fold, respectively, of the d5-LNA diet group).

Unlabeled LNA and d5-LNA composition of rat pup organs

LNA is the initial precursor n-3 PUFA for the biosynthesis of DHA; thus, under conditions of a diet replete in DHA (d5-LNA + DHA diet), tissue pools of unlabeled LNA and d5-LNA would be anticipated to be less actively utilized. In this experiment, all dietary LNA fed to the animals from p8 to p28 consisted of d5-labeled LNA, which

TABLE 7. Skeletal muscle fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total skeletal muscle	
Saturates			
10:0	0.3 ± 0.3	1.3 ± 0.4	1.6 ± 0.3
12:0	2.5 ± 1.7	10 ± 2	11 ± 2
14:0	2.8 ± 1.4	9.1 ± 1.4	9.0 ± 1.5
16:0	7.9 ± 2.6	49 ± 7	46 ± 6
18:0	4.6 ± 1.0	46 ± 5	39 ± 6.4[a]
20:0	0.07 ± 0.01	0.36 ± 0.06	0.32 ± 0.06
22:0	0.06 ± 0.01	0.24 ± 0.03	0.28 ± 0.09
24:0	0.09 ± 0.01	0.68 ± 0.10	0.52 ± 0.23
Total saturates	18 ± 7	116 ± 13	107 ± 14
Monounsaturates			
16:1n-7	0.7 ± 0.3	1.4 ± 0.5	1.7 ± 0.4
18:1n-7	1.0 ± 0.3	3.6 ± 1.0	2.4 ± 0.4[a]
18:1n-9	6.1 ± 3.3	137 ± 20	125 ± 24
20:1n-9	0.12 ± 0.04	1.7 ± 0.2	1.4 ± 0.2[b]
22:1n-9	0.03 ± 0.01	0.25 ± 0.02	0.24 ± 0.05
24:ln-9	0.12 ± 0.01	1.5 ± 0.1	1.3 ± 0.4
Total monounsaturates	8.0 ± 3.9	145 ± 21	132 ± 25
n-6 PUFA			
18:2n-6	3.2 ± 1.0	62 ± 7	50 ± 8[a]
18:3n-6	0.06 ± 0.03	0.35 ± 0.08	0.25 ± 0.06[a]
20:2n-6	0.26 ± 0.05	1.6 ± 0.1	1.3 ± 0.2[a]
20:3n-6	0.42 ± 0.10	3.0 ± 0.6	2.6 ± 0.5
20:4n-6	3.8 ± 0.6	37 ± 5	22 ± 3[b]
22:2n-6	0.02 ± 0.004	0.16 ± 0.05	0.14 ± 0.02[b]
22:4n-6	0.77 ± 0.12	3.9 ± 0.6	1.2 ± 0.2[b]
22:5n-6	0.24 ± 0.05	12 ± 1	1.2 ± 0.2[b]
Total n-6 PUFA	8.8 ± 1.9	121 ± 14	78 ± 11[b]
n-3 PUFA			
d5-18:3n-3	–	1.2 ± 0.2	1.3 ± 0.3
18:3n-3	0.20 ± 0.09	0.08 ± 0.02	0.08 ± 0.02
d5-20:5n-3	–	0.28 ± 0.05	0.28 ± 0.06
20:5n-3	0.13 ± 0.05	0.07 ± 0.01	0.55 ± 0.09[b]
d5-22:5n-3	–	1.77 ± 0.31	0.67 ± 0.13[b]
22:5n-3	0.64 ± 0.12	0.64 ± 0.11	1.3 ± 0.3[b]
d5-22:6n-3	–	11 ± 1	4.3 ± 0.8[b]
22:6n-3	1.1 ± 0.2	4.3 ± 0.6	46 ± 9[b]
Total n-3 PUFA	2.1 ± 0.4	19 ± 2	54 ± 10[b]
Total fatty acids	37 ± 13	401 ± 45	371 ± 51

Total body skeletal muscle values were determined from fatty acid concentration data (mg/g tissue) derived from thigh muscle samples. Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA + DHA diet groups of $P < 0.010$.

TABLE 8. Bone fatty acid composition of the two experimental diet and 8 day-old dam-related reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total bones	
Saturates			
10:0	0.03 ± 0.03	0.01 ± 0.02	0.01 ± 0.01
12:0	0.44 ± 0.2	1.0 ± 0.7	1.0 ± 0.5
14:0	0.65 ± 0.3	1.6 ± 0.6	1.6 ± 0.7
16:0	3.2 ± 1.0	11 ± 2	12 ± 4
18:0	1.9 ± 0.4	9.1 ± 1.3	9.6 ± 3.4
20:0	0.03 ± 0.01	0.11 ± 0.02	0.12 ± 0.04
22:0	0.05 ± 0.01	0.13 ± 0.01	0.14 ± 0.05
24:0	0.09 ± 0.02	0.26 ± 0.03	0.28 ± 0.09
Total saturates	6.4 ± 1.8	23 ± 4	24 ± 9
Monounsaturates			
16:1n-7	0.30 ± 0.10	0.68 ± 0.14	0.78 ± 0.25
18:1n-7	0.47 ± 0.11	1.1 ± 0.2	1.1 ± 0.4
18:1n-9	2.0 ± 0.5	26 ± 9	27 ± 12
20:1n-9	0.04 ± 0.01	0.61 ± 0.12	0.57 ± 0.25
22:1n-9	0.01 ± 0.002	0.14 ± 0.02	0.14 ± 0.05
24:1n-9	0.09 ± 0.02	1.1 ± 0.1	1.2 ± 0.4
Total monounsaturates	2.9 ± 0.7	30 ± 9	30 ± 13
n-6 PUFA			
18:2n-6	1.0 ± 0.3	6.2 ± 1.7	7.5 ± 2.8
18:3n-6	0.02 ± 0.01	0.07 ± 0.03	0.04 ± 0.01
20:2n-6	0.14 ± 0.03	0.43 ± 0.05	0.51 ± 0.18
20:3n-6	0.14 ± 0.03	0.49 ± 0.03	0.72 ± 0.20[a]
20:4n-6	1.9 ± 0.4	9.4 ± 1.4	8.5 ± 3.0[b]
22:2n-6	0.01 ± 0.002	0.05 ± 0.005	0.05 ± 0.01[b]
22:4n-6	0.32 ± 0.05	1.6 ± 0.2	1.1 ± 0.5[a]
22:5n-6	0.08 ± 0.02	1.3 ± 0.2	0.18 ± 0.05[b]
Total n-6 PUFA	3.6 ± 0.8	20 ± 3	18 ± 7
n-3 PUFA			
d5-18:3n-3	–	0.22 ± 0.11	0.24 ± 0.11
18:3n-3	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
5-20:5n-3	–	0.04 ± 0.01	0.07 ± 0.02[b]
20:5n-3	0.05 ± 0.01	0.02 ± 0.00	0.13 ± 0.03[b]
d5-22:5n-3	–	0.26 ± 0.03	0.19 ± 0.06[a]
22:5n-3	0.20 ± 0.04	0.04 ± 0.01	0.28 ± 0.10[b]
d5-22:6n-3	–	0.89 ± 0.14	0.35 ± 0.14[b]
22:6n-3	0.40 ± 0.08	0.21 ± 0.04	3.8 ± 1.1[b]
Total n-3 PUFA	0.69 ± 0.14	1.7 ± 0.3	5.1 ± 1.5[b]
Total fatty acids	78 ± 30	74 ± 16	78 ± 30

