Whole-Body Docosahexaenoic Acid Synthesis-Secretion Rates in Rats Are Constant across a Large Range of Dietary \(\alpha\)-Linolenic Acid Intakes\(^1\)\(^–\)\(^3\)

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Abstract

Background: Docosahexaenoic acid (DHA) is an \(\omega\)-3 (n–3) polyunsaturated fatty acid (PUFA) thought to be important for brain function. Although the main dietary source of DHA is fish, DHA can also be synthesized from \(\alpha\)-linolenic acid (ALA), which is derived from plants. Enzymes involved in DHA synthesis are also active toward \(\omega\)-6 (n–6) PUFAs to synthesize docosapentaenoic acid n–6 (DPA–n–6). It is unclear whether DHA synthesis from ALA is sufficient to maintain brain DHA.

Objective: The objective of this study was to determine how different amounts of dietary ALA would affect whole-body DHA and DPA–n–6 synthesis rates.

Methods: Male Long-Evans rats were fed an ALA-deficient diet (ALA-D), an ALA-adequate (ALA-A) diet, or a high-ALA (ALA-H) diet for 8 wk from weaning. Dietary ALA concentrations were 0.07%, 3%, and 10% of the fatty acids, and ALA was the only dietary PUFA that differed between the diets. After 8 wk, steady-state stable isotope infusion of labeled ALA and linoleic acid (LA) was performed to determine the in vivo synthesis-secretion rates of DHA and DPA–n–6.

Results: Rats fed the ALA-A diet had an ~2-fold greater capacity to synthesize DHA than did rats fed the ALA-H and ALA-D diets, and a DHA synthesis rate that was similar to that of rats fed the ALA-H diet. However, rats fed the ALA-D diet had a 750% lower DHA synthesis rate than rats fed the ALA-A and ALA-H diets. Despite enrichment into arachidonic acid, we did not detect any labeled LA appearing as DPA–n–6.

Conclusions: Increasing dietary ALA from 3% to 10% of fatty acids did not increase DHA synthesis rates, because of a decreased capacity to synthesize DHA in rats fed the ALA-H diet. Tissue concentrations of DPA–n–6 may be explained at least in part by longer plasma half-lives. J Nutr 2017;147:37–44.

Keywords: \(\alpha\)-linolenic acid, docosahexaenoic acid, kinetics, \(\omega\)-3 PUFA, synthesis

Introduction

The \(n\)-3 PUFA DHA (22:6n–3) is highly concentrated in the brain (1–4), where it regulates numerous functions, including cell survival and neuroinflammation (5–9). The brain takes up DHA from the blood, which is maintained directly via the diet or from DHA that has been synthesized in the liver (10). The main precursor to DHA in Western diets is \(\alpha\)-linolenic acid (18:3n–3) (ALA)\(^6\) (11–14). DHA synthesis rates are believed to be low and insufficient to maintain optimal brain DHA during

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\(^3\) Supplemental Tables 1 and 2 and Supplemental Figure 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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\(^6\) Abbreviations used: ALA, \(\alpha\)-linolenic acid; ALA-A, \(\alpha\)-linolenic–adequate; ALA-D, \(\alpha\)-linolenic–deficient; ALA-H, high–\(\alpha\)-linolenic acid; ARA, arachidonic acid; DPA–n–6, docosapentaenoic n–6; LC–MS/MS, LC tandem MS; LA, linoleic acid; LC–tandem MS; LCPUFA, long-chain PUFA; TLE, total lipid extract; \(V_{serum}\), serum volume.
early development (15–18). However, a recent study performed in rodents with the use of a novel steady-state infusion to measure DHA synthesis concluded that DHA synthesis from ALA appears sufficient to supply the brain (19, 20).

How dietary components affect DHA synthesis is not completely understood. The enzymes that synthesize DHA from ALA are also thought to be used by the n-6 PUFA linoleic acid (18:2n-6) (LA) to synthesize arachidonic acid (20:4n-6) (ARA) and docosapentaenoic acid n-6 (22:5n-6) (DPA-n-6), the n-6 PUFA homolog to DHA; therefore, it is possible that competition exists between n-6 and n-3 PUFAs for these enzymes. It is generally accepted that the most competition exists for the Δ6 desaturase enzyme, which is believed to be rate limiting for DHA synthesis, because it is reactive to both 18- and 24-carbon FAs of the n-3, n-6, and n-9 family (21, 22). In addition, it has been shown that dietary DHA can decrease the expression of enzymes involved in its own synthesis (23), thereby decreasing the rate of its synthesis (19). Similar evidence has been reported for ALA, with studies showing that, compared with rats fed an n-3 PUFA–deficient diet, rats consuming dietary ALA have decreased expression of enzymes involved in DHA synthesis (24) and decreased DHA synthesis capacity (25). Despite the fact that dietary ALA decreases DHA synthesis capacity, however, in vivo DHA synthesis rates were higher in rats fed ALA than they were in those fed a diet deficient in n-3 PUFAs because of a lack of substrate in the deficient rats (19, 25).

