

Distribution, depletion and recovery of docosahexaenoic acid are region-specific in rat brain

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The present study examined: (i) age-induced regional changes in fatty acid composition of brain phospholipids; (ii) α -linolenic acid deficiency-induced regional depletion and recovery of DHA in the brain. DHA and arachidonic acid (AA) did not distribute evenly in the brain. In weaning and adult rats, the region with the highest DHA percentage was the cortex whereas the medulla had the lowest DHA percentage. In the aged rats, both the cortex and cerebellum were the regions with the highest DHA percentage whereas in the neonatal rats, the striatum had the greatest percentage of DHA, and the hypothalamus and hippocampus had the least percentage of DHA. Regarding AA, the hippocampus was the region that had the highest percentage whereas the medulla was the region with the lowest percentage except for the neonatal rats, whose cerebellum, hypothalamus, striatum and midbrain had AA percentage lower than hippocampus and cortex. DHA was not proportionally depleted in various regions of brain when the rats were maintained on an n -3-deficient diet for two generations. The results demonstrated that the cortex, hippocampus, striatum, cerebellum and hypothalamus had DHA depleted by >71%, whereas the midbrain and medulla had only 64 and 57% DHA depleted, respectively. The most important observation was that the diet reversal for 12 weeks resulted in complete DHA recovery in all regions except for the medulla where the recovery was only 62%. It was concluded that the location of DHA, n -3 deficiency-induced DHA depletion and reversibility of DHA deficiency across the brain were region-specific.

Arachidonic acid: Brain: Cerebellum: Cortex: Docosahexaenoic acid

DHA (22:6 n -3) and arachidonic acid (AA; 20:4 n -6) are important for the structural development of the mammalian central nervous system and are accumulated in large amounts in the developing brain and retina. DHA and AA can be synthesised from their respective short-chain precursors, α -linolenic acid (LNA; 18:3 n -3) and linoleic acid (18:2 n -6), via the desaturase and elongase pathway. LNA and linoleic acid are regarded as essential fatty acids, since mammals cannot synthesise them and have to obtain them from the diet. However, LNA and linoleic acid are quantitatively minor in any mammalian brain (Sastry, 1985). DHA and AA contents in the growing brain are largely determined by dietary fatty acid composition. Depletion of DHA can be induced by a diet deficient in LNA. Fortunately, the deficiency is not permanent and is reversible by re-supplementation of LNA or preformed DHA in the diet (Connor *et al.* 1990; Moriguchi *et al.* 2001).

Previous research has shown that the addition of DHA or DHA and AA combined into infant formula are associated with an improved performance in visual acuity, visual recognition memory, learning ability, Bayley mental developmental index, and developmental quotient scores of infants (Uauy *et al.* 1990; Carlson *et al.* 1993; Agostoni *et al.* 1995; Carlson & Werkman, 1996; Willatts *et al.* 1998; Birch *et al.* 2000; Innis, 2004). However, the beneficial effect associated with supplementation of DHA and AA on neural functions of infants has not always been consistently

observed in other trials (Innis *et al.* 1994; Auestad *et al.* 1997; Jorgensen *et al.* 1998; Makrides *et al.* 1995; Lucas *et al.* 1999, 2002). An association between DHA deficiency and functional deficits in the nervous system has also been demonstrated in animal studies. DHA deficiency was typically induced by feeding a diet low in n -3 fatty acids during development. DHA depletion in the brain led rodents to show poor performance in the Morris water maze, shock avoidance, spatial task performance, olfactory discrimination, and exploratory behaviour (Bourre *et al.* 1989; Enslin *et al.* 1991; Greiner *et al.* 1999; Moriguchi *et al.* 2000). DHA deficiency induced by a low-LNA diet also caused abnormal electroretinograms in rats, cats and rhesus monkeys (Wheeler *et al.* 1975; Neuringer *et al.* 1986; Pawlosky *et al.* 1997). However, other trials found no difference in performance for spatial tasks between the control and DHA-depleted mice (Wainwright *et al.* 1994, 1997).

Fatty acid composition in various species is well characterised but regional distribution of DHA and AA in the brain has not been thoroughly examined (Carrie *et al.* 2000). The present study was to: (i) examine differences in fatty acid composition of sub-brain regions including the cerebellum, medulla, hypothalamus, striatum, hippocampus, cortex and midbrain of rats; (ii) investigate whether LNA deficiency-induced DHA depletion was region-specific in these sub-regions; (iii) most importantly, exam-

ine the regional difference in DHA recovery in the brain after diet reversal from an *n*-3-deficient diet to an *n*-3-adequate diet.

Materials and methods

Age and regional distribution of fatty acids in rat brain

Female Sprague–Dawley rats (Laboratory Experimental Animal Service, The Chinese University of Hong Kong, Shatin, Hong Kong) were housed (two rats per cage) in an animal room at 23°C with 12–12 h light–dark cycles. The rats consumed a rodent chow diet (PMI Nutrition International, LLC, Brentwood, MO, USA) that contained 60 g fat/kg. The fatty acid composition of the chow diet is shown in Table 1. The rats were killed under CO₂ anaesthesia at day 3 (8.6 (SD 0.3) g), day 21 (55.0 (SD 2.6) g), week 10 (231.6 (SD 19.0) g) and month 18 (412.5 (SD 29.9) g). The whole brain was retained and then dissected into cerebellum, medulla, hypothalamus, striatum, hippocampus, cortex and midbrain according to the procedure previously described by Glowinski & Iversen (1966).