Total body bone values were determined from fatty acid concentration data (mg/g tissue) derived from femur bone samples. Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA + DHA diet groups of $P < 0.010$.

leads to the formation of biosynthesized d5-DHA (10). Any unlabeled LNA present in the tissues at p28 is endogenous LNA that accumulated in the p8 dam-reared animals before the start of the feeding experiment. There were no tissues that displayed a significant increase in d5-LNA and/or unlabeled LNA content in the d5-LNA + DHA compared with d5-LNA diet group; but the white adipose content approached a significant increase in d5-LNA content (1.2-fold; $P = 0.025$).

Unlabeled EPA and d5-EPA composition of rat pup organs

EPA is one of the principal stable intermediates and follows LNA in the biosynthetic pathway toward DHA; thus, d5-LNA would be converted to d5-EPA. In the experimental diet replete in DHA (d5-LNA + DHA diet), the formation of unlabeled EPA and biosynthesized d5-EPA would be anticipated to be decreased. In none of the tissues, however, was there a significant or near-significant decrease in the amount of biosynthesized d5-EPA content in the d5-LNA + DHA compared with the d5-LNA diet group. In fact, plasma, lung, kidney, brain, and liver all exhibited significant increases in biosynthesized d5-EPA on the d5-LNA + DHA diet; whereas heart, skin, bone, brown adipose, and carcass approached a significant increase in d5-EPA content ($P = 0.020$, 0.015, 0.014, 0.013, and 0.011, respectively). For the internal organs and various other

TABLE 9. Brown adipose fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total brown adipose	
Saturates			
10:0	0.1 ± 0.1	1.6 ± 0.5	1.4 ± 0.3
12:0	1.4 ± 0.9	23 ± 4	21 ± 2
14:0	1.6 ± 1.0	26 ± 5	25 ± 4
16:0	4.1 ± 1.8	64 ± 14	70 ± 15
18:0	3.5 ± 0.9	27 ± 5	30 ± 4
20:0	0.07 ± 0.03	0.35 ± 0.06	0.39 ± 0.06
22:0	0.04 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
24:0	0.08 ± 0.02	0.11 ± 0.01	0.10 ± 0.05
Total saturates	11 ± 5	142 ± 26	148 ± 23
Monounsaturates			
16:1n-7	0.25 ± 0.16	2.6 ± 0.7	2.9 ± 1.4
18:1n-7	0.57 ± 0.20	2.3 ± 0.5	2.3 ± 0.6
18:1n-9	4.4 ± 1.9	207 ± 25	208 ± 16
20:1n-9	0.16 ± 0.06	2.4 ± 0.4	2.6 ± 0.3
22:1n-9	0.03 ± 0.01	0.16 ± 0.03	0.18 ± 0.03
24:1n-9	0.07 ± 0.02	0.29 ± 0.04	0.31 ± 0.06
Total monounsaturates	5.5 ± 2.3	215 ± 26	216 ± 18
n-6 PUFA			
18:2n-6	3.4 ± 0.3	45 ± 5	49 ± 5
18:3n-6	0.03 ± 0.01	0.3 ± 0.07	0.22 ± 0.03
20:2n-6	0.2 ± 0.1	1.1 ± 0.20	1.3 ± 0.1
20:3n-6	0.5 ± 0.2	1.3 ± 0.29	1.5 ± 0.2
20:4n-6	2.7 ± 0.6	4.9 ± 1.2	5.2 ± 0.9
22:2n-6	0.03 ± 0.01	0.14 ± 0.05	0.13 ± 0.02
22:4n-6	0.5 ± 0.1	1.7 ± 0.4	1.7 ± 0.2
22:5n-6	0.21 ± 0.05	2.2 ± 0.6	0.8 ± 0.1[b]
Total n-6 PUFA	7.6 ± 2.0	57 ± 7	60 ± 5
n-3 PUFA			
d5-18:3n-3	–	2.7 ± 0.4	2.9 ± 0.2
18:3n-3	0.11 ± 0.06	0.41 ± 0.18	0.40 ± 0.15
d5-20:5n-3	–	0.08 ± 0.03	0.11 ± 0.01[a]
20:5n-3	0.07 ± 0.02	0.06 ± 0.03	0.20 ± 0.02[b]
d5-22:5n-3	–	0.23 ± 0.11	0.26 ± 0.02
22:5n-3	0.43 ± 0.09	0.59 ± 0.15	0.94 ± 0.17[b]
d5-22:6n-3	–	1.3 ± 0.3	1.2 ± 0.1
22:6n-3	0.89 ± 0.19	1.8 ± 0.5	15 ± 1[b]
Total n-3 PUFA	1.5 ± 0.3	7.1 ± 1.5	21 ± 1[b]
Total fatty acids	25 ± 9	421 ± 57	445 ± 45

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA + DHA diet group of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA + DHA diet group of $P < 0.010$.

