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Distribution of omega-6 and omega-3 polyunsaturated fatty acids in the whole rat body and 25 compartments ☆

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ABSTRACT

The steady state compositions of omega-6 and omega-3 polyunsaturated fatty acids (PUFA) throughout the various viscera and tissues within the whole body of rats have not previously been described in a comprehensive manner. Dams consumed diets containing 10 wt% fat (15% linoleate and 3% α-linolenate). Male offspring (n=9) at 7-week of age were euthanized and dissected into 25 compartments. Total lipid fatty acids for each compartment were quantified by GC/FID and summed for the rat whole body; total $n-6$ PUFA was 12 wt% and total $n-3$ PUFA was 2.1% of total fatty acids. 18:2$n-6$ accounted for 84% of the total $n-6$ PUFA, 20:4$n-6$ was 12%, 18:3$n-3$ was 59% of the total $n-3$ PUFA, 20:5$n-3$ was 2.1%, and 22:6$n-3$ was 32%. The white adipose tissue contained the greatest amounts of 18:2$n-6$ (1.5 g) and 18:3$n-3$ (0.2 g). 20:4$n-6$ was highest in muscle (60 mg) and liver (57 mg), while 22:6$n-3$ was greatest in muscle (46 mg), followed by liver (27 mg) and carcass (20 mg). In terms of fatty acid composition expressed as a percentage, 18:2$n-6$ was the highest in the heart (13 wt%), while 18:3$n-3$ was about 1.3 wt% for skin, white adipose tissue and fur. 20:4$n-6$ was highest (21–25 wt%) in the circulation, kidney, and spleen, while 22:6$n-3$ was highest in the brain (12 wt%), followed by the heart (7.9 wt%), liver (5.9 wt%), and spinal cord (5.1 wt%). Selectivity was greatest when comparing 22:6$n-3$ in brain (12%) to white adipose (0.08%) (68-fold) and 22:5$n-6$ in testes (15.6%) compared to white adipose (0.02%), 780-fold.

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1. Introduction

Omega-6 ($n-6$) and omega-3 ($n-3$) polyunsaturated fatty acids (PUFA) are essential fatty acids (EFA) and play a vital role in cellular and physiological functions [1,2]. They serve key functions in various organ systems and contribute to growth and development, cardiovascular health, immune responses, psychological health as well as the prevention for many diseases [3]. The precursors, both linoleic acid and α-linolenic acid, cannot be synthesized *de* novo in animals, but must be supplied from the diet. Their longer chain and more unsaturated metabolites are then synthesized from their respective precursors though the synthesis rates are quite low [4,5].

Despite decades of interest in essential fatty acids, little is known about the complete profiles of PUFA distribution within viscera and tissues throughout the whole body. It has long been appreciated that particular PUFAs are selectively concentrated in particular organs and tissues, some examples being linoleic acid enrichment in liver [6,7], α-linolenic acid enrichment in skin and fur [8], docosahaexenoic acid (DHA) in brain [9], and $n-6$ docosapentaenoic acid (DPA$n-6$) in testes [10]. Many studies have described PUFA profiles in major organs in young rats [11] such as, rat blood, muscle and some viscera [12], as well as autopsy studies in human subjects [13]. In addition, some studies have investigated the PUFA as a whole in animals with a variety of methods, applying whole body balance methods to determine the oxidation of two precursors [14–16].

Our previous stable isotope tracer study [10] described the uptake of precursors, deuterated-18:2$n-6$ and -18:3$n-3$, as well as their deuterated metabolites *de novo* into viscera and other tissues in rats. However, to our knowledge, a thorough and

Abbreviations: ATL, adrenal gland, thyroid and mandibular lymph nodes; ADB, brown adipose; ADW, white adipose; BHT, butylated hydroxytoluene; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FID, flame ionization detector; GC, gas–liquid chromatography; HUFA, highly unsaturated fatty acids; MS, mass spectrometry; PUFA, polyunsaturated fatty acids; RBC, red blood cell; SG, salivary glands; SPC, spinal cord; TFA, total fatty acid; wt%, weight percent

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quantitative description of the endogenous fatty acid composition in various compartments throughout the whole body of any mammal has not been conducted. In this steady state study, fatty acids ranging from C10 to C24, including the saturated, monounsaturated, $n-6$ and $n-3$ PUFA, were quantified in 25 compartments of the rat body, detailing both essential PUFA families. We investigated rats that were fed to equilibrium on a defined diet and quantified the tissue selectivity for each tissue when the same fatty acid substrates were available to the tissues via the diet.

2. Materials and methods

2.1. Animals, diet, and tissue collection

All animal procedures were carried out in accordance with the NIH animal care and welfare guidelines; the protocol was approved by the NIAAA Animal Care and Use Committee. The animals in this study were the same as those used in a previous study [10] on PUFA metabolism using a stable isotope tracer technique coupled with a GC/MS negative chemical ionization assay. The details of the animals, diets and tissue collection were thus reported previously in Lin et al. [10]. Briefly, male Long-Evans hooded rats were weaned onto the same defined, pelleted custom diet as their parents, which was modified from the AIN-93G formulation [17] as previously described as an $n-3$ adequate diet [18]. Lipid extracted casein was used; carbohydrate sources were modified and fat sources (10 wt%) were controlled. Fat sources were 77 g of hydrogenated coconut oil, 18 g of safflower oil and 5 g flaxseed oil per kg of diet. The fatty acid distribution of the diet was as follows: 77% saturates, 4% monounsaturates, 15% linoleate, 3% α-linolenate and only traces of longer chain C20 and C22 EFAs. The animals were euthanized and the tissues were dissected out into 25 compartments when the animals were seven to eleven weeks of age, with a mean body weight of 246 ± 25 g (mean ± SEM).