n-3 Deficiency-induced regional depletion of docosahexaenoic acid from rat brain

DHA deficiency was induced by feeding an *n*-3-deficient diet for two generations. In brief, the experiment started with weaning Sprague–Dawley female rats. They were divided into two groups (ten per group) and placed on either an *n*-3 fatty acid-adequate (*n*-3 Adq) diet or an *n*-3 fatty acid-deficient (*n*-3 Def) diet. The rats were allowed free access to diet and tap water. When the rats were 10 weeks old (*n*-3 Adq, 286.2 (SD 19.4) g; *n*-3 Def, 272.2 (SD 23.1) g), they (F1 generation) were mated with Sprague–Dawley male rats (12 weeks). After weaning, the pups (F2 generation) were placed on their corresponding maternal diet. At week 5, the F2 female rats (*n*-3 Adq, 110.0 (SD 26.2) g; *n*-3 Def, 111.2 (SD 13.4) g) were killed under CO₂ anaesthesia. The brain was removed and dissected into cerebellum, medulla, hypothalamus, striatum, hippocampus, cortex and midbrain for the fatty acid analysis.

Reversibility of docosahexaenoic acid deficiency

The F2 *n*-3 Def female rats at week 5 were switched to the *n*-3 Adq diet for 12 weeks whereas the F2 *n*-3 Adq female rats were maintained on the *n*-3 Adq diet for the same period of

time. Five rats from each group were killed at every 3 weeks and sub-regions were dissected for fatty acid analysis.

Diets

The composition of the *n*-3 Adq diet and *n*-3 Def diet was similar except for fat (Table 2). All diet ingredients were purchased from Harlan Teklad (Madison, WI, USA) except for the maize starch, oils and sucrose, which were obtained from a local supermarket. Both diets contained 60 g fat/kg. The *n*-3 Adq diet contained (per kg diet): 42.3 g coconut oil, 8.4 g rapeseed oil, 6.8 g sunflower-seed oil and 2.5 g flaxseed oil. The *n*-3 Def diet contained only 44.2 g coconut oil and 15.8 g sunflower-seed oil (Table 2). The fatty acid composition of the two diets was similar except for LNA (18:3*n*-3), which accounted for 3.49% in the *n*-3 Adq diet but was less than 0.04% of total fatty acids in the *n*-3 Def diet (Table 3).

Fatty acid analysis

Total lipids of brain regions were extracted using chloroform–methanol (2:1, v/v) containing 2% butyrate hydroxytoluene (Sigma Chemical Co., St Louis, MO, USA) as an antioxidant. L-Phosphatidylcholine diheptadecanoyl (Sigma Chemical Co.) was added as an internal standard to quantify total phospholipids (PL). Neutral lipid TLC (20 × 20 cm plates pre-coated with 250 μm silica gel 60A; Macherey-Nagel GmbH & Co. KG, Düren, Germany) was applied to separate total PL by using a developing solvent system of hexane–diethyl ether–acetic acid (80:20:1, by vol.). Total PL were recovered from the TLC plate, and their fatty acids were converted to the corresponding methyl esters using 14% boron trifluoride in methanol (Sigma Chemical Co.) under N₂ gas at 90°C for 60 min.

The fatty acid methyl esters were analysed on a flexible silica capillary column (Innowax 19091N-213, 30 m × 0.32 mm internal diameter; J&W Scientific, Folsom, CA, USA) in an HP 5980 series II gas-liquid chromatograph equipped with a flame-ionisation detector (Hewlett Packard, Palo Alto, CA, USA). Column temperature was programmed from 180 to 230°C at a rate of 2°C/min and then held for 5 min. Injector and detector temperature were set at 250 and 300°C, respectively. The carrier gas used was He at a head pressure of 100 kPa. Identification of each fatty acid methyl ester was made by comparison of retention time of authentic standards (Sigma Chemical Co.).

Table 1. Fatty acid composition (%) of rodent chow diet (Data averaged from three determinations)

Fatty acids	Mean	SD
16:0	18.9	0.1
18:0	7.0	0.1
16:1 <i>n</i> -7	1.8	0.1
18:1 <i>n</i> -9	27.5	1.0
20:1 <i>n</i> -9	0.7	0.1
18:2 <i>n</i> -6	35.0	0.1
20:2 <i>n</i> -6	0.2	0.0
20:3 <i>n</i> -6	0.2	0.0
20:4 <i>n</i> -6	<0.1	0.0
18:3 <i>n</i> -3	3.1	0.1
20:5 <i>n</i> -3	0.8	0.0
22:5 <i>n</i> -3	0.1	0.0
22:6 <i>n</i> -3	0.6	0.0

Table 2. Composition (g/kg diet) of the *n*-3 adequate diet and *n*-3-deficient diet

	<i>n</i> -3-Adequate diet	<i>n</i> -3-Deficient diet
Maize starch	571.0	571.0
Casein	235.0	235.0
Sucrose	50.0	50.0
Mineral mix (AIN-76)*	35.0	35.0
Cellulose	32.0	32.0
Vitamin mix (AIN-76A)*	10.0	10.0
Choline bitartrate	4.0	4.0
DL-Methionine	3.0	3.0
Total fat	60.0	60.0
Coconut oil	42.3	44.2
Rapeseed oil	8.4	0
Sunflower-seed oil	6.8	15.8
Flaxseed oil	2.5	0

* Supplied by Harlan Teklad, Madison, WI, USA.