TABLE 10. White adipose fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total white adipose	
Saturates			
10:0	9.8 ± 8.1	59 ± 9	62 ± 8
12:0	52 ± 33	279 ± 51	302 ± 32
14:0	44 ± 25	166 ± 33	182 ± 22
16:0	89 ± 38	280 ± 62	347 ± 54
18:0	18 ± 6	81 ± 14	90 ± 7
20:0	0.21 ± 0.06	1.1 ± 0.2	1.2 ± 0.4
22:0	0.08 ± 0.02	0.41 ± 0.14	0.49 ± 0.47
24:0	0.02 ± 0.01	0.29 ± 0.11	2.0 ± 1.4[a]
Total saturates	213 ± 109	867 ± 163	986 ± 117
Monounsaturates			
16:1n-7	13 ± 7	29 ± 11	38 ± 10
18:1n-7	72 ± 24	14 ± 4	14 ± 4
18:1n-9	8.1 ± 2.2	2096 ± 341	2228 ± 161
20:1n-9	1.2 ± 0.3	10 ± 2	10 ± 1
22:1n-9	0.10 ± 0.02	0.46 ± 0.09	0.44 ± 0.05
24:1n-9	0.12 ± 0.05	0.65 ± 0.29	0.71 ± 0.16
Total monounsaturates	94 ± 32	2,150 ± 354	2,291 ± 172
n-6 PUFA			
18:2n-6	36 ± 10	384 ± 59	438 ± 30
18:3n-6	0.90 ± 0.24	2.5 ± 0.48	2.2 ± 0.4
20:2n-6	2.0 ± 0.6	4.6 ± 1.30	5.1 ± 0.7
20:3n-6	2.1 ± 0.5	3.3 ± 1.12	4.2 ± 0.9
20:4n-6	5.8 ± 1.7	15 ± 4	12 ± 1
22:2n-6	0.13 ± 0.03	0.75 ± 0.20	0.70 ± 0.52
22:4n-6	2.0 ± 0.5	2.5 ± 0.8	1.6 ± 0.4[a]
22:5n-6	0.75 ± 0.23	5.6 ± 1.4	2.1 ± 0.5[b]
Total n-6 PUFA	50 ± 14	418 ± 67	466 ± 33
n-3 PUFA			
d5-18:3n-3	–	28 ± 4	33 ± 3[a]
18:3n-3	3.2 ± 1.5	1.7 ± 0.7	1.7 ± 0.7
d5-20:5n-3	–	0.47 ± 0.18	0.53 ± 0.11
20:5n-3	0.71 ± 0.37	0.16 ± 0.08	0.79 ± 0.20[b]
d5-22:5n-3	–	0.92 ± 0.42	0.78 ± 0.16
22:5n-3	1.8 ± 0.6	0.34 ± 0.17	1.2 ± 0.2[b]
d5-22:6n-3	–	2.2 ± 0.5	2.9 ± 0.4[a]
22: 6n-3	3.4 ± 1.3	0.86 ± 0.28	34 ± 7[b]
Total n-3 PUFA	9.1 ± 3.7	34 ± 6	74 ± 10[b]
Total fatty acids	492 ± 361	3,469 ± 587	3,818 ± 315

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

tissues, liver and bone exhibited the greatest increases in biosynthesized d5-EPA, at 3.2- and 1.7-fold, respectively, of the d5-LNA diet group. Plasma, however, was found to have a much larger increase in biosynthesized d5-EPA, at 4.8-fold of the d5-LNA diet group. Likewise, in all of the whole organs/tissues, unlabeled EPA was found to be significantly increased in the d5-LNA + DHA compared with d5-LNA diet group. Compared with the other internal organs and body tissues, kidney and skeletal muscle showed the greatest increases in unlabeled EPA content at 98- and 8-fold, respectively. Conversely, plasma showed a more marked response than these tissues in that unlabeled EPA was undetectable in the d5-LNA diet group, but was present in the d5-LNA + DHA diet group at 22% of the levels found in the liver.

Unlabeled DPA and d5-DPA composition of rat pup organs

DPA is the elongation product of EPA in the biosynthetic pathway toward DHA, and thus biosynthesized d5-EPA would be directly converted to d5-DPA. In the experimental diet replete in DHA (d5-LNA + DHA diet), the formation of unlabeled DPA and biosynthesized d5-DPA would be anticipated to be decreased. Consistent with this, in all tissues except brown, white, and visceral adipose, there was a significant or near-significant (bone, spleen, and testes; $P = 0.022$, 0.012, and 0.037, respectively) decrease in the amount of biosynthesized d5-DPA in the d5-LNA + DHA compared with the d5-LNA diet group. Brown, white, and visceral adipose showed no significant differences in biosynthesized d5-DPA content between the two diet groups. For the internal organs and various other tissues,