Current North American ALA intake is supplied primarily by consumption of vegetable oils (26); however, the food industry is increasingly producing high–ω3 acid varieties of vegetable oil. These varieties of vegetable oil contain reduced amounts of ALA (27–29), which could lead to a population-wide decrease in ALA intake. Therefore, this study aimed to assess the potential impact of decreased dietary ALA intake by performing a dietary dose-response to determine how different amounts of dietary ALA affect the rates of synthesis-secretion of ALA and LA to their long-chain PUFA (LCPUFA) products in rats. With the use of a novel steady-state infusion technique the synthesis secretion rates of DHA, EPA (20:5n-3; an n-3 PUFA also found in fish), ARA, and DPA-n-6 were determined in rats fed diets with markedly different amounts of dietary ALA.

Methods

Rats. All procedures were performed in accordance with the policies set out by the Canadian Council on Animal Care and were approved by the Animal Ethics Committee at the University of Toronto. Male 21-d-old Long-Evans rats were randomly assigned to an α-linolenic acid-deficient (ALA-D) diet (n = 8), an α-linolenic acid–adequate (ALA-A) diet (n = 8), or a high-α-linolenic acid (ALA-H) (n = 6) diet (containing 0.7%, 3%, or 9.9% of FAs as ALA). Rats consumed these diets ad libitum with ad libitum access to water for 8 wk, at which time they underwent surgery to implant catheters in the carotid artery and jugular vein. After 3 d recovery from surgery, rats were subjected to an infusion of 2H5-ALA and 13C18-LA (described below). Food intake and weight gain throughout the study were not measured, thus limiting any interpretation of the effect of these diets on body composition.

Diets. The diets were custom-synthesized on the basis of the AIN-93G custom low-n-3 diet (Dyets Inc.) (19, 30). Diets were isocaloric, and each diet contained 90% of FAs (by weight) as hydrogenated coconut oil (57.2% by weight) and safflower oil (32.8% by weight). Ten percent of the FAs (by weight) consisted of added ethyl esters, which allowed for manipulation of the ALA content of the diet without changing the whole FA composition of the diet [full dietary composition is reported in Supplemental Table 1 and Reeves et al. (31)]. The only FAs that differed between the 3 diets used in this study were ALA and oleate (18:1n-9). The ALA-D diet contained ethyl ester oleate (Nu-Chek Prep) added at a concentration of 10% of the FAs (by weight). The only n-3 PUFA in this diet was the residual ALA found in the safflower oil. The ALA-A diet contained ethyl ester oleate added at 7% of the FAs and ethyl ester ARA [generously donated by BASF Pharma (Callanish, Isle of Lewis)] added at 3% of the FAs (by weight). The ALA-H diet contained ethyl ester ALA added at 10% of the FAs (by weight) and no ethyl ester oleate. The full dietary FA composition as measured by GC-flame ionization detection is reported in Supplemental Table 2.

Surgery. Similar to our previous reports (19, 32), after the rats consumed their respective diets for 8 wk, they were subjected to surgery to implant catheters in the jugular vein and carotid artery [surgical method and baseline blood draw method reported elsewhere (32)].

2H5-ALA and 13C18-LA infusion. As reported previously, U-13C18-LA ethyl ester (Cambridge Isotope Laboratories) was converted to free FA by hydrolysis (32). As modified from Domenichiello et al. (19), infusion was made by dissolving 4.5 μmol U-13C18-LA free acid and 2H5-ALA free acid/100 g body weight (Cambridge Isotope Laboratories) into 5 mM HEPES buffer (pH 7.4) containing 100 g FA-free BS/AL. The infusate was mixed by sonication at 37°C for 45 min.

Three days after the surgery, catheters were connected to a dual channel stainless steel swivel that was connected to a single channel counterbalance arm (Instech Laboratories) and placed in a cage. A stainless steel tether (Instech Laboratories) was secured to the swivel and to a skin button that was surgically implanted on the rat. This protected the catheters from being tangled or chewed by the rat, allowing for the infusion to be performed in a completely free-living environment. Throughout the infusion, the rats had ad libitum access to food and water.

