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Whole-body DHA synthesis-secretion kinetics from plasma eicosapentaenoic acid and alpha-linolenic acid in the free-living rat

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ABSTRACT

Whole body docosahexaenoic acid (DHA, 22:6n-3) synthesis from α-linolenic acid (ALA, 18:3n-3) is considered to be very low, however, the daily synthesis-secretion of DHA may be sufficient to supply the adult brain. The current study aims to assess whether whole body DHA synthesis-secretion kinetics are different when comparing plasma ALA versus eicosapentaenoic acid (EPA, 20:5n-3) as the precursor. Male Long Evans rats ($n = 6$) were fed a 2% ALA in total fat diet for eight weeks, followed by surgery to implant a catheter into each of the jugular vein and carotid artery and 3 h of steady-state infusion with a known amount of ^{2}H-ALA and ^{13}C-eicosapentaenoic acid (EPA, 20:5n3). Blood samples were collected at thirty-minute intervals and plasma enrichment of ^{2}H- and ^{13}C EPA, n − 3 docosapentaenoic acid (DPAn-3, 22:5n-3) and DHA were determined for assessment of synthesis-secretion kinetic parameters. Results indicate a 13-fold higher synthesis-secretion coefficient for DHA from EPA as compared to ALA. However, after correcting for the 6.6 fold higher endogenous plasma ALA concentration, no significant differences in daily synthesis-secretion (nmol/day) of DHA (97.6 ± 28.2 and 172 ± 62), DPAn-3 (853 ± 279 and 1139 ± 484) or EPA (1587 ± 592 and 1628 ± 366) were observed from plasma unesterified ALA and EPA sources, respectively. These results suggest that typical diets which are significantly higher in ALA compared to EPA yield similar daily DHA synthesis-secretion despite a significantly higher synthesis-secretion coefficient from EPA.

1. Introduction

Docosahexaenoic acid (DHA, 22:6n-3) is an omega-3 (n − 3) PUFA highly enriched in the brain [1] and the heart [2]. n − 3 PUFA cannot be synthesized by mammals de novo, and DHA must therefore be consumed directly or synthesized from shorter chain n − 3 PUFA consumed in the diet. The most prominent source of n − 3 PUFA in the diet comes from α-linolenic acid (ALA, 18:3n-3) that is highly enriched in flaxseeds, representing >50% of the total fatty acids present [3]. The majority of ALA in our diet, however, is derived from cooking oil sources such as vegetable oil in Canada and soybean oil in the United States [4,5]. Alternatively, EPA, a product of ALA metabolism, is consumed in our diet primarily from marine-based foods, particularly in fatty fish such as trout, salmon, sardines and herring [6]. Both ALA and EPA are metabolic precursors to DHA, another n − 3 PUFA found primarily in seafood. Although high in fatty fish, the relative consumption in North America for EPA (50 mg per day) [7] is very low relative to ALA (approximately 1500 mg per day) [4,8–10]. Even so, only a small proportion of consumed ALA enters the DHA synthesis pathway with the majority being metabolized elsewhere in the body [11].

Oral administration of stable-isotope labeled ALA for determination of DHA synthesis rates is a popular method that assesses the appearance of labeled DHA in blood lipids over time, and these methods have been reviewed recently [12]. The appearance of DHA in blood from an oral ALA dose ranges from non-detectable to 9.8%, with the majority of studies estimating conversion at less than 1% [13–22]. Fractional DHA synthesis from ALA using this method ranges from 0.01 to 0.08% of orally ingested stable-isotope ALA [18,21–23], nevertheless, recent evidence suggests that although the daily conversion rates are low, it remains at least 3-fold higher than brain uptake requirements in rats [24]. Although much of the orally ingested tracers are either oxidized or stored in adipose tissue [20], compartmental modelling is an extremely useful tool for comparing multiple experimental groups or multiple tracers within the same study. One previous study has compared co-ingestion of stable-isotope labeled ALA and EPA using compartmental modelling to determine fractional conversion rates in infants [25], demonstrating 3.5 fold and 1.6 fold higher fractional conversion rates to DHA for EPA