TABLE 11. Visceral adipose fatty acid composition of the two experimental diet and 8-day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total visceral adipose	
Saturates			
10:0	0.22 ± 0.16	6.8 ± 1.1	4.7 ± 1.4[a]
12:0	1.9 ± 1.0	34 ± 6	33 ± 4
14:0	1.9 ± 0.9	22 ± 4	23 ± 3
16:0	4.2 ± 1.3	44 ± 9	52 ± 8
18:0	0.94 ± 0.19	13 ± 2	13 ± 1
20:0	0.01 ± 0.002	0.19 ± 0.07	0.16 ± 0.03
22:0	0.006 ± 0.002	0.05 ± 0.02	0.09 ± 0.03[a]
24:0	0.006 ± 0.001	0.05 ± 0.01	0.04 ± 0.03
Total saturates	9.2 ± 3.5	121 ± 19	126 ± 16
Monounsaturates			
16:1n-7	0.54 ± 0.24	3.8 ± 1.3	4.6 ± 1.0
18:1n-7	0.43 ± 0.06	0.19 ± 0.07	0.18 ± 0.03
18:1n-9	3.4 ± 0.6	332 ± 53	315 ± 30
20:1n-9	0.07 ± 0.01	1.1 ± 0.7	1.5 ± 0.2
22:1n-9	0.007 ± 0.001	0.08 ± 0.02	0.13 ± 0.04[a]
24:1n-9	0.01 ± 0.004	0.08 ± 0.02	0.14 ± 0.06
Total monounsaturates	4.5 ± 0.9	337 ± 54	322 ± 31
n-6 PUFA			
18:2n-6	1.8 ± 0.3	58 ± 9	60 ± 6
18:3n-6	0.04 ± 0.01	0.37 ± 0.08	0.27 ± 0.05[a]
20:2n-6	0.11 ± 0.02	0.60 ± 0.14	0.63 ± 0.09
20:3n-6	0.02 ± 0.003	0.37 ± 0.29	0.59 ± 0.11
20:4n-6	0.31 ± 0.05	2.0 ± 0.4	1.5 ± 0.2[a]
22:2n-6	0.01 ± 0.001	0.06 ± 0.01	0.07 ± 0.01
22:4n-6	0.11 ± 0.01	0.42 ± 0.12	0.23 ± 0.05[b]
22:5n-6	0.04 ± 0.01	0.86 ± 0.17	0.26 ± 0.07[b]
Total n-6 PUFA	2.4 ± 0.3	63 ± 10	63 ± 6
n-3 PUFA			
d5-18:3n-3	–	4.4 ± 0.7	4.2 ± 0.5
18:3n-3	0.16 ± 0.05	0.18 ± 0.05	0.15 ± 0.03
d5-20:5n-3	–	0.07 ± 0.04	0.09 ± 0.04
20:5n-3	0.04 ± 0.01	0.02 ± 0.01	0.12 ± 0.03[b]
d5-22:5n-3	–	0.11 ± 0.04	0.12 ± 0.05
22:5n-3	0.10 ± 0.02	0.03 ± 0.01	0.16 ± 0.04[b]
d5-22:6n-3	–	0.35 ± 0.07	0.42 ± 0.08
22:6n-3	0.19 ± 0.03	0.11 ± 0.02	4.5 ± 1.1[b]
Total n-3 PUFA	0.49 ± 0.10	5.3 ± 0.8	9.8 ± 1.8[b]
Total fatty acids	17 ± 5	526 ± 84	520 ± 52

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

heart and skeletal muscle showed the greatest decreases in biosynthesized d5-DPA for the d5-LNA + DHA diet group, at 3.8- and 2.6-fold, respectively, of the d5-LNA diet group. Plasma also exhibited a comparatively large decrease in d5-DPA at 2.5-fold of the d5-LNA diet group. In contrast to the findings for biosynthesized d5-DPA, in all of the whole organs/tissues, unlabeled DPA was found to be significantly increased in the d5-LNA + DHA compared with the d5-LNA diet group. For the internal organs, liver showed the greatest increase in unlabeled DPA, at 12-fold of the d5-LNA diet group. With respect to the various other tissues, bone and visceral adipose exhibited the largest increase in unlabeled DPA, both at ~6.3-fold of the d5-LNA diet group. Plasma had a comparatively larger increase in unlabeled DPA at 13-fold of the d5-LNA diet group.

Unlabeled DHA and d5-DHA composition of rat pup organs

DHA is the terminal product in the biosynthetic pathway that begins with dietary LNA. For the experimental diet replete in preformed unlabeled DHA (d5-LNA + DHA diet), the formation of biosynthesized d5-DHA would be anticipated to be decreased, but the tissue accumulation of unlabeled DHA, from the constant input of dietary DHA, would be large. Consistent with this, in all tissues except brown, white, and visceral adipose, there was a significant decrease in the amount of biosynthesized d5-DHA content in the d5-LNA + DHA compared with the d5-LNA diet group. Whereas brown and visceral adipose showed no significant or near-significant differences between the two diet groups, the effect on biosynthesized d5-DHA in white adipose, although only of near significance ($P = 0.022$), was in the opposite direction to that observed for the other tissues, inasmuch as it displayed increased d5-DHA in the d5-LNA + DHA diet group. The retina showed the most marked and significant decrease in biosynthesized d5-DHA, at 5.1-fold of the d5-LNA diet group. With respect to the major internal organs, however, brain, liver, and kidneys showed the greatest decrease in the amount of biosynthesized d5-DHA present, with each at ~3.9-fold of the d5-LNA diet group. For the other tissues, bone and skeletal muscle exhibited the greatest decrease, with each at ~2.5-fold of the d5-LNA diet group. Plasma exhibited a decrease in unlabeled DPA of 2.9-fold that of the d5-LNA diet group.

As anticipated, all tissues showed a dramatic increase in unlabeled DHA content in the d5-LNA + DHA compared with the d5-LNA diet group. Of the internal organs, digestive tract and spleen showed the largest increases, both at ~21-fold of the d5-LNA diet group. Of the other tissues, visceral fat exhibited the greatest increase, at 43-fold of the d5-LNA diet group. Plasma exhibited an increase in unlabeled DHA of 20-fold that of the d5-LNA diet group. As a direct result of the marked accumulation of unlabeled DHA in the d5-LNA + DHA diet group, there was a corresponding significant increase in the total n-3 PUFA content of all organs/tissues within this diet group.

Percent distribution of d5-DHA in rat pup organs

Table 14 shows the biosynthesized d5-DHA in the tissues of the d5-LNA + DHA versus d5-LNA diet groups (n = 6 and 7, respectively) expressed as a percentage of the total-body d5-DHA that was accumulated. The total-body amount of d5-DHA found in the d5-LNA + DHA diet group was 2.3-fold lower than that of the d5-LNA diet group; whereas the corresponding amount of total-body unlabeled DHA was 12-fold higher than the d5-LNA diet group. Of the internal organs and other tissues, the greatest percent of whole-body distribution of d5-DHA was found in the liver and skeletal muscle, respectively, for both diet groups. Although skeletal muscle did not differ between the diet groups in the percent distribution of d5-DHA, there were differences detected for many of the other tissues examined. The differing internal organs as represented by brain, retina, digestive tract, liver, kidneys, and testes all had a significantly lower percentage (1.7-, 2.2-, 1.3-, 1.8-, 1.7-, and