The infusion and blood sampling was performed according to our previously published reports (32). After 180 min of the infusion, 1 mL blood was drawn from the carotid artery and the rats were killed with a lethal injection of T-61 into the carotid artery, while tracer was still being infused into the jugular vein. Livers were collected immediately and flash-frozen in liquid nitrogen. Rats were killed while still being infused in an effort to ensure an accurate measurement of labeled fatty acyl-CoA products in the liver. Blood samples were processed and serum collected as previously described (19).

Serum lipid extraction. Lipids were extracted from 50 μL serum that had been spiked with internal standard with the use of the method by Folch et al. (33). For baseline serum samples, a known amount of heptadecenoic acid (17:0; Nu-Chek Prep) and di-17:0 phosphatidylcholine (Nu-Chek Prep) internal standard were added. For serum samples collected during infusions, a known amount of 2H5-ARA was added as internal standard. Total lipid extract (TLE) was stored under nitrogen gas at ~80°C.

TLC. Esterified and unesterified lipids from baseline serum TLE were separated by TLC and prepared for transmethylation as described previously (19, 32).

Transmethylation and gas chromatography-flame ionization detection. Baseline samples were transmethylated with the use of 14% boron trifluoride in methanol, and FAME were extracted with hexane and quantified with the use of GC-flame ionization detection as previously described (19).

Preparation of serum infusion samples. TLE from serum samples taken during the infusion were evaporated completely under nitrogen and reconstituted in 1 mL hexane. The TLE was then split into 2 aliquots. One aliquot of the TLE was analyzed by LC tandem MS (LC-MS/MS) to determine the concentration of the infusion (unesterified 2H5-ALA and 13C18-LA) in the serum throughout the infusion. The other aliquot of TLE was used to determine the concentration of labeled, longer-chain n-3 and n-6 PUFAs that were synthesized from 2H5-ALA and 13C18-LA throughout the infusion. Because most of these products were found in the esterified fraction of the serum, this aliquot was

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The optimized variables were as follows: declustering potential, (100 acyl-CoA in liver samples were measured by LC-MS/MS. One-half of LC-MS/MS. Labeled PUFAs in serum and labeled and unlabeled fatty LC-MS/MS (described below).

Acyl-CoA extraction. Acyl-CoA was extracted from ~0.7 g liver while following the method of Deutsch et al. (34) as modified by Chen et al. (35). Acyl-CoA concentrations were measured with the use of LC-MS/MS (described below).

LC-MS/MS. Labeled PUFAs in serum and labeled and unlabeled fatty acyl-CoA in liver samples were measured by LC-MS/MS. One-half of each sample was evaporated under nitrogen gas and reconstituted in acetonitrile (80:20, vol/vol), were detected with the use of an Agilent HPLC 1290 (Agilent Technologies) equipped with an Agilent Zorbax SB-Phenyl column (3 × 50 mm, 3.5 μm; Agilent). The initial HPLC conditions of elution were set at a 500 μL/min gradient system consisting of 1) 50% H2O and 2) 50% acetonitrile. The gradient started with 50% (1) and 50% (2), and was maintained for 1.5 min, was increased to 100% (2) from 1.5 to 6 min, and was maintained at 100% (2) for 4 min to complete the total run of 10 min. MS analyses were carried out on a QTRAP 5500 triple quadrupole mass spectrometer (AB SCIEX) in electrospray ionization, negative ion mode. The source temperature was 600°C and the ion spray voltage was ~4500 electron V. The optimized variables were as follows: declustering potential, ~40; entrance potential, ~10; collision energy, ~20; and collision cell exit potential, ~11. Mass transitions for [2H5-H2]-ALA, [2H5-H2]-EPA, and [2H5-H2]-DHA were 282.2–39.0, 306.2–262.2, and 332.2–288.2 m/z, respectively. Concentration was quantified by comparing the peak area ratios (peak of interest:internal standard) and correcting for a response factor that was determined for each FA of interest. Response factors were determined by analyzing a standard mixture of 100 ng each DHA, EPA, ALA, DPAn–6, and H2-DHA per milliliter by LC-MS/MS and comparing peak areas for each of the 4 FAs in relation to the peak area for [2H5-H2]-ALA to generate response factors. The response factors were 10, 0.75, and 0.75 for ALA, EPA, and DHA, respectively.

Serum volume determination. Serum volume (Vserum) was previously determined in our laboratory to equal 0.018 mL/g body weight in Long-Evans rats at 8 wk postweaning (32). This factor was used to estimate Vserum on the basis of the rat weights from the current study.

Steady-state infusion kinetics. The steady-state infusion method used to measure LCPUFA synthesis was designed by Rapoport et al. (36), and requires that labeled ALA and LA, in the unesterified form, be infused at a steady rate such that the concentration of these tracers achieves steady-state in the serum. Throughout the infusion, newly synthesized, labeled LCPUFAs were measured by LC-MS/MS (described below).