Table 3. Fatty acid composition (% of total fatty acids) of the *n*-3 adequate diet and *n*-3-deficient diet

Fatty acid	<i>n</i> -3-Adequate diet	<i>n</i> -3-Deficient diet
12:0	33.3	34.6
14:0	13.5	14.6
16:0	9.8	8.7
18:0	3.5	3.2
18:1 <i>n</i> -9	15.0	17.1
18:2 <i>n</i> -6	17.5	17.7
18:3 <i>n</i> -3	3.5	<0.1
Others	3.9	4.1

Statistics

Data are expressed as means and standard deviations. Where applicable, one-way ANOVA was used followed by using Fisher's least significant difference method to statistically evaluate

significant differences in fatty acid composition among the various regions in the brain using SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant when *P*<0.05.

Results

Regional distribution of phospholipid fatty acids

The regional distribution of fatty acids in PL of sub-brain regions of neonatal rats (day 3) is shown in Table 4. It appeared that the striatum had the greatest percentage of DHA (12.6) while the hypothalamus and hippocampus had the least percentage of DHA (10.7 and 10.8, respectively). AA (20:4*n*-6) in all the regions was similar, but statistically in the hippocampus and cortex it was greater than that in the cerebellum, hypothalamus, striatum and midbrain.

A regional difference in DHA distribution was observed in weaning rats aged 21 d (Table 4). The cortex had the greatest

Table 4. Major fatty acids (% of total) of phospholipids of sub-regions including the cerebellum, medulla, hypothalamus, striatum, hippocampus, cortex and mid-brain of rats aged 3 d, 21 d, 10 weeks and 18 months (*n* 10)

(Mean values and standard deviations for ten rats)