TABLE 12. Brain fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total brain	
Saturates			
10:0	0.02 ± 0.01	0.05 ± 0.03	0.01 ± 0.003[b]
12:0	0.01 ± 0.003	0.01 ± 0.01	0.008 ± 0.005
14:0	0.30 ± 0.03	0.13 ± 0.02	0.21 ± 0.10
16:0	4.2 ± 0.3	11 ± 1	10 ± 0
18:0	2.0 ± 0.1	9.3 ± 0.8	9.5 ± 0.5
20:0	0.01 ± 0.001	0.20 ± 0.04	0.22 ± 0.03
22:0	0.007 ± 0.001	0.20 ± 0.04	0.24 ± 0.03
24:0	0.007 ± 0.002	0.39 ± 0.09	0.45 ± 0.04
Total saturates	7.1 ± 0.6	23 ± 2	23 ± 1
Monounsaturates			
16:1n-7	0.21 ± 0.01	0.13 ± 0.01	0.16 ± 0.01[b]
18:1n-7	0.35 ± 0.03	1.4 ± 0.1	1.4 ± 0.1
18:1n-9	1.5 ± 0.1	7.6 ± 0.7	7.7 ± 0.5
20:1n-9	0.03 ± 0.003	0.25 ± 0.26	0.50 ± 0.07[a]
22:1n-9	0.002 ± 0.0004	0.06 ± 0.01	0.06 ± 0.01
24:1n-9	0.01 ± 0.02	0.66 ± 0.30	0.65 ± 0.09
Total monounsaturates	2.2 ± 0.2	11 ± 1	11 ± 1
n-6 PUFA			
18:2n-6	0.13 ± 0.02	0.35 ± 0.04	0.45 ± 0.04[b]
18:3n-6	0.01 ± 0.001	0.004 ± 0.001	0.004 ± 0.001
20:2n-6			
20:3n-6	0.06 ± 0.01	0.21 ± 0.02	0.31 ± 0.05[b]
20:4n-6	1.8 ± 0.1	5.0 ± 0.3	4.7 ± 0.2
22:2n-6			
22:4n-6	0.39 ± 0.03	1.5 ± 0.1	1.3 ± 0.1[b]
22:5n-6	0.21 ± 0.02	1.2 ± 0.1	0.25 ± 0.02[b]
Total n-6 PUFA	2.6 ± 0.2	8.3 ± 0.5	7.0 ± 0.3[b]
n-3 PUFA			
d5-18:3n-3	–	0.002 ± 0.0002	0.002 ± 0.0002
18:3n-3	0.0008 ± 0.0003	ND	ND
d5-20:5n-3	–	0.002 ± 0.001	0.004 ± 0.001[b]
20:5n-3	0.007 ± 0.001	0.001 ± 0.0001	0.01 ± 0.004[b]
d5-22:5n-3	–	0.03 ± 0.002	0.02 ± 0.01[b]
22:5n-3	0.05 ± 0.004	0.02 ± 0.003	0.06 ± 0.01[b]
d5-22:6n-3	–	2.4 ± 0.3	0.63 ± 0.03[b]
22:6n-3	1.6 ± 0.1	3.1 ± 0.4	6.3 ± 0.3[b]
Total n-3 PUFA	1.6 ± 0.1	5.6 ± 0.4	7.1 ± 0.3[b]
Total fatty acids	13 ± 1	46 ± 3	48 ± 2

ND, not detected(i.e., < 0.0001 mg/total brain). Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet). 20:2n-6 and 22:2n-6 were not reported; and this table was originally found in Ref. 10.

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

1.4-fold, respectively) of the total d5-DHA in the d5-LNA + DHA compared with the d5-LNA diet group. In contrast, the other tissues, as represented by skin and brown, white, and visceral adipose, all had a significantly higher percentage (1.3-, 2.1-, 3.0-, and 2.7-fold, respectively) of the total d5-DHA in the d5-LNA + DHA diet group. This may indicate that a redistribution of biosynthesized DHA out of these latter tissues, which are key sites of fat storage, and into the major internal organs occurs when DHA is absent from the diet, as in the d5-LNA diet group.

Table 15 shows the unlabeled DHA content, in milligrams, of the whole organs and tissues of the 8 day-old dam-reared reference group compared with 28 days in the d5-LNA and d5-LNA + DHA dietary groups (n = 7, 7, and 6, respectively). These data indicate how much DHA has been accumulated in each organ over the 3 week period of the experiment. Table 15 indicates that most organs had a substantial accumulation of DHA when animals were fed the d5-LNA + DHA diet. In contrast, most organs had a net loss of DHA when animals were fed the d5-LNA diet.

Fatty acid composition of rear leg muscle subtypes

Supplementary Tables VII–IX show the fatty acid compositional results obtained for red gastrocnemius (fast-oxidative), white gastrocnemius (fast-glycolytic), and soleus (slow-oxidative) leg muscle fiber types, respectively. Because these muscle fiber types are not a distinct whole-organ compartment, and for purposes of comparison, the data are expressed as milligrams fatty acid/gram tissue. Of the three muscle types, red gastrocnemius displayed the most significant differences between the d5-LNA + DHA and d5-LNA diet groups. Red gastrocnemius in the d5-LNA + DHA diet group had significantly decreased amounts of 18:1n-9, 20:1n-9, total monounsaturates, LA, 20:2n-6, AA, 22:4n-6, 22:5n-6, total n-6 PUFA, d5-LNA, unlabeled LNA, d5-DPA, and d5-DHA, with d5-LNA and unlabeled LNA

TABLE 13. Liver fatty acid composition of the two experimental diet and 8 day-old dam-reared reference groups