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DPAn-3, n − 3 docosapentaenoic acid; FAME, fatty acid methyl ester; F_n, turnover rate; GC-FID, gas chromatography-flame ionization detection; J_n, synthesis-secretion rate; $k_{1,n}$, synthesis-secretion coefficient; LCPUFA, long chain polyunsaturated fatty acid; LNA, linoleic acid; n − 3, omega-3; SDA, stearidonic acid; S_{max}, maximum first derivative; $t_{1/2}$, halflife; THA, tetracosahexaenoic acid; TLE, total lipid extract; TPA, tetracosapentaenoic acid.

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compared to ALA from oral and plasma compartments, respectively. Although these finding were from infants only, with fewer metabolic steps DHA synthesis rates from EPA compared to ALA is likely higher across the life cycle.

A steady-state infusion model developed by Rapoport et al. [26] and modified by our lab [24] includes infusing labeled ALA to steady-state and measures the appearance of labeled esterified DHA in the plasma. This method minimizes effects of adipose storage and tissue uptake thereby allowing for the determination of a whole body DHA synthesis-secretion rate from plasma ALA directly. In the present study, we are the first to utilize this steady state infusion protocol for the determination of DHA, DPAn-3 and EPA synthesis-secretion rates following the co-infusion of $^{2}H_5$-ALA and uniformly labeled (U)-^{13}C-EPA. From this we measured the rate of appearance of esterified $^{2}H_5$-DHA and ^{13}C-DHA to determine the daily rates of n−3 PUFA synthesis-secretion from the two fatty acid precursors.

2. Materials and methods

2.1. Animals

All experimental procedures were performed in agreement 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. One Long Evans dam with six 18-day-old male non-littermate Long Evans rats were ordered from Charles River Laboratories (St. Constant, QC, Canada). Following arrival at the University of Toronto, the dam and pups were acclimated for 3 days and then weaned at 21 days old. The dam was placed on a DHA-free, 2% ALA diet immediately upon arriving at the University of Toronto and the pups were placed on the same diet for 8 weeks following weaning. During this time and prior to cannulation, rats were handled frequently and housed in pairs [24].

2.2. Diets

The diet was modified from the AIN-93G custom low n−3 rodent diet (Dyets, Inc., Bethlehem, PA) [27]. The diet contained 10% lipids by weight, and the fat content of the diet by weight was 32.8% safflower oil, 63.2% hydrogenated coconut oil and 4% added oils. The added oils were 2% oleate ethyl ester (Nu-Chek Prep, Inc., Elysian, MN) and 2% ALA ethyl ester as the only n−3 fatty acid present in the diet as confirmed by GC-FID (Table 1) (gift from BASF Pharma Callanish Ltd., Isle of Lewis, UK). Each oil was determined to be >98% pure by gas chromatography-flame ionization detection (GC-FID). The custom 2% ALA AIN-93G diet is designed to be sufficient in ALA and free of other n−3 PUFA present, thereby modelling expected n−3 intakes. Other research studies from our lab include 2% DHA in the diet, therefore, oleate ethyl ester was added to the diet to keep total fat content of the diets between studies consistent, and to ensure a constant n−6 PUFA level across all studies. This allows for easier comparisons between various studies. The fatty acid composition of the diet as measured by GC-FID is shown in Table 1.