	Cerebellum		Medulla		Hypothalamus		Striatum		Hippocampus		Cortex		Midbrain	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0														
3 d	32.4 ^{A,c}	0.5	31.6 ^{A,d}	0.8	32.4 ^{A,b,c}	0.5	33.2 ^{A,a}	0.1	33.0 ^{A,a,b}	0.4	32.9 ^{A,a,b}	0.2	33.3 ^{A,a}	0.4
21 d	20.5 ^{B,d}	0.6	17.2 ^{B,f}	0.7	24.5 ^{B,c}	0.7	23.4 ^{B,d}	0.5	25.2 ^{B,b}	0.5	26.0 ^{B,a}	0.6	21.9 ^{B,e}	0.8
10 weeks	20.2 ^{B,b,c}	0.3	14.5 ^{C,e}	0.3	21.0 ^{C,b}	0.9	19.5 ^{C,c}	0.9	21.6 ^{C,a,b}	0.5	22.4 ^{C,a,b}	0.5	17.9 ^{C,d}	0.1
18 months	19.2 ^{C,c}	0.9	13.9 ^{C,e}	0.4	19.1 ^{D,c}	0.9	18.6 ^{C,c}	1.2	20.3 ^{C,b}	0.5	21.8 ^{C,a}	0.4	17.3 ^{C,d}	0.2
18:0														
3 d	16.8 ^{D,c}	0.2	17.4 ^{B,b}	0.5	18.0 ^{B,a}	0.5	17.4 ^{B,b,c}	0.2	17.6 ^{B,a,b}	0.4	17.1 ^{B,c}	0.3	17.1 ^{C,c}	0.3
21 d	20.3 ^{A,d}	0.3	20.5 ^{A,c}	0.3	21.9 ^{A,a}	0.2	21.8 ^{A,a}	0.5	21.4 ^{A,b}	0.3	21.5 ^{A,b}	0.4	21.6 ^{A,a,b}	0.3
10 weeks	19.8 ^{B,d}	0.2	17.7 ^{B,e}	0.3	21.3 ^{A,b,c}	0.1	22.1 ^{A,a}	0.2	21.9 ^{A,a,b}	0.6	21.5 ^{A,b}	0.7	20.7 ^{B,c}	0.2
18 months	18.7 ^{C,d}	0.1	17.3 ^{B,e}	0.1	21.4 ^{A,b}	0.6	21.0 ^{A,b,c}	0.2	21.9 ^{A,a}	0.2	21.6 ^{A,a,b}	0.1	20.1 ^{B,c}	0.1
18:1 <i>n</i> -9														
3 d	13.2 ^{C,a}	0.5	13.2 ^{C,a}	0.4	12.9 ^{C,a}	0.3	11.2 ^{C,c}	0.1	12.0 ^{C,b}	0.4	11.3 ^{D,c}	0.2	11.9 ^{D,b}	0.3
21 d	18.0 ^{B,b}	0.5	22.3 ^{B,a}	0.7	15.7 ^{B,c}	0.5	15.9 ^{B,c}	0.4	14.6 ^{B,d}	0.5	13.1 ^{C,e}	0.6	17.9 ^{C,b}	1.0
10 weeks	20.7 ^{A,b}	0.5	26.5 ^{A,a}	0.4	20.4 ^{A,c}	0.9	20.2 ^{A,c}	1.4	18.7 ^{A,d}	0.7	17.8 ^{B,d}	0.9	23.9 ^{B,b}	0.5
18 months	21.8 ^{A,c,d}	0.5	26.6 ^{A,a}	0.2	22.2 ^{A,c}	1.0	21.1 ^{A,d}	0.6	19.6 ^{A,e}	0.4	19.0 ^{A,e}	0.6	25.1 ^{A,b}	0.6
18:2 <i>n</i> -6														
3 d	1.3 ^{A,a}	0.2	1.1 ^{A,b,c}	0.1	0.9 ^{A,c}	0.1	0.9 ^{A,c}	0.1	1.1 ^{A,b,c}	0.1	1.2 ^{A,b}	0.1	1.0 ^{A,c}	0.1
21 d	1.4 ^{A,a}	0.1	1.3 ^{A,b}	0.1	0.9 ^{A,e}	0.2	1.0 ^{A,d}	0.1	0.9 ^{B,e}	0.1	1.0 ^{A,c}	0.1	0.9 ^{A,d}	0.1
10 weeks	1.0 ^{B,a}	0.1	0.6 ^{B,b}	0.0	0.5 ^{B,b,c}	0.0	0.6 ^{B,b}	0.0	0.5 ^{C,c}	0.1	0.6 ^{B,b,c}	0.2	0.6 ^{B,b}	0.0
18 months	0.9 ^{B,a}	0.1	0.6 ^{B,b}	0.1	0.5 ^{B,c}	0.1	0.6 ^{B,c}	0.0	0.5 ^{C,c}	0.1	0.7 ^{B,b}	0.0	0.7 ^{B,b,c}	0.0
20:4 <i>n</i> -6														
3 d	12.4 ^{A,b}	0.4	12.8 ^{A,a,b}	0.6	12.3 ^{A,b}	0.5	12.3 ^{A,b}	0.2	12.9 ^{A,a}	0.2	12.9 ^{A,a}	0.2	12.4 ^{A,b}	0.4
21 d	9.2 ^{B,e}	0.2	8.0 ^{B,f}	0.3	11.4 ^{B,c}	0.3	12.3 ^{A,b}	0.2	13.0 ^{A,a}	0.3	12.8 ^{A,a}	0.5	10.2 ^{B,d}	0.7
10 weeks	7.2 ^{C,d}	0.2	5.6 ^{C,e}	0.2	9.2 ^{C,c}	0.7	10.3 ^{B,b}	0.3	11.4 ^{B,a}	0.4	10.0 ^{B,b}	0.4	7.6 ^{C,b}	0.2
18 months	6.1 ^{D,e}	0.2	5.0 ^{D,f}	0.2	7.9 ^{D,c}	0.2	9.0 ^{C,b}	0.2	10.4 ^{C,a}	0.3	9.1 ^{C,b}	0.3	6.8 ^{D,d}	0.0
22:4 <i>n</i> -6														
3 d	3.1 ^{A,b}	0.2	3.3 ^{A,a}	0.2	3.5 ^{B,a}	0.3	2.6 ^{B,c,a}	0.2	2.8 ^{C,c}	0.2	2.7 ^{B,c,a}	0.2	2.9 ^{B,b,c}	0.2
21 d	3.2 ^{A,c,d}	0.9	3.1 ^{A,d}	0.1	4.4 ^{A,a}	0.2	3.1 ^{A,d}	0.1	3.4 ^{A,c}	0.1	3.3 ^{A,c,d}	0.1	3.6 ^{A,b}	0.1
10 weeks	2.4 ^{B,f}	0.1	2.7 ^{B,e}	0.1	4.3 ^{A,a}	0.1	3.3 ^{A,b,c}	0.1	3.5 ^{A,b}	0.1	3.0 ^{A,d}	0.1	3.2 ^{A,c}	0.1
18 months	1.7 ^{C,f}	0.1	1.9 ^{C,e}	0.1	3.7 ^{C,a}	0.1	2.8 ^{C,c}	0.1	3.2 ^{A,b}	0.1	2.5 ^{B,d}	0.3	2.5 ^{B,d}	0.1
22:5 <i>n</i> -6														
3 d	1.4 ^{A,b}	0.2	1.5 ^{A,a,b}	0.2	1.8 ^{A,a}	0.2	1.8 ^{A,a}	0.2	1.7 ^{A,a}	0.2	1.8 ^{A,a}	0.2	1.7 ^{A,b,c}	0.2
21 d	0.6 ^{B,c}	0.1	0.3 ^{B,d}	0.1	0.8 ^{B,a}	0.1	0.6 ^{B,c}	0.1	0.7 ^{B,b}	0.1	0.7 ^{B,b}	0.1	0.6 ^{B,c}	0.2
10 weeks	0.2 ^{C,c,d}	0.0	0.1 ^{C,d}	0.0	0.8 ^{B,b}	0.6	0.4 ^{C,c}	0.0	0.6 ^{C,b}	0.1	0.4 ^{C,b,c}	0.1	0.2 ^{C,c}	0.0
18 months	0.1 ^{D,d}	0.1	0.1 ^{D,d}	0.1	0.2 ^{C,b,c}	0.1	0.3 ^{C,b,c}	0.0	0.4 ^{D,a}	0.1	0.3 ^{D,b}	0.1	0.2 ^{D,c}	0.0
22:6 <i>n</i> -3														
3 d	10.9 ^{C,b,c}	0.6	11.6 ^{B,b}	0.8	10.7 ^{B,c}	0.8	12.6 ^{C,a}	0.4	10.8 ^{B,c}	0.4	11.4 ^{B,b,c}	0.5	11.5 ^{B,b}	0.7
21 d	13.8 ^{B,b}	0.4	11.7 ^{B,d}	0.3	13.1 ^{A,c}	0.6	13.5 ^{B,b,c}	0.3	13.9 ^{A,b}	0.7	15.3 ^{A,a}	0.6	13.6 ^{A,b}	0.5
10 weeks	14.2 ^{B,b}	0.4	10.7 ^{C,e}	0.3	12.2 ^{A,d}	1.0	12.5 ^{C,c,d}	0.7	13.2 ^{A,c}	0.5	15.9 ^{A,a}	0.4	11.7 ^{B,d}	0.1
18 months	16.3 ^{A,a}	0.5	12.7 ^{A,c}	0.2	13.7 ^{A,b}	0.6	14.4 ^{A,b}	0.1	14.0 ^{A,b}	0.3	16.1 ^{A,a}	1.0	13.4 ^{A,b,c}	0.6