Fatty Acid	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total liver	
Saturates			
10:0	0.3 ± 0.5	0.08 ± 0.09	0.01 ± 0.03
12:0	1.3 ± 1.3	0.40 ± 0.29	0.14 ± 0.04
14:0	1.7 ± 1.5	1.7 ± 1.1	0.7 ± 0.3
16:0	7.2 ± 4.0	23 ± 8	20 ± 2
18:0	2.7 ± 1.0	28 ± 5	28 ± 2
20:0	0.02 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
22:0	0.02 ± 0.004	0.13 ± 0.02	0.14 ± 0.01
24:0	0.05 ± 0.01	0.41 ± 0.09	0.33 ± 0.04
Total saturates	13 ± 8	53 ± 14	49 ± 3
Monounsaturates			
16:1n-7	0.31 ± 0.25	0.55 ± 0.50	0.22 ± 0.06
18:1n-7	0.65 ± 0.38	1.4 ± 1.0	0.61 ± 0.13
18:1n-9	3.9 ± 2.4	40 ± 26	18 ± 7
20:1n-9	0.07 ± 0.03	0.65 ± 0.26	0.51 ± 0.16
22:1n-9	0.01 ± 0.004	0.05 ± 0.01	0.05 ± 0.005
24:1n-9	0.04 ± 0.007	0.55 ± 0.08	0.68 ± 0.06[b]
Total monounsaturates	5.0 ± 3.1	43 ± 28	20 ± 8
n-6 PUFA			
18:2n-6	3.8 ± 2.0	8.5 ± 3.3	7.6 ± 1.8
18:3n-6	0.08 ± 0.05	0.26 ± 0.14	0.09 ± 0.02[a]
20:2n-6			
20:3n-6	0.36 ± 0.15	0.64 ± 0.20	1.2 ± 0.2[b]
20:4n-6	3.5 ± 1.1	26 ± 5	20 ± 2[a]
22:2n-6			
22:4n-6	0.67 ± 0.23	0.8 ± 0.2	0.21 ± 0.05[b]
22:5n-6	0.40 ± 0.14	6.7 ± 0.8	0.19 ± 0.03[b]
Total n-6 PUFA	8.8 ± 3.5	43 ± 9	30 ± 1[b]
n-3 PUFA			
d5-18:3n-3	–	0.17 ± 0.08	0.16 ± 0.07
18:3n-3	0.21 ± 0.17	ND	ND
d5-20:5n-3	–	0.05 ± 0.02	0.17 ± 0.04[b]
20:5n-3	0.27 ± 0.14	0.01 ± 0.007	0.71 ± 0.22[b]
d5-22:5n-3	–	0.47 ± 0.09	0.15 ± 0.04[b]
22:5n-3	1.1 ± 0.4	0.04 ± 0.02	0.53 ± 0.12[b]
d5-22:6n-3	–	9.9 ± 1.6	2.5 ± 0.2[b]
22:6n-3	3.6 ± 1.0	1.5 ± 0.3	22 ± 2[b]
Total n-3 PUFA	5.2 ± 1.7	12 ± 2	26 ± 2[b]
Total fatty acids	32 ± 16	145 ± 51	124 ± 11

ND, not detected(i.e., < 0.0001 mg/total liver). Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet). 20:2n-6 and 22:2n-6 were not reported; and this table was originally found in Ref. 10.

[a] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P = 0.010–0.050$.

[b] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.010$.

TABLE 14. d5-DHA accumulated in rat tissues expressed as percentage of total-body d5-DHA

Tissues	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
	% mg/total body d5-DHA	
Plasma	2.1 ± 0.4	1.7 ± 0.6
Brain	7.5 ± 1.2	4.4 ± 0.4[d]
Retina	0.15 ± 0.03	0.07 ± 0.01[d]
Heart	1.2 ± 0.1	1.0 ± 0.2
Lung	0.45 ± 0.06	0.44 ± 0.05
Spleen	0.15 ± 0.03	0.13 ± 0.02
Digestive tract[a]	5.6 ± 0.3	4.3 ± 0.2[d]
Liver	30 ± 3	17 ± 1[d]
Kidney	1.1 ± 0.1	0.66 ± 0.11[d]
Testes	0.35 ± 0.06	0.24 ± 0.03[d]
Skin	5.0 ± 0.4	6.6 ± 0.8[d]
Brown adipose	3.8 ± 0.9	8.2 ± 1.0[d]
White adipose	6.7 ± 1.3	20 ± 2[d]
Visceral adipose	1.1 ± 0.1	2.9 ± 0.4[d]
Skeletal muscle[b]	32 ± 2	30 ± 4
Bones[c]	2.7 ± 0.3	2.4 ± 1.0
Total-body d5-DHA, mg	33 ± 5	14 ± 2[d]

Values were calculated by dividing the d5-DHA content of each tissue (Tables 1–13 and supplementary Tables III–IX) by the total-body d5-DHA as shown here. Data are given as percent total-body d5-DHA ± SD (n = 7 for d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Digestive tract includes the stomach, small and large intestines, and pancreas.

[b] Skeletal muscle was determined from fatty acid concentration data derived from thigh muscle samples.

[c] Bone was determined from fatty acid concentration data derived from femur bone samples.

[d] Statistically different values between d5-LNA and d5-LNA + DHA diet groups of $P < 0.01$.

approaching significance ($P = 0.041$ and 0.018, respectively); but there were significantly increased amounts of unlabeled EPA, unlabeled DPA, unlabeled DHA, and total n-3 PUFA. White gastrocnemius and soleus leg muscles were nearly identical in their significant differences, both displaying in the d5-LNA + DHA diet group significantly decreased amounts of 22:4n-6, 22:5n-6, d5-DPA, and d5-DHA, but there were significantly increased amounts of unlabeled DPA, unlabeled DHA, and total n-3 PUFA. White gastrocnemius additionally showed significantly decreased amounts of AA in the d5-LNA + DHA diet group, whereas soleus had significantly decreased amounts of total n-6 PUFA, as well as increased amounts of d5-EPA approaching significance ($P = 0.044$). The significant differences between the diet groups as noted above for red gastrocnemius were the most similar of the three muscle types to that reported for total-body skeletal muscle (Table 7), whose data were derived by extrapolation from analysis of mixed thigh muscle tissue.