2.2. Chemicals

Methanol and chloroform were purchased from Burdick & Jackson (Muskegon, MI); hexane from EMD chemicals Inc. (Gibbstown, NJ); boron trifluoride in methanol (14 g/L) was from Sigma Chemical (St. Louis, MO); docosatrienoic ethyl ester ($22{:}3n-3$) and the GC reference standard GLC-462 were purchased from Nu-Chek Prep (Elysian, MN). All chemicals were of analytical grade and used without further purification.

2.3. Homogenization and lipid extraction of various tissues and derivatization reactions

The processing of tissues was as previously described [10]. In brief, large organs/tissues were dissected and thoroughly diced into fine pieces at 4 °C prior to extraction. Organs were homogenized in methanol containing 0.2 mM BHT (10 mL for 1 g of tissue). One aliquot of homogenate (about 100 mg of tissue wet weight) was used for total lipid extraction, following a Folch total fatty acid lipid extraction [19]. The total lipid extract was dried under a stream of nitrogen and transmethylated using boron trifluoride in methanol as described by Morrison and Smith [20] and modified by Salem et al. [21]. The internal standard, $22{:}3n-3$ ethyl ester (20 µg) was added to each sample prior to lipid extraction.

2.4. Gas–liquid chromatographic analysis

HP-5890 (series II) gas chromatograph (Hewlett-Packard, Palo Alto, CA) coupled with a flame ionization detector was employed to quantify fatty acids. An aliquot of fatty acid methyl ester (FAME) from each sample was injected onto a DB-FFAP fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm, J&W Scientific, Folsom, CA) through a split/splitless inlet (50:1). The oven temperature was programmed as previously reported [22]. A reference standard GLC-462 containing 28 fatty acid methyl esters was used to identify the retention time of FAME peaks on GC chromatograms. PUFA in a mixture of adrenal gland, thyroid gland and mandibular lymph nodes (ATL), and bladder were quantified employing gas chromatography–mass spectrometry, negative chemical ionization as reported previously [10].

2.5. Calculation

Unless indicated in the text, data were expressed as mean ± SEM ($n=9$) in concentrations, µg of fatty acid per mg of wet tissue weight (µg/mg) or per mL of plasma or red blood cell (µg/mL), or the proportion of each fatty acid in the weight of the total fatty acids in each sample (wt%). The concentrations were calculated by comparing the integrated areas of each fatty acid peak in the gas chromatograms with that of the known amount of internal standard ($22{:}3n-3$) added in the sample prior to total lipid extraction. The total amount of each fatty acid in rat whole body

Table 1
Fatty acid composition in rat whole body (wt%).

Fatty acids	Mean		SEM
10:0	**0.53**	±	0.06
12:0	**9.08**	±	0.56
14:0	**7.93**	±	0.49
16:0	**25.8**	±	0.3
18:0	**4.87**	±	0.09
20:0	**0.08**	±	0.00
22:0	**0.04**	±	0.00
24:0	**0.09**	±	0.01
14:1	**0.54**	±	0.02
16:1$n-7$	**6.92**	±	0.22
18:1$n-9$	**24.0**	±	1.0
18:1$n-7$	**3.05**	±	0.16
20:1$n-9$	**0.17**	±	0.01
22:1$n-9$	**0.02**	±	0.00
24:1$n-9$	**0.15**	±	0.02
18:2$n-6$	**10.1**	±	0.30
18:3$n-6$	**0.06**	±	0.01
20:2$n-6$	**0.09**	±	0.01
20:3$n-6$	**0.09**	±	0.01
20:4$n-6$	**1.45**	±	0.07
22:4$n-6$	**0.14**	±	0.01
22:5$n-6$	**0.09**	±	0.01
18:3$n-3$	**1.23**	±	0.03
20:5$n-3$	**0.04**	±	0.01
22:5$n-3$	**0.14**	±	0.01
22:6$n-3$	**0.66**	±	0.03
20:3$n-9$	**0.09**	±	0.00
Summary			
Saturated	**48.4**	±	0.9
Monounsaturated	**34.8**	±	1.3
$n-6$ PUFA	**12.0**	±	0.3
$n-3$ PUFA	**2.07**	±	0.05
$n-6$% HUFA	**67.6**	±	0.5
$n-6$/$n-3$ PUFA	**5.8**	±	0.1
Total identified fatty acids	**97.4**	±	0.2
Total fatty acids (g)	**20.6**	±	4.4

Data were presented as mean ± SEM ($n=8$–9). “0.00” indicates values were less than 0.005. Data were not included for saturated and monounsaturated fatty acids in *adrenal gland*, *thyroid gland*, *mandibular lymph nodes*, *and bladder*. The total fatty acid accounted for all fatty acid detected by gas chromatography. HUFA was defined as fatty acids with 20 or more carbons and at least 3 carbon–carbon double bonds, including $20{:}3n-6$, $20{:}4n-6$, $22{:}4n-6$, $22{:}5n-6$, $20{:}5n-3$, $22{:}5n-3$, and $22{:}6n-3$.

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