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ScienceDirect
Journal of Nutritional Biochemistry 22 (2011) 835–842
Journal of Nutritional Biochemistry

Deposition of docosahexaenoic acid (DHA) is limited in forebrain of young obese *fa/fa* Zucker rats fed a diet high in α-linolenic acid but devoid of DHA☆

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Received 3 February 2010; received in revised form 20 June 2010; accepted 30 June 2010

Abstract

Docosahexaenoic acid (DHA) is required for neurotransmitter synthesis and learning. Conversion of α-linolenic acid (ALA) to DHA is considered adequate to support brain function in youth, but it is unknown if brain DHA can be maintained in insulin resistant states. This study investigated brain fatty acid and desaturase activities in young insulin resistant Zucker rats on diets with and without DHA. Male *fa/fa* and lean rats were fed diets enriched with flaxseed (FXO, ALA: 35.5% fatty acids), menhaden (MO, DHA: 9.2%) or safflower oil (SO, linoleic acid: 54.1%) for 9 weeks, $n=8$ per diet per genotype. Compared to lean, the 15 week old *fa/fa* rats were obese (56% heavier) and insulin-resistant (>18-fold in homeostasis model assessment of insulin resistance). The forebrain of *fa/fa* rats had higher palmitoleic (16:1n-7) and dihomo-γ-linolenic (20:3n-6) acids, and higher Δ9, Δ6 but lower Δ5 (all $P\leq.006$) desaturase indices than lean. The Δ9 and Δ6 desaturase indices positively, while the Δ5 negatively (all $P\leq.01$) correlated with insulin resistance. The Δ9 desaturase index positively correlated with adiposity index. The percentage of forebrain DHA of *fa/fa* rats was lower ($P=.011$) than lean rats when fed FXO diet while there was no difference ($P>.05$) between *fa/fa* and lean rats fed MO or SO diet. Thus, the alterations in the fatty acid and desaturase indices in the brain were consistent inhibited forebrain synthesis of DHA in the *fa/fa* rats. ALA may not have potential to effectively serve as a precursor for synthesizing DHA for youth forebrain during insulin resistance since Δ5 desaturase activity is limited.

Keywords: Hyperinsulinemia; Forebrain; DHA; α-linolenic acid; Young Zucker rats

1. Introduction

The rising prevalence of childhood obesity is a major public health concern worldwide in view of the associated insulin resistance [1]. A US population-based study revealed a prevalence of insulin resistance of 3.1% among normal weight, 15.0% among overweight and 52.1% among obese adolescents [2]. The insulin resistance syndrome is highly prevalent in Canadian youth as well [3]. Academic achievement [4] and intelligence [5] are lower in obese children, the cause of which is multifactorial. Lipids such as DHA are postulated to affect cognition in obesity [6]. The brain is rich in DHA as a fundamental component of neural cell membranes [7] where it modulates functional properties such as fluidity, permeability for metabolite exchange, activity of membrane-bound enzymes and receptors, and electrical and humoral signal transduction [8]. To date, whether DHA is implicated in the reduced academic achievement of children has not been addressed.

Numerous studies have reported on plasma and tissue fatty acids in adults [9,10] and youth [11–13] with insulin resistance and related disorders. This pattern is characterized by a decrease in linoleic acid (18:2n-6) and an increase in palmitoleic (16:1n-7), γ-linolenic (18:3n-6) and dihomo-γ-linolenic (20:3n-6) acids. Since the content of these increased fatty acids is normally very small in the diet, this augmentation in plasma lipids is thus indicative of increasing endogenous desaturation of palmitic acid (16:0) by Δ9 desaturase (leading to increased 16:1n-7) and of linoleic acid by Δ6 desaturase (increasing the proportions of 18:3n-6 and 20:3n-6) [14]. This may reflect a high intake of 16:0 and low intake of 18:2n-6 in the diet and a concomitant increase in Δ9 and Δ6 desaturases but low Δ5 desaturase activity [15,16], the latter which is required for the synthesis of DHA. Indeed, Pima Indians, a population with the highest reported incidence of insulin resistance in the world, have low skeletal muscle DHA [17]. This striking difference cannot be explained by diet since among the Australian population, even individuals with little or no discernible DHA intake had muscle DHA much higher than the Pima study group [17,18].

Under normal physiological conditions, the adult liver has ample capacity to synthesize DHA from circulating ALA. In adult rats the

☆ Supported by grants from the Flax Council of Canada, Canada-Manitoba Agri-Food Research and Development Initiative and Natural Sciences and Engineering Research Council of Canada.
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0955-2863/$ - see front matter.
doi:10.1016/j.jnutbio.2010.06.012