DISCUSSION

This study focused upon the issue of whether inclusion of preformed DHA in the diet decreases the net accretion of DHA derived from dietary LNA in growing rat pups. We have expanded upon our previous work (10) that examined only in the brains and livers of rat pups during development (p8–p28) whether inclusion of preformed DHA in the diet decreases the net accretion of DHA derived from dietary LNA. We have now obtained similar data for all the other major tissues of this same set of animals. Our study is novel in that it is able to examine the total accumulation of biosynthesized d5-DHA in various tissues over an extended time period. We achieved this result due to the complete and continuous replacement of LNA in the animals' diet with the stable isotope-labeled LNA (d5-LNA). If the animals are not provided any unlabeled n-3 PUFA during the feeding experiment, any unlabeled n-3 PUFA present in the tissues at the end of the study must be that remaining from prior accumulated stores of these fatty acids. Our study is also unique in that the experimental feeding and development time period examined, p8–p28, is one in

TABLE 15. Unlabeled DHA present in dam-reared rat tissues at 8 days and after 28 days on d5-LNA and d5-LNA + DHA diets

Tissues	8 Day Dam-reared	28 Day-old d5-LNA Diet	28 Day-old d5-LNA + DHA Diet
		mg/total tissue	
Plasma	0.11 ± 0.02	0.10 ± 0.03	2.1 ± 0.9[e]
Brain	1.6 ± 0.1	3.1 ± 0.4[e]	6.3 ± 0.3[e]
Retina	0.022 ± 0.004	0.053 ± 0.005[e]	0.091 ± 0.003[e]
Heart	0.16 ± 0.03	0.12 ± 0.02[d]	1.5 ± 0.2[e]
Lung	0.27 ± 0.03	0.04 ± 0.01[e]	0.70 ± 0.08[e]
Spleen	0.020 ± 0.007	0.010 ± 0.003[e]	0.20 ± 0.05[e]
Digestive tract[a]	0.60 ± 0.13	0.34 ± 0.06[e]	7.6 ± 0.8[e]
Liver	3.6 ± 1.0	1.5 ± 0.3[e]	22 ± 2[e]
Kidney	0.13 ± 0.03	0.07 ± 0.01[e]	1.0 ± 0.2[e]
Testes	0.009 ± 0.002	0.024 ± 0.01[e]	0.29 ± 0.04[e]
Skin	2.4 ± 0.5	0.50 ± 0.10[e]	12 ± 3[e]
Brown adipose	0.89 ± 0.19	1.8 ± 0.5[e]	15 ± 1[e]
White adipose	3.4 ± 1.3	0.86 ± 0.28[d]	34 ± 7[e]
Visceral adipose	0.19 ± 0.03	0.11 ± 0.02[e]	4.5 ± 1.1[e]
Skeletal muscle[b]	1.1 ± 0.2	4.3 ± 0.6[e]	46 ± 9[e]
Bones[c]	0.40 ± 0.08	0.21 ± 0.04[e]	3.8 ± 1.1[e]
Total body unlabeled DHA, mg	15 ± 4	13 ± 2	156 ± 26[e]

Data represent means ± SD (n = 7 for 8 day dam-reared and d5-LNA diet, n = 6 for d5-LNA + DHA diet).

[a] Digestive tract includes the stomach, small and large intestines, and pancreas.

[b] Sketetal muscle was determined from fatty acid concentration data derived from thigh muscle samples.

[c] Bone was determined from fatty acid concentration data derived for femur bone samples.

[d] Statistically different values between the 8 day dam-reared versus the d5-LNA or d5-LNA + DHA diet group of $P = 0.010–0.050$.

[e] Statistically different values between the 8 day dam-reared versus the d5-LNA or d5-LNA + DHA diet group of $P < 0.010$.

which the rat pups are undergoing an intense spurt in brain growth (61). Accelerated growth of all other tissues throughout the body is also clearly evident in the data presented in supplementary Table I, which show an increase in organ/tissue masses of 2- to 24-fold between 8 and 28 days of age. Overall, the p8–p28 rat pup model is somewhat analogous to the growth period of brain and other organs that occurs in humans from ~28 week gestation to 2–3 years of age (61); however, it must be taken into account that the basal metabolic rate of infant rats and humans differs greatly, which may produce major differences in lipid metabolism within some tissues.

One central question in human infant nutrition is whether the DHA demands of the developing brain, retina, and other organs can be supported by providing sufficient dietary LNA alone or if the inclusion of dietary DHA is necessary. It is known that young mammals provided with diets sufficient in LNA (>1% of energy intake) do not attain the brain or retinal levels of DHA associated with animals that consume preformed dietary DHA (>0.25% of energy intake) (12, 35–37). Several stable-isotope studies have supported this contention by demonstrating that dietary LNA is not bioequivalent to preformed dietary DHA in supplying DHA to various tissues (62–64). The conversion efficiency of LNA to DHA appears to be rather low as measured within the plasma, with the highest human values reportedly found in pregnant women and term infants at 9% and 4%, respectively, and the lowest in adult males at ≤0.1% (63–70); however, this is a combined reflection of the liver synthesis, plasma secretion, catabolic rate, and uptake of DHA by the peripheral tissues such as the brain (40, 42). In our previous study (10), we found that accretion of unlabeled DHA in the liver and brain of the d5-LNA + DHA diet group was 2- and 3-fold, respectively, higher than the d5-DHA accreted in the d5-LNA diet group, which is basically consistent with the amount of dietary unlabeled DHA being twice that of the d5-LNA included in both diets. In our present study, however, we have now found that the total-body unlabeled DHA accumulation in the d5-LNA + DHA diet group was 5-fold higher than the total d5-DHA found in the LNA diet group (Tables 14 and 15); and this strongly suggests that preformed DHA is a much more efficient source for tissue accretion of DHA than is dietary LNA in developing rat pups, as has been reported for infant primates (62, 63). A very high accumulation of the total d5-DHA in both diet groups was seen for the connective tissues (skin, adipose, skeletal muscle, and bones combined), at 50–70% of the total body distribution, with skeletal muscle containing the most at ~30% (Table 14). The brain and liver in both diets, however, each contained 4–7% and 17–30%, respectively, of the total biosynthesized d5-DHA. Preferential accumulation of dietary DHA has been noted before in the whole skin and carcass (adipose, skeletal muscle, and bones) of rats (71). Overall, our results suggest that the principal source of DHA for rat pup tissues is preformed dietary DHA and not DHA synthesized from dietary LNA. Although in our study, on a whole-body basis, dietary preformed DHA is more efficient than dietary LNA for supplying DHA to tissues in the developing rat pup, this does not imply that a much higher dietary level of LNA than that used in our study would provide the same tissue content of DHA, inasmuch as it has been shown that even extremely high intakes of LNA cannot support the nervous system levels observed for much smaller DHA dietary content (35–37).