Serum volume determination. Serum volume (Vserum) was previously determined in our laboratory to equal 0.018 mL/g body weight in Long-Evans rats at 8 wk postweaning (32). This factor was used to estimate Vserum on the basis of the rat weights from the current study.

The maximum first derivative [Smax (nanomoles per minute)], is assumed to be the point at which the disappearance coefficient is equal to 0, or negligible. According to this assumption, Smax is determined solely by the synthesis-secretion rate for a given PUFA as described by Equations 3 and 4.

\[ S_{\text{max}} = k_{1,0 \rightarrow 3 \text{LCPUFA}} (\text{H}_5 - \text{ALA})_{\text{monomethyl}} - 0 \] (3)

\[ S_{\text{max}} = k_{1,0 \rightarrow 6 \text{LCPUFA}} (\text{C}_{18} - \text{LA})_{\text{monomethyl}} - 0 \] (4)

Therefore, if Smax is known, the synthesis-secretion coefficient can be determined. The actual synthesis rate of an LCPUFA from its plasma precursor FAs [JLCPUFA (nanomoles per minute)] is the product of the synthesis-secretion coefficient for that LCPUFA multiplied by the serum unesterified concentration of its precursor, and can be described by Equations 5 and 6.

\[ J_{\text{1-3LCPUFA}} = k_{1,0 \rightarrow 3 \text{LCPUFA}} (\text{ALA})_{\text{monomethyl}} = \frac{S_{\text{max}} (\text{ALA})_{\text{monomethyl}}}{(\text{H}_5 - \text{ALA})_{\text{monomethyl}}} \] (5)

\[ J_{\text{1-6LCPUFA}} = k_{1,0 \rightarrow 6 \text{LCPUFA}} (\text{LA})_{\text{monomethyl}} = \frac{S_{\text{max}} (\text{LA})_{\text{monomethyl}}}{(\text{C}_{18} - \text{LA})_{\text{monomethyl}}} \] (6)

In order for labeled LCPUFAs to be synthesized, the labeled precursor must be incorporated into the fatty acyl-CoA pool of the liver. The dilution of our infused tracers in the liver fatty acyl-CoA pool (λ) can be calculated with the use of Equations 7 and 8.

\[ \lambda_{\text{ALA}} = \frac{(\text{H}_5 - \text{ALA})_{\text{C}_9 \text{LCPUFA}}}{(\text{H}_5 - \text{ALA})_{\text{monomethyl}}} \] (7)

\[ \lambda_{\text{LNA}} = \frac{(\text{C}_{18} - \text{LA})_{\text{C}_9 \text{LCPUFA}}}{(\text{C}_{18} - \text{LA})_{\text{monomethyl}}} \] (8)

The DHA synthesis rate from the hepatic fatty acyl-CoA precursor in the liver (J_{\text{3-6LCPUFA-LA-CoA}} and J_{\text{4-6LCPUFA-LA-CoA}}) is described by Equations 9 and 10.

\[ J_{\text{3-6LCPUFA-LA-CoA}} = J_{\text{3-6LCPUFA}} / \lambda_{\text{ALA}} \] (9)

\[ J_{\text{4-6LCPUFA-LNAs-CoA}} = J_{\text{4-6LCPUFA}} / \lambda_{\text{LNA}} \] (10)

The diets consumed for this study contained no LCPUFAs, and it can be assumed that the serum esterified lipids were constant during the infusion period. Therefore, the turnover rate [F_{LCPUFA} (per minute) and half-life [t_{1/2,LCPUFA} (minutes)] of esterified LCPUFAs in the serum can be determined by Equations 11 and 12, respectively.

\[ F_{\text{LCPUFA}} = \frac{J_{\text{LCPUFA}}}{V_{\text{plasma}} (\text{LCPUFA})_{\text{monomethyl}}} \] (11)

\[ t_{1/2,LCPUFA} = \frac{0.693}{F_{\text{LCPUFA}}} \] (12)

Turnover rates and half-lives are the proportion of the plasma lipids that are replaced by newly synthesized PUFA over time.

Statistics. All data are presented as means ± SEMs and were compared by f-factor ANOVA with the use of Tukey’s test for multiple comparison if P ≤ 0.05 by ANOVA (GraphPad Prism, version 4.0). When data were not normally distributed as determined by Bartlett’s test for equal variances, then data were log-transformed and then compared by
1-factor ANOVA with the use of Tukey’s test for multiple comparisons. A \( P \leq 0.05 \) with Tukey’s multiple comparison test was considered to be significant.

**Results**

**Body weight.** Rats consuming the ALA-D diet had significantly lower body weights (420 ± 11 g) than rats consuming the ALA-A diet (478 ± 14 g, \( P < 0.05 \)), and the body weight of rats consuming the ALA-D diet did not differ from that of rats fed the ALA-H diet (435 ± 23 g). No differences in body weight were observed between rats fed the ALA-A and ALA-H diets.

\( V_{\text{serum}} \), \( V_{\text{serum}} \) was significantly higher in rats fed the ALA-A diet (8.6 ± 0.2 mL) than in those consuming the ALA-D diet (7.6 ± 0.2 mL, \( P < 0.05 \)). \( V_{\text{serum}} \) in rats fed the ALA-H diet (7.8 ± 0.4 mL) was not significantly different from the \( V_{\text{serum}} \) of rats fed the ALA-A and ALA-D diets.

**Serum n–3 and n–6 PUFA concentrations.** Serum unesterified ALA concentrations (Figure 1A) were higher in rats fed the ALA-H diet than those in rats fed the ALA-D and ALA-A diets, whereas serum unesterified LA concentrations (Figure 1B) did not differ significantly. Rats fed the ALA-H and ALA-A diets had significantly higher concentrations of serum esterified ALA, EPA, and DHA than did rats fed the ALA-D diet (Figure 2A–C). No differences were found for serum esterified LA and ARA (Figure 2D and E). Serum esterified DPAn–6 concentrations were ~7-fold greater in the ALA-D diet group than those in the ALA-A group, and they were ~10-fold greater in the ALA-A diet group than those in the ALA-H diet group (\( P < 0.05 \)) (Figure 2F).

**Serum \( ^2\text{H}_5 \text{n–3 PUFA and } ^{13}\text{C}_{18} \text{n–6 PUFA.** Mean concentrations of the n–3 PUFA tracer (\( ^2\text{H}_5\)-ALA) in the unesterified serum lipid fraction throughout the infusion did not differ between rats fed the 3 diets. Mean concentrations of the n–6 PUFA tracer (\( ^{13}\text{C}_{18}\)-LA) in the unesterified serum lipid fraction throughout the infusion were higher in the ALA-H diet group than those in the ALA-A diet group. Mean serum unesterified \( ^{13}\text{C}_{18}\)-LA concentrations throughout the infusion in rats fed the ALA-D diet did not differ from those in rats fed the other diets.

**n–3 PUFA synthesis-secretion coefficients, rates, turnover rates, and half-life.** Synthesis-secretion variables for DHA, EPA, and ARA synthesis are found in Table 1. For both EPA and DHA, there were no significant differences with respect to \( S_{\text{max}} \) between rats fed any of the diets. \( k_{1,\text{EPA}} \) was not significantly different across the diet groups; however, the EPA synthesis-secretion rate was higher in rats fed the ALA-H diet than it was in those fed the ALA-D and ALA-A diets. \( F_{\text{EPA}} \) was significantly higher in rats fed the ALA-D diet than it was in those fed the ALA-A diet, and \( t_{1/2,\text{EPA}} \) was significantly lower in rats fed the ALA-D diet than it was in rats fed the ALA-A diet.

The daily synthesis-secretion rate for DHA was lower in the ALA-D diet group than it was in rats fed the ALA-A and ALA-H diets. \( k_{1,\text{DHA}} \) was significantly higher in rats fed the ALA-A diet than it was in those fed the ALA-H and ALA-D diets. \( F_{\text{DHA}} \) and \( t_{1/2,\text{DHA}} \) did not differ between rats consuming the 3 diets.

**n–6 PUFA synthesis-secretion coefficients, rates, turnover rates, and half-life.** No labeled DPAn–6 was detected in the plasma of rats at any time during the infusion (Supplemental Figure 1), and, therefore, none of the synthesis-secretion variables could be calculated for DPAn–6. None of the synthesis-secretion variables measured for ARA were significantly different between rats fed the 3 diets (Table 1). Furthermore, measures of ARA turnover (\( F_{\text{ARA}} \) and \( t_{1/2,\text{ARA}} \)) did not differ between rats consuming the 3 diets.

\( \lambda_{\text{ALA}}, \lambda_{\text{LA}}, J_{\text{ALA}-3\text{PUFA}}, \lambda_{\text{ALA}}, \lambda_{\text{LA}}, J_{\text{ALA}-6\text{PUFA}}, \lambda_{\text{ALA}} \) was not different between rats fed either diet, and equaled 0.0586 ± 0.0198, 0.0619 ± 0.0286, and 0.0712 ± 0.0103 in rats fed the ALA-D, ALA-A, and ALA-H diets, respectively. \( \lambda_{\text{LA}} \) also did not differ by diet and equaled 0.183 ± 0.0532, 0.102 ± 0.0450, and 0.134 ± 0.0469 in rats fed the ALA-D, ALA-A, and ALA-H diets, respectively. With the use of the mean \( \lambda_{\text{ALA}} \) and \( \lambda_{\text{LA}} \) to correct the daily synthesis rate for dilution (Equations 9 and 10), the corrected DHA synthesis-secretion rates (\( J_{\text{DHA}}(\lambda_{\text{ALA}},\lambda_{\text{LA}}) \)) were 106 ± 27, 756 ± 203, and 906 ± 232 nmol/d in rats fed the ALA-D, ALA-A, and ALA-H diets, respectively (\( P > 0.05 \)). The EPA synthesis-secretion rates, when corrected for tracer dilution (\( J_{\text{EPA}}(\lambda_{\text{ALA}},\lambda_{\text{LA}}) \)) were 1460 ± 383, 4760 ± 1440, and 12200 ± 4830 nmol/d for rats fed the ALA-D, ALA-A, and ALA-H diets, respectively (\( P > 0.05 \)), and the corrected ARA synthesis-secretion rates (\( J_{\text{ARA}}(\lambda_{\text{ALA}},\lambda_{\text{LA}}) \)) were 4370 ± 1490, 6610 ± 2170, and 1980 ± 659 nmol/d for rats in the ALA-D, ALA-A, and ALA-H diet groups, respectively (\( P > 0.05 \)).

**Discussion**

We used a stable isotope steady-state infusion method to directly measure, for the first time, DHA, EPA, and ARA synthesis-secretion rates in rats, in response to dietary ALA in free-living unanesthetized rats. Interestingly, DHA synthesis-secretion rates followed the same pattern as serum esterified DHA concentrations, because we found that DHA synthesis was lowest in rats fed the ALA-D diet, and no significant difference was observed between rats fed the ALA-A and ALA-H diets. This indicates that the higher serum DHA concentrations observed in rats fed the ALA-A and ALA-H diets were at least in part due to increased DHA synthesis from ALA. The finding of no significant difference in the daily synthesis-secretion rate between rats fed the ALA-A and ALA-H diets was surprising; however, it is in agreement with the lack of significant difference in serum esterified DHA between rats fed these 2 diets. Moreover, \( k_{1,\text{DHA}} \) was significantly lower in rats fed the ALA-H diet, meaning that rats fed the ALA-H diet had a lower capacity to synthesize DHA than did those fed the ALA-A diet. The lower \( k_{1,\text{DHA}} \) in rats fed the ALA-H diet, therefore, contributed to the similar DHA synthesis-secretion rates between rats fed the ALA-A and ALA-H diets, despite the fact that rats fed the ALA-H diet had significantly higher concentrations of serum unesterified ALA (the substrate for DHA synthesis). However, rats fed the ALA-D diet did not have an increased \( k_{1,\text{DHA}} \) compared with rats fed the ALA-H diet, despite having lower serum unesterified ALA concentrations. This finding indicates that the body adapts to low substrate availability for DHA synthesis by increasing the capacity to synthesize DHA, therefore maintaining a stable DHA synthesis rate across a wide range of ALA intake amounts; however, there appears to be a minimum threshold of ALA intake below which this compensation no longer occurs and DHA synthesis is significantly reduced. Although this finding is not consistent with the generally accepted view on PUFA synthesis, it is important to note that most of the work in this area uses in vitro kinetic models, in which substrates are not rate-limited.
limiting; therefore, the PUFA synthesis pathways appear to be more complicated and controlled in vivo.

EPA synthesis rates were higher in rats fed the ALA-H diet than they were in those fed the ALA-A and ALA-D diets. EPA turnover was faster and its half-life was shorter in rats fed the ALA-D diet, indicating rapid uptake of EPA from the serum. Future studies should aim to confirm this finding with the use of a method that can directly measure PUFA turnover within the serum, because the steady-state infusion method can only estimate PUFA turnover indirectly, when that particular PUFA is absent from the diet, based on the synthesis rate of that PUFA.

Similar to serum esterified concentrations, ARA synthesis-secretion rates were unaffected by diet, though it should be noted that the estimates for the synthesis-secretion for ARA and the n–3 PUFAs were highly variable and limited our statistical power. Future studies should consider adjustments to the experimental protocol (such as performing infusions under fasting conditions) that will decrease variability in the measure and strengthen statistical power. Furthermore, none of the synthesis-secretion variables for ARA were statistically different between rats fed the 3 diets. Because there were no differences in serum concentrations of ARA, it would be unexpected to have found differences in ARA synthesis-secretion rates between rats fed the different diets. It is known that both n–6 and n–3 PUFAs are substrates for the desaturase and elongase enzymes involved in the synthesis of n–6 and n–3 LCPUFAs (21, 22); however, the results of this study do not indicate that competition between these 2 families of PUFAs affected synthesis rates.

Despite large differences and high concentrations of serum esterified DPAn–6, we were unable to detect any labeled DPAn–6.
ALA-CoA and LA-CoA, respectively, were composed of infused tracers. We found that there was significant dilution of the tracers in the pool available for hepatic DHA synthesis, should be performed. To more accurately measure the net hepatic DHA synthesis-secretion of LCPUFAs from serum unesterified precursors, the appearance curve may occur below the limit of detection. However, this is unlikely, because our instruments is high, then we may not be able to measure possible contributions from other secretory organs. However, this correction implies that LCPUFA synthesis-secretion from serum unesterified ALA or LA represents a very small percentage of total hepatic synthesis-secretion. Moreover, it is possible that differences in synthesis-secretion rates were due to tracer retention. Although we were unable to measure tracer retention in the current study, a previous study showed that diet does not affect this variable (32); however, another group reported that whichever tissues an FA is retained in the liver, we determined synthesis-secretion capacity by measuring labeled n–3 PUFAs that were secreted into the blood. An unexpected observation was that $k_{1,DHA}$ was similar between rats fed the ALA-H and ALA-D diets, whereas $k_{1,DHA}$ was higher in rats fed the ALA-A diet. It has previously been reported that the expression of the desaturase and elongase enzymes involved in DHA synthesis is upregulated and DHA synthesis capacity is increased in rats consuming a diet deficient in n–3 PUFAs (24, 25). Whereas Igarashi et al. (25) found that rats fed an n–3 PUFA–deficient diet had increased DHA synthesis capacity compared with rats fed adequate n–3 PUFAs, we found that rats fed the ALA-D diet (n–3 PUFA–deficient diet) in this study, which contained no added n–3 PUFAs, had a lower synthesis-secretion capacity than did rats fed a diet with ALA added at a concentration of 3% of the FAs (ALA-A diet). This might be explained by experimental differences, for instance, although the diets in this study were designed to be similar to those used by Igarashi et al. (25); diets in this study were formulated by adding pure ALA ethyl esters, whereas Igarashi et al. (25) formulated their diets with vegetable oils. Therefore, future studies should use a methodology that will reduce the variability of the λ measurement so that LCPUFA, half-life.

### TABLE 1  
Synthesis-secretion variables for ARA, EPA, and DHA synthesis as determined from a 3-h steady-state infusion of $^{2}H_{2}$-ALA and $^{13}$C$_{18}$-LA in rats fed the ALA-D, ALA-A, or ALA-H diet for 8 wk

<table>
<thead>
<tr>
<th>Diet</th>
<th>$S_{\text{max}}$, nmol/min</th>
<th>$k_{1,\text{PUFA}}$, mL/min</th>
<th>$J_{\text{PUFA}}$, nmol/min</th>
<th>Daily synthesis-secretion rate, nmol</th>
<th>$F_{\text{PUFA}}$/min</th>
<th>$t_{1/2,\text{PUFA}}$, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA-D</td>
<td>0.025 ± 0.006</td>
<td>0.004 ± 0.0008</td>
<td>0.0043 ± 0.0011</td>
<td>6.2 ± 1.6$^{a}$</td>
<td>0.0002 ± 0.00005</td>
<td>79,900 ± 31,000</td>
</tr>
<tr>
<td>ALA-A</td>
<td>0.066 ± 0.016</td>
<td>0.013 ± 0.003$^{b}$</td>
<td>0.032 ± 0.008$^{b}$</td>
<td>46.8 ± 12.5$^{b}$</td>
<td>0.0002 ± 0.00004</td>
<td>109,000 ± 45,000</td>
</tr>
<tr>
<td>ALA-H</td>
<td>0.027 ± 0.007</td>
<td>0.005 ± 0.0014$^{a}$</td>
<td>0.045 ± 0.012$^{b}$</td>
<td>64.5 ± 18.0$^{d}$</td>
<td>0.0003 ± 0.000010</td>
<td>76,900 ± 39,700</td>
</tr>
<tr>
<td>EPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA-D</td>
<td>0.29 ± 0.052</td>
<td>0.049 ± 0.009</td>
<td>0.059 ± 0.016$^{a}$</td>
<td>85.7 ± 22.9$^{a}$</td>
<td>0.04 ± 0.02$^{b}$</td>
<td>216 ± 98$^{b}$</td>
</tr>
<tr>
<td>ALA-A</td>
<td>0.40 ± 0.090</td>
<td>0.084 ± 0.023</td>
<td>0.20 ± 0.062$^{ab}$</td>
<td>295 ± 89.2$^{ab}$</td>
<td>0.002 ± 0.0007$^{a}$</td>
<td>1330 ± 500$^{ab}$</td>
</tr>
<tr>
<td>ALA-H</td>
<td>0.37 ± 0.15</td>
<td>0.068 ± 0.032</td>
<td>0.60 ± 0.24$^{b}$</td>
<td>869 ± 34$^{b}$</td>
<td>0.003 ± 0.002$^{b}$</td>
<td>1580 ± 970$^{b}$</td>
</tr>
<tr>
<td>ARA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA-D</td>
<td>0.030 ± 0.006</td>
<td>0.014 ± 0.003</td>
<td>0.055 ± 0.018</td>
<td>798 ± 271</td>
<td>0.00004 ± 0.00001</td>
<td>43,300 ± 15,000</td>
</tr>
<tr>
<td>ALA-A</td>
<td>0.039 ± 0.02</td>
<td>0.017 ± 0.007</td>
<td>0.47 ± 0.15</td>
<td>673 ± 220</td>
<td>0.00003 ± 0.00008</td>
<td>45,600 ± 13,900</td>
</tr>
<tr>
<td>ALA-H</td>
<td>0.023 ± 0.008</td>
<td>0.008 ± 0.004</td>
<td>0.18 ± 0.06</td>
<td>264 ± 80.4</td>
<td>0.00001 ± 0.00004</td>
<td>63,000 ± 12,200</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs, $n = 8$ or 6 (ALA-H). Labeled means for a given variable without a common superscript letter differ, $P \leq 0.05$. ALA, α-linolenic acid; ALA-A, α-linolenic acid–adequate; ALA-D, α-linolenic acid–deficient; ALA-H, high–α-linolenic acid; ARA, arachidonic acid; $F_{\text{PUFA}}$, turnover; $J_{\text{PUFA}}$, synthesis rate; $k_{1,\text{PUFA}}$, synthesis-secretion coefficient; LA, linoleic acid; $S_{\text{max}}$, maximum first derivative; $t_{1/2,\text{PUFA}}$, half-life.
due to a decreased capacity for the liver to secrete lipoproteins containing DHA when rats are fed a diet that is low in n–3 PUFAs. It is also possible that in situations of n–3 PUFA deficiency, the liver preferentially accumulates DHA to maintain a required hepatic DHA concentration. Although it is unlikely that the livers of rats fed the ALA-D diet selectively accumulated DHA, because there were no increases in tracer enrichment, future studies should investigate whether liver secretion of DHA containing lipoproteins is impaired or whether the liver selectively acquires DHA in other FA pools under conditions of n–3 PUFA deprivation. Previously, we reported that rats fed a diet containing 2% of the FAs as ALA had a higher DHA synthesis-secretion capacity than did rats fed a control diet containing 0.2% of the FAs as ALA (19). Therefore, the likely explanation is that rats fed the ALA-D diet lack sufficient substrate to maintain synthesis-secretion.

Current intake of ALA in North America ranges from 0.6% to 1.2% of calories (1.7% to 6% of total FAs) (14), with the majority of ALA being consumed in vegetable oils (canola in Canada and soybean in the United States) (26, 38). Recently, the food industry has been moving toward producing largely high–oleic acid varieties of vegetable oils, which can reduce the ALA content up to 400% (27–29). In light of the results of this study, lowering dietary ALA may negatively affect rates of DHA synthesis. This study found that rats fed the ALA-D diet, which contained 0.02% of calories as ALA, had a significantly lower DHA synthesis-secretion rate than did rats fed the ALA-A and ALA-H diets (which provided 0.7% and 2.3% of calories as ALA, respectively). It should be noted that the ALA-D diet is an n–3 PUFA–deficient diet that provides 2000% fewer n–3 PUFAs than is considered to be sufficient (39, 40). Such an extreme diet is not reflective of normal ALA dietary intake. However, this study was intended only to provide proof of concept that lowering the intake of ALA in the population may decrease DHA synthesis rates and potentially decrease serum DHA concentrations in a proportion of the population. Future studies should aim to elucidate a level of dietary ALA consumption between the levels in the ALA-D and ALA-A diet at which DHA synthesis rates are not maintained.

In conclusion, DHA synthesis-secretion remained constant in rats fed diets consisting of 3% and 10% of the FAs as ALA. However, rats fed 0.07% of FAs as ALA had lower DHA synthesis-secretion rates, suggesting that there is a level of ALA intake below 3% of the FAs at which DHA synthesis cannot be maintained, because of either decreased substrate availability or decreased synthesis-secretion capacity.

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References