2.3. Surgery and $^{2}H_5$-ALA and U-^{13}C-EPA infusion

At 8 weeks post-weaning, rats were subjected to surgery to implant a catheter into each of the jugular vein and the carotid artery, as previously described in detail [28]. Modified from the method of Rapoport, Igarashi, and Gao [26]; 0.563 μmol/100 g body weight of $^{2}H_5$-ALA (purity >95% confirmed by GC-FID and GC–MS; Cayman Chemical, Ann Arbor, MI) and 0.563 μmol/100 g body weight of U-^{13}C-EPA (purity determined to be approximately 35% $^{13}C_{20}$, 30% $^{13}C_{19}$, 20% $^{13}C_{18}$ and 15% $^{13}C_{16/17}$ by GC–MS; generously provided by Dr. Joseph Hibbeln, National Institutes of Health, Bethesda, MD) was infused into the jugular vein for 180 min. Due to the presence of non-uniformly labeled ^{13}C-EPA all calculations were performed using the $^{13}C_{19}$ isotope as this isotope demonstrated minimal background interference during HPLC–MS analysis, and will henceforth continue to be referred to as ^{13}C-EPA. Infusate preparation, 3-hour steady state infusions and blood collections (0, 30, 60, 90, 120, 150 and 180 min) were performed as previously described in detail [28]. All blood samples were centrifuged for 10 min (PC-100 microcentrifuge; Diamed, ON, Canada) and the plasma was collected and stored at −80 °C.

Table 1
Composition of 2% α-linolenic acid diet.

Fatty acid	Weight %
C 10:0	1.03 ± 0.19
C 12:0	27.9 ± 0.8
C 14:0	13.9 ± 0.11
C 16:0	10.0 ± 0.1
C 18:0	9.29 ± 0.14
C 18:1n-9	8.97 ± 0.17
C 18:2n-6	25.6 ± 0.4
C 18:3n-3	1.95 ± 0.09

Values expressed as % weight of fatty acid in total fatty acids (mean ± S.D.), n = 3.

2.4. Determination of plasma volume

Plasma volume was determined using the method of Schreihofer, Hair and Stepp and modified by our lab [24,29]. Briefly, a known amount of Evans Blue dye was injected into the jugular vein of the rats. 15 min following injection, 1 mL of blood was drawn from the carotid artery, twice. The plasma was collected as described above and 100 μL of plasma was diluted into 1 mL of saline. Absorbance of plasma in saline was determined at 604 nm with a Nanodrop 1000 and compared to a standard curve, and the concentration of the Evans Blue dye was determined. Concentration of the dye was then used to determine plasma volume.

2.5. Lipid extraction

Total lipid extracts (TLE) were obtained from plasma by the method of Folch, Lees, and Sloane Stanley [30]. Briefly, lipids from thawed plasma was extracted with of 2:1:0.8 chloroform:methanol:0.88% potassium chloride (by volume). For baseline total esterified fatty acid determinations, extraction solvents contained 10.9 μg of heptadecanoic acid (17:0, NuCheck Prep Inc., Elysian, MN, USA) and 28.3 μg of di:17:0 phosphatidylcholine (Avanti Polar Lipids Inc., Alabaster, AL, USA) as internal standards. For plasma unesterified and esterified $^{2}H_5$ and ^{13}C fatty acid enrichment and unlabeled baseline plasma unesterified fatty acid determinations, extraction solvents contained 272 ng of $^{2}H_8$-arachidonic acid (ARA, 20:4n-6, Cayman Chemical, Ann Arbor, MI, USA) as an internal standard. The mixtures were vortexed, centrifuged at 500 *g* for 10 min, and the lower, chloroform lipid-containing layer was pipetted into a new test tube. For unesterified fatty acid determinations, half of the stable isotope enriched plasma and all of the baseline plasma TLE were evaporated under N_2 gas, reconstituted in 100 μL of water/acetonitrile (50:50 v/v) and stored at −80 °C until HPLC–MS determination of the enrichment of $^{2}H_5$-ALA and ^{13}C-EPA isotopes in the unesterified fatty acid lipid fraction of infused blood, and baseline plasma unesterified ALA and EPA, respectively. The remaining half of the TLE for total fatty acid ^{2}H and ^{13}C enrichment was stored at −80 °C until further analysis.

2.6. Transesterification and GC-FID

Baseline plasma TLE for determination of total unlabeled esterified fatty acids was performed by transesterification to fatty acid methyl esters (FAMEs) using 2 mL 14% boron trifluoride in methanol with