^{a,b,c,d,e,f} Mean values within the same row with unlike superscript letters were significantly different (*P*<0.05).
^{A,B,C,D} Mean values within the same column with unlike superscript letters were significantly different (*P*<0.05).

DHA (15.3%), while the medulla had the least (11.7%) among the seven regions examined. Similarly, AA was the highest (12.8%) in the cortex while it was the lowest in the medulla (8.0%). The difference in 16:0 was also significant among the seven regions, with the cortex having 26.0% while the medulla had only 17.2%. The medulla had the highest percentage of 18:1n-9 (22.3) while the cortex had the lowest percentage of 18:1n-9 (13.13).

The distribution pattern of brain fatty acids in adult rats (10 weeks) is shown in Table 4. DHA was the highest in the cortex (15.9%) followed by that in the cerebellum (14.2%), hippocampus (13.2%), striatum (12.5%), hypothalamus (12.2%), mid-brain (11.7%) and medulla (10.7%). AA was the greatest in the hippocampus (11.4%) and the least in medulla (5.6%). The fatty acid 18:1n-9 was accumulated most in the medulla (26.5%) and least in the cortex (17.8%).

Distribution pattern was assessed for aged rats (18 months). Regarding DHA, the cerebellum (16.3%) and cortex (16.1%) accumulated most while the medulla (12.7%) and midbrain (13.4%) had the least. In the case of AA, the hippocampus had most (10.4%) whereas the medulla contained the least (5.0%). Proportionally, the cortex had the greatest percentage of 16:0 (21.8) in contrast to the least percentage in the medulla (13.9). In contrast, the medulla accumulated 18:1n-9 the most (26.6%) whereas in the cortex it was only 19.0%.

Ageing-induced change in regional docosahexaenoic acid distribution in brain

Ageing affected DHA distribution most in the cerebellum, where DHA percentage increased with ageing. In contrast, age-induced change in DHA distribution was not so obvious in the other regions. Regarding other fatty acids, ageing led to a decrease in percentages of AA, 22:4n-6, 22:5n-6 and 16:0 in all seven regions. The reverse was true for that of 18:1, which increased with ageing.

α -Linolenic acid deficiency-induced docosahexaenoic acid depletion in brain sub-regions

Feeding the *n*-3-deficient diet caused depletion of DHA in the brain of F2 female rats (aged 5 weeks). As shown in Table 5, DHA was reduced in various regions by 57.1–75.7%. The cortex, hippocampus, cerebellum and striatum appeared to have DHA depleted the most (73.2–75.7%) followed by the hypothalamus (71.5%), midbrain (64.4%) and medulla (57.1%). Depletion of DHA was mainly compensated by an increase in 22:5n-6 by 7–14-fold across various regions of rat brain. Feeding the *n*-3 deficient diet led to the medulla accumulating 22:5n-6 the most (14-fold) while the hypothalamus accumulated 22:5n-6 the least (only 8-fold).

Table 5. Alteration in fatty acid composition of brain sub-regions in F2 *n*-3-deficient rats (*n*-3 Def) compared with that of F2 *n*-3-adequate rats (*n*-3 Adq) aged 5 weeks (*n* 5)

(Mean values and standard deviations for five rats)

	Cerebellum		Medulla		Hypothalamus		Striatum		Hippocampus		Cortex		Midbrain	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0														
<i>n</i> -3 Adq	21.2	0.8	15.2	0.3	21.2	1.4	21.5	0.4	22.9	0.3	23.8	0.3	19.3	0.9
<i>n</i> -3 Def	21.6	0.8	15.5	0.6	22.6	0.9	22.4	1.1	24.1	1.1	22.5	0.9	19.4	0.5
% Change	2.2	0.1	1.5	0.1	6.7	0.3	4.2	0.2	5.5	0.3	-5.2	0.2	0.7	0.0
18:0														
<i>n</i> -3 Adq	20.9	0.5	20.5	0.8	22.4	0.7	23.5	1.4	22.5	0.7	22.6	0.3	22.3	0.3
<i>n</i> -3 Def	20.2	0.5	19.8	0.4	22.7	1.0	23.6	1.6	23.2	1.0	23.2	1.0	22.0	0.3
% Change	-3.4 ^b	0.0	-3.6 ^b	0.1	1.0 ^a	0.0	0.8 ^a	0.1	3.3 ^a	0.1	3.3 ^a	0.1	-1.5 ^b	0.0
18:1n-9														
<i>n</i> -3 Adq	19.1	0.5	24.4	0.6	19.2	0.4	17.7	0.5	17.3	0.2	16.0	0.7	20.6	1.2
<i>n</i> -3 Def	18.1	0.3	22.5	0.7	17.7	0.6	16.5	0.8	16.1	0.8	14.6	0.8	19.3	0.8
% Change	-5.1	0.1	-7.7	0.2	-8.1	0.3	-6.7	0.3	-6.9	0.3	-8.4	0.5	-6.4	0.3
18:2n-6														
<i>n</i> -3 Adq	1.0	0.1	0.8	0.1	0.6	0.1	0.7	0.0	0.6	0.0	0.6	0.1	0.6	0.1
<i>n</i> -3 Def	0.9	0.1	0.7	0.1	0.6	0.1	0.5	0.3	0.4	0.3	0.7	0.1	0.5	0.1
% Change	-13.5	1.7	-8.6	1.2	-8.2	1.0	-25.8	17.4	-32.7	17.4	9.4	0.8	-15.9	3.6
20:4n-6														
<i>n</i> -3 Adq	7.4	0.2	6.5	0.3	9.2	0.5	10.3	0.3	11.1	0.3	10.3	1.1	8.5	0.8
<i>n</i> -3 Def	7.9	0.1	7.0	0.3	9.0	0.2	10.4	0.7	10.4	0.7	10.7	0.5	8.4	0.8
% Change	7.5	0.1	7.2	0.3	-1.8	0.1	0.2	0.0	-3.4	0.2	-3.2	0.2	-0.6	0.1
22:4n-6														
<i>n</i> -3 Adq	2.5	0.1	3.1	0.2	4.3	0.2	3.0	0.2	3.3	0.1	3.1	0.3	3.4	0.1
<i>n</i> -3 Def	3.4	0.1	3.6	0.4	4.0	0.1	3.1	0.4	3.2	0.4	3.3	0.3	3.7	0.3
% Change	35.7 ^a	2.0	14.9 ^b	1.5	-17.9 ^c	0.2	6.4 ^{b,c}	0.8	-2.7 ^c	0.3	7.8 ^{b,c}	0.7	11.0 ^b	0.9
22:5n-6														
<i>n</i> -3 Adq	0.8	0.1	0.5	0.0	1.1	0.2	0.9	0.1	1.0	0.1	1.0	0.2	0.8	0.2
<i>n</i> -3 Def	10.6	0.8	7.0	0.4	9.4	0.9	10.6	1.1	10.0	0.3	11.6	1.0	9.4	0.5
% Change	1256 ^{a,b}	39	1420 ^a	8	790 ^c	74	1041 ^{b,c}	105	873 ^c	29	1058 ^b	94	1117 ^b	54
22:6n-3														
<i>n</i> -3 Adq	13.8	3.7	10.0	0.4	12.2	0.6	12.5	0.7	12.3	0.7	13.9	0.5	12.2	0.6
<i>n</i> -3 Def	3.7	0.6	4.3	0.2	3.5	0.3	3.4	0.4	3.1	0.4	3.4	0.6	4.4	0.2
% Change	-73.4 ^c	12.6	-57.1 ^a	2.5	-71.5 ^c	6.8	-73.2 ^c	8.7	-74.4 ^c	9.5	-75.7 ^c	12.9	-64.4 ^b	3.0

a,b,c,d Mean values within the same row with unlike superscript letters were significantly different ($P < 0.05$).

Reversibility of docosahexaenoic acid deficiency

DHA depletion in the brain was reversible after the *n*-3 Def rats were switched to the *n*-3 Adq diet (Fig. 1). There was about a 50% recovery of DHA in seven regions within the first 3 weeks. After 12 weeks of diet reversal, DHA recovery was almost complete in all regions except for the medulla where DHA percentage was only 62 of that in the *n*-3 Adq rats. Fatty acid 22:5*n*-6 was an indicator of DHA deficiency. It rose by 7–14-fold across various regions in the *n*-3 Def rats compared with that in the *n*-3 Adq rats (Table 5). Fatty acid 22:5*n*-6 in the *n*-3 Def group gradually decreased and approached that of the *n*-3 Adq group after 12 weeks of the diet reversal, but statistically it was still higher in the *n*-3 Def group than that in the *n*-3 Adq control (Fig. 2).

Discussion

The present study examined fatty acid composition across various regions in the rat brain with its focus entirely on DHA and AA.

The results clearly demonstrated that fatty acid composition varied with various regions and it was evident that DHA and AA did not distribute evenly in the brain. The present study found that in weaning and adult rats the region with the highest DHA percentage was the cortex whereas the medulla had the lowest DHA percentage. This observation was similar to the report by Carrie *et al.* (2000), who examined the fatty acid composition of PL present in various regions in adult mice. The present study was carried out to investigate further the age-related changes in DHA levels of various brain regions, demonstrating that in aged rats (18 months), the cerebellum and cortex were the regions that equally had the highest DHA percentages compared with other regions in the brain (Table 4). Regarding AA, the hippocampus was the region that always had the highest percentage whereas the medulla was the region with the lowest AA percentage except for the neonatal rats (aged 3 d), whose difference in AA across various regions was not so obvious and apparent, although statistically the hippocampus and cortex had an AA percentage higher than the cerebellum, hypothalamus, striatum

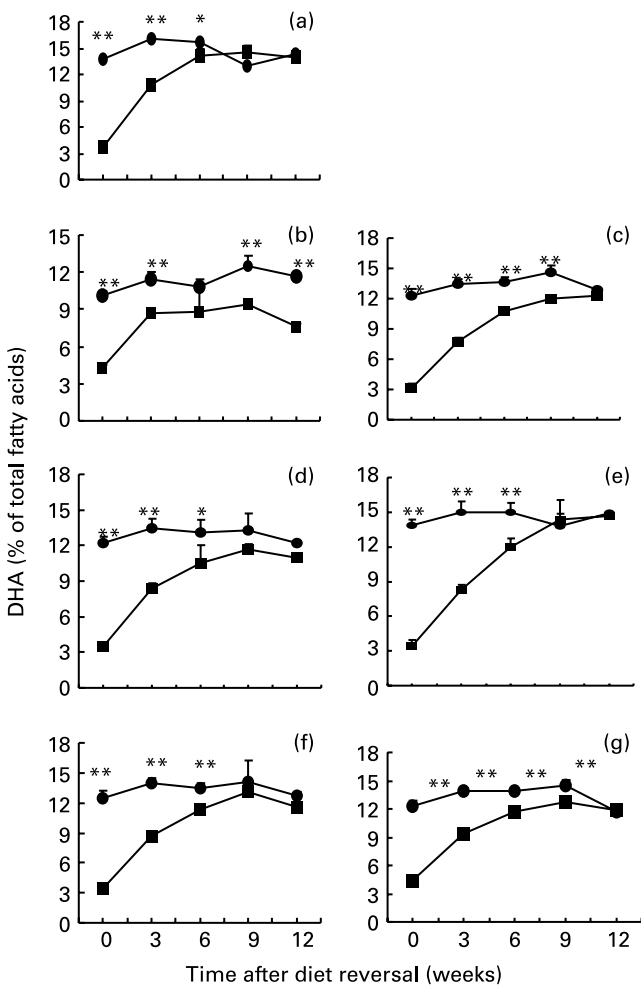


Fig. 1. Regional changes in DHA (% of total fatty acids) in the cerebellum (a), medulla (b), hippocampus (c), hypothalamus (d), cortex (e), striatum (f) and midbrain (g) of F2 female rats after switching from an *n*-3-deficient diet (*n*-3 Def; ■) to an *n*-3-adequate diet (*n*-3 Adq; ●). At each time point, the values are means for five rats, with standard deviations represented by vertical bars. Mean value was significantly different from that of the *n*-3 Def rats: **P* < 0.05; ***P* < 0.01.

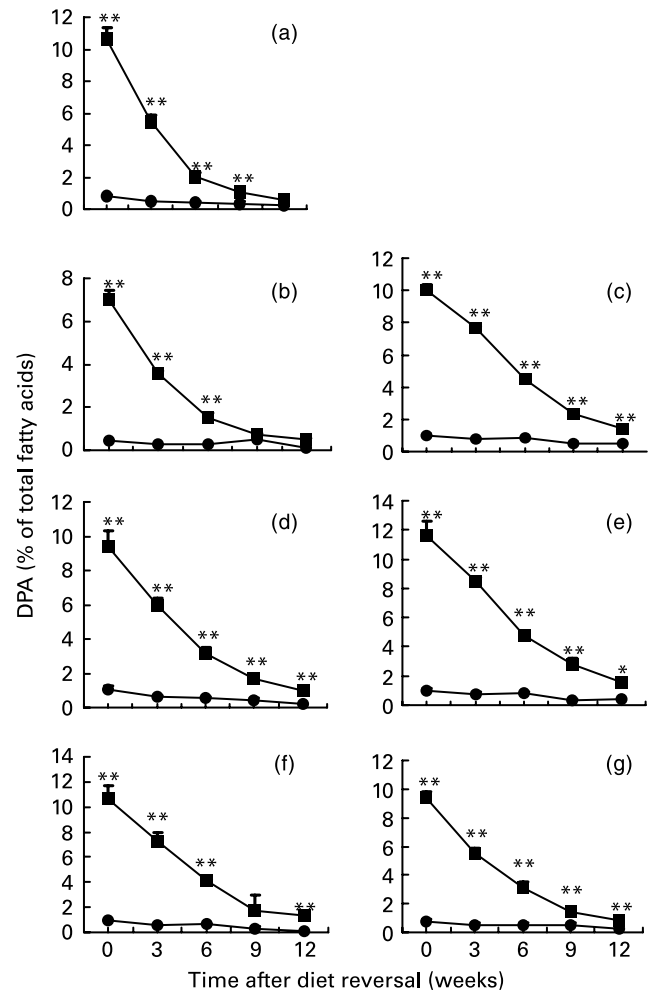


Fig. 2. Regional changes in docosapentaenoic acid (DPA, % of total fatty acids) in the cerebellum (a), medulla (b), hippocampus (c), hypothalamus (d), cortex (e), striatum (f) and midbrain (g) of F2 female rats after switching from an *n*-3-deficient diet (*n*-3 Def; ■) to an *n*-3-adequate diet (*n*-3 Adq; ●). At each time point, the values are means for five rats, with standard deviations represented by vertical bars. Mean value was significantly different from that of the *n*-3 Def rats: **P* < 0.05; ***P* < 0.01.

and midbrain. The present result agreed with that reported by Carrie *et al.* (2000) on 4-month-old mice, but it had no comparison with the data presented by Marteinsdottir *et al.* (1998) and Favreliere *et al.* (1998), who measured fatty acid composition in other regions rather than the medulla.

The difference in distribution of DHA and AA across various regions was apparently greater when the neonatal rats were compared with the weaning and adult rats. In the neonatal rats (aged 3 d), the difference in DHA content was less than 15.1% between the striatum with the highest percentage (12.6) and the hypothalamus that had the lowest percentage (10.7). The weaning rats had a difference of 23.5% when DHA content in the cortex (15.3%) was compared with that in the medulla (11.7%). It was true that the difference in DHA content was even greater (32.7%) between the cortex (15.9%) and medulla (10.7%). This difference could be greater (>59.3%) in mice as reported by Carrie *et al.* (2000), who found that in 4-month-old mice, the medulla had a DHA content of 9.0% while front cortex had a DHA content of 22.1%. It was more evident if the difference in AA content in a region that had the highest percentage was compared with that with the lowest percentage. The neonatal rats (day 3) had a difference less than 4.7% (hippocampus, 12.9%; striatum, 12.3%). In contrast, the weaning rats (day 21) had a difference increased to 37.5% (cortex, 12.84%; medulla, 8.02%) while the adult rats (10 weeks) had a difference greater than 50.9% (hippocampus, 11.4%; medulla, 5.6%). Similarly, Carrie *et al.* (2000) observed that, in mice, the medulla had the lowest AA percentage (4.7) while hippocampus had the highest AA percentage (10.5).

The present study showed that DHA was not proportionally depleted in various regions of brain in response to an *n*-3 deficient diet (Table 5). The cortex, hippocampus, striatum, cerebellum and hypothalamus had DHA depleted by more than 71% followed by the midbrain, which had 64% depletion. In contrast, the medulla appeared to be a region that was relatively resistant to depletion, having DHA depleted only by 57% (Table 5). The present study also showed that *n*-3 deficiency was characterised by not only a decrease in brain DHA percentage but also an increase in 22:5*n*-6 (Table 5). This observation is in agreement with a report on rats by Moriguchi *et al.* (2001), mice by Carrie *et al.* (2000) and monkeys by Connor *et al.* (1990). Compared with the *n*-3 adequate diet, 22:5*n*-6 increased by 7–14-fold in various brain regions of rats maintained on the *n*-3-deficient diet, suggesting that the depletion of DHA was compensated by an accumulation of 22:5*n*-6 in the brain. In this regard, the medulla had 22:5*n*-6 accumulated most although it had DHA lost least (Table 5). In contrast, the hypothalamus and hippocampus were the two regions that gained 22:5*n*-6 least.

The present study is the first to examine the regional difference in DHA recovery in rat brain. The major finding was that the medulla exhibited a much slower time course for DHA recovery compared with the other regions (Fig. 1). The present study is in agreement with that by Moriguchi *et al.* (2001), who only examined the rat brain as a whole and found that full recovery of DHA in F2 *n*-3-deficient rats was not possible until 8 weeks after switching from an *n*-3-deficient diet to an *n*-3 adequate diet. The similar DHA reversible rate in retina was also observed, but DHA recovery in the liver and serum was complete within 2 weeks after the diet reversal (Moriguchi *et al.* 2001). Connor *et al.* (1990) studied reversibility of *n*-3 fatty acid deficiency in the brain of rhesus monkeys and found that DHA level in

the cerebral cortex could be up regulated by switching to feeding a fish oil diet.

Each brain region is associated with its specific functions (Bennett, 1977). For example, the cerebellum is the site related to the control of balance in body movement, and the storage of conditional learning. The hypothalamus has been implicated in the mediation of a number of behaviours including sleep, waking, emotion, reproductive activity, hunger and thirst (Bennett, 1977). The cerebral cortex and hippocampus are the sites that are involved in information storage and control of memory, intelligence, thoughts and other higher mental functions. It has been shown that the level of brain DHA falling is associated with brain functional changes. In human infants, the addition of DHA or DHA and AA in combination into infant formula improves performance in visual acuity, visual recognition memory, learning ability, Bayley mental developmental index, and developmental quotient scores (Uauy *et al.* 1990; Carlson *et al.* 1993; Agostoni *et al.* 1995; Carlson & Werkman, 1996; Willatts *et al.* 1998; Birch *et al.* 2000; Innis, 2004). When maintained on an *n*-3-deficient diet, rats or mice had a poorer performance in a Y-maze (Lamprey & Walker, 1976), shock avoidance (Bourre *et al.* 1989) and spatial learning (Moriguchi *et al.* 2000). When the rats or mice were re-fed with the *n*-3 fatty acid-supplemented diet, the DHA level was partially recovered and so were spatial learning (Moriguchi & Salem, 2003) and the performance behaviour in a maze (Lim & Suzuki, 2001). The present results demonstrated that the cortex and hippocampus were the two sites where DHA was depleted most (Table 5). This correlates well with the observation that the rats showed poorer performance in a maze, shock avoidance and spatial learning when fed the *n*-3-deficient diet.

In summary, the present results demonstrated that DHA and AA in the brain were not proportionally distributed in various regions. The distribution of DHA and AA was age-dependent and region-specific. Most important was that DHA in the brain was not proportionally depleted in response to an LNA-deficient diet. Most interesting was that complete recovery of DHA was possible in all sub-regions except for the medulla, where DHA recovery reached only 62% after 12 weeks of diet reversal.

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