In the present study, we found that the presence of dietary preformed DHA decreases the net biosynthesis/accretion of d5-DHA from d5-LNA in the tissues of growing rat pups by 2- to 5-fold, in comparison to those receiving a diet containing LNA alone. As anticipated, the nervous system, brain, and retina were among the tissues most sensitive to the inclusion of DHA in the diets (5.1- and 4.0-fold decrease, respectively), but we also found that the kidneys, liver, and testes ranked rather high in their responses (3.9-, 3.9-, and 3.3-fold decrease, respectively). These findings are consistent with liver being the major site of DHA biosynthesis in the body (72), with the kidneys and testes also having a high importance for DHA content for renal function and spermatogenesis (73–76). Interestingly, the heart, lungs, and bones were found to have somewhat lower responses in this respect (2.6-, 2.4-, and 2.5-fold, respectively). This is somewhat unexpected, because DHA has been implicated in maintaining cardiac rhythm, respiratory surfactant formation, and mineralization by osteoclasts (77–82), but it should be noted that these tissues still exhibit a marked effect (more than 2-fold) of dietary DHA upon d5-DHA accretion.

The decreases in d5-DHA accumulation that we noted for the tissues are probably due primarily to changes in DHA biosynthesis in liver hepatocytes (72), with the other tissues more reflective of changes in DHA uptake from the plasma thereafter. It is known, however, that skin fibroblasts, lung/bronchial epithelium, retinal pigmented epithelium, and brain astrocytes show some capability to synthesize DHA from LNA (83–86). Thus a portion of the biosynthesized d5-DHA may possibly have arisen from tissues outside of the liver.

Inside the liver hepatocytes, expression of the Δ-5 and Δ-6 desaturases, utilized in the biosynthesis of DHA from LNA, is positively controlled by the transcription factors SREBP-1 and NF-Y (sterol regulatory element binding protein-1 and nuclear factor-Y), which are both turned off when dietary DHA is abundant (87, 88). Anticipated decreases did occur in d5-DPA and d5-DHA content of most tissues in the d5-LNA + DHA diet group, providing support for the existence of this product feedback inhibition mechanism in the DHA biosynthetic pathway of the developing rat pup. In contrast, there were substantial increases in tissue d5-EPA, unlabeled EPA, and unlabeled DPA content, but this may be due in part to retro-conversion processes acting on these fatty acids and DHA (89, 90). Unexpectedly, tissue levels of d5-LNA were not increased in the d5-LNA + DHA diet group, an anticipated end result of decreased utilization of LNA toward DHA synthesis; however, some excess d5-LNA could have been lost through increased gene expression of enzymes involved in fatty acid β-oxidation by PPAR (peroxisomal proliferation-activated receptor) transcription factors that use DHA as an activating ligand (7, 91, 92).

Surprisingly, in brown, white, and visceral adipose tissues, we found that the accumulation of d5-DHA as well as d5-DPA was insensitive to the inclusion of preformed DHA in the diet. This unresponsiveness was not observed for any of the other tissues examined, in that they all showed significant decreases in d5-DPA and d5-DHA content for the d5-LNA + DHA diet group relative to the d5-LNA diet group. Although we cannot rule out possible tissue-specific differences in fatty acid uptake and transport, the insensitivity of d5-DHA incorporation into adipose to the presence of dietary DHA is likely to be due to adipose tissue storing fatty acids primarily in triglycerides, whereas other tissues store this fatty acid mainly in the phospholipid form. The activities of enzymes controlling the acylation of DHA into phospholipids are sensitive to tissue levels of DHA (43, 93, 94). In contrast, it has been shown that dietary DHA does not influence the activity of triglyceride acylation; thus, plasma d5-DHA would be more equally incorporated into adipose triglycerides in both diet groups (95, 96).

In our study, we examined the tissue fatty acid composition of 8 day-old dam-reared rat pups (Tables 1–13 and supplementary Tables III–IX) prior to the 20 day (p8–p28) administration of the d5-LNA and d5-LNA + DHA diets, as a baseline reference group. This allowed us to quantify the changes in unlabeled endogenous fatty acids at 20 days over baseline (p28 versus p8), especially that of unlabeled DHA. As anticipated, providing dietary preformed DHA dramatically increased unlabeled DHA in all tissues by 3- to 41-fold above the p8 baseline group level (Table 15). Coinciding with its large mass, skeletal muscle had the greatest increase in unlabeled DHA, at 41-fold above the p8 baseline group. Of all the internal organs, the testes had the largest elevation in unlabeled DHA, at 32-fold above the p8 baseline group, and this agrees with rapid sexual maturation of the p8–p28 male animals (75, 76). On a total-body basis, the d5-LNA + DHA diet group had a 10-fold increase in unlabeled DHA over the p8 baseline group. In the d5-LNA diet group, an absence of preformed dietary DHA apparently led to the redistribution among tissues of body stores of unlabeled DHA (Table 15). Although on a total-body basis there was no difference detected in unlabeled DHA content between the d5-LNA diet and the p8 baseline groups, all organs/tissues except plasma showed significant alterations in unlabeled DHA content over baseline. The lack of a difference in total-body unlabeled DHA content between the two groups, however, suggests that a balanced reshuffling of unlabeled DHA occurred between the various organs/tissues. Brain, retina, testes, skeletal muscle, and brown adipose all showed an increase of unlabeled DHA of 2- to 3-fold above the p8 baseline group, suggesting movement of unlabeled DHA into these highly important organs and tissues. In contrast, liver, digestive tract, bone, skin, white adipose (near significance; $P = 0.021$), and visceral adipose incurred net decreases in unlabeled DHA at 2- to 5-fold less than the p8 baseline group, which implies an efflux of DHA from these organs and tissues, which are known to process and/or store fatty acids. Even more troubling, however, were the decreases noted in unlabeled DHA content of the heart (near significance; $P = 0.010$), lungs, kidneys, and spleen at 1.3-, 7.6-, 1.9-, and 2.0-fold less than the p8 baseline group, respectively. The optimal functioning of these latter organs is no doubt critical for early development in infant mammals, and decreases in DHA content during rapid growth may potentially lead to a compromise in their functioning in order to allow the developing brain, retina, skeletal muscle, and testes to accumulate DHA. Movement of DHA into brown adipose, which is lost as infants mature, may be critical for supporting thermogenesis. Thus, our findings may have unforeseen health implications for human infants being continually fed formula that contains LNA as the sole source of n-3 PUFA.

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