estimated rate of DHA synthesis by liver is about 10× the DHA consumption rate in brain tissue [19]. This high synthesis rate is supported by the high expression and high activity of the elongase and desaturase enzymes in the liver [19,20]. However, in humans below normal blood levels of DHA are common in liver disease, diabetes and aging [21,22]. Such reductions are postulated to be associated with reduced desaturase or elongase activity and are a risk factor for brain disease in the absence of dietary DHA supplementation [23]. It is not clear if brain DHA can be sustained in obesity and insulin resistant states but it is suspected to be inadequate since people with insulin resistance have greater cognitive decline [24,25]. In fact, insulin activity is required elongase and desaturase enzymes in liver [26]. Very few studies have examined the effect of insulin resistance on brain fatty acid metabolism [27]. Although one study in the Zucker model of insulin resistance observed no difference in brain DHA compared with non-insulin-resistant rats, the diet provided preformed eicosapentaenoic acid (EPA) and DHA which bypasses Δ5 desaturase [27]. It is well known that deposition of DHA is higher in brain when it is provided in the diet compared to ALA [28]. Thus, it is unknown if Δ5 desaturase activity is sufficient to compensate for a diet devoid of DHA. The purpose of the present study was to compare the effects of dietary n-6 polyunsaturated fatty acid (PUFA) (linoleic acid, 18:2n-6), n-3 PUFA (ALA, 18:3n-3) and n-3 long chain-PUFA (LC-PUFA; DHA, 22:6n-3) on the fatty acid composition and desaturases activities of three brain regions in obese and hyperinsulinemic young rats. It is particularly important to study this association in young rats as brain responds to n-3 fatty acid supplementation in an age- and time-dependent manner [29,30]. This study was designed to advance understanding of the impact of hyperinsulinemia on brain fatty acid profile and the effectiveness of different dietary PUFA supplementation to prevent the postulated aberrations in fatty acid status in a youth model of insulin resistance.

2. Methods and materials

2.1. Animals and diets

The study design and diet composition have been published in detail [31,32]. Briefly, 5-week-old male *fa*/*fa* and lean Zucker rats (Charles River Laboratories, St. Constant, QC) were randomly assigned to diets containing 10% (w/w) fat mixtures with flaxseed oil (FXO, ALA: 35.5% fatty acids), menhaden oil (MO, DHA: 9.2%) or safflower oil (SO, linoleic acid: 54.1%) as the primary oils (n=8 per group per genotype). To control for possible effects of the n-3 diets (FXO or MO) on body weight, there were weight-matched (WM) groups within each genotype with rats fed SO control diet in an amount to maintain body weight similar to FXO or MO groups, whichever weighed less. All diets had similar total saturated fatty acids (SFA), monounsaturated fatty acid (MUFA), and PUFA, and thus, the PUFA-to-SFA ratio was close to 2.1 for all groups. The n-6 to n-3 ratios for the MO, FXO and SO control diets were 1.0, 0.5 and 58.6, respectively (Table 1). Both the MO and FXO diets met the minimum suggested requirements for rodents according to the AIN-93 diets for growth and maintenance; ALA of at least 2 g/kg diet [33], while the SO diet provided 0.9 g/kg diet. Previously diets with 0.7 and 1.1 g ALA/kg diet supported brain DHA in rats at amounts not different from diets with ALA at 2 g/kg diet [34].

The rats were given a 1 week adaptation while fed the SO control diet, and then received their respective test diets ad libitum, except the WM group, for 9 weeks. The duration of 9 weeks was used since in rats 8 weeks is required for brain fatty acid status to fully respond to changes in dietary fat [30]. The diet was made fresh weekly and stored at −20°C until fed. Fresh feed was provided daily and feed intake was recorded, while rats were weighed weekly. Throughout the entire study period, rats were housed individually in standard hanging cages with controlled temperature (21–23°C), humidity (55%), and 12-h light cycle. The experiment was conducted in accordance with the guidelines of the Canadian Council for Animal Care and was approved by the University of Manitoba Protocol Management and Review Committee.

2.2. Tissue collection

At the end of the 9 week feeding trial, rats (15 weeks of age) were food deprived overnight and euthanized by CO_2 asphyxiation. Trunk blood was collected, stored on ice and centrifuged at 4°C for 15 minutes. Serum for biochemical measurements was stored at −80°C. The brains were quickly removed, weighed and dissected on ice into forebrain, cerebellum and hippocampus. Samples were flash frozen in liquid nitrogen and stored at −80°C until fatty acid analysis.

Table 1
Fatty acid composition of lipids in diets

Fatty acids (g/100 g)	FXO	MO	SO
Σ SFA	25.5	25.2	26.2
Σ MUFA	19.4	20.5	18.5
Σ PUFA	55.1	54.3	55.3
18:2 n-6	19.2	23.2	54.1
18:3 n-6	0	0.4	0
20:4 n-6 (AA)	0	0.5	0
Σ (n-6) PUFA	19.2	24.4	54.1
18:3 n-3 (ALA)	35.5	1.1	0.9
20:5 n-3	0	8.0	0
22:6 n-3 (DHA)	0	9.2	0
Σ (n-3) PUFA	35.5	24.3	0.9
n-6/n-3 PUFA	0.5	1.0	58.6
PUFA/SFA	2.2	2.1	2.1

2.3. Brain fatty acid analyses

Total lipids were measured in forebrain, cerebellum and hippocampus since the proportion of free fatty acids and phospholipid-bound fatty acids is modified by CO_2 asphyxiation [35]. In brief, fatty acids were extracted using chloroform: methanol 2:1 containing 0.01% butylated hydroxytoluene (BHT) according to a method [36] adapted from Folch et al. [37]. After adding an internal standard, C17:0, brain tissues were homogenized and the crude lipid extracts were transmethylated in 1.2 ml of methanolic HCl (3 mol/L, Supelco, Bellefonte, PA, USA) at 80°C for 1 h. Fatty acids were identified by comparison of retention times with standards and expressed as g/100 g of total fatty acids. For comparison of the results to similar studies [27,38], eight fatty acids including the most prominent peaks on gas chromatography (Varian Star 3400, Mississauga, Canada) analysis were included in the calculation: SFA (16:0 and 18:0), MUFA (16:1n-7 and 18:1n-9), PUFA (18:2n-6), LC-PUFA (20:3n-6, 20:4n-6 and 22:6n-3). Fatty acids including 18:3n-3, 18:3n-6 and 22:5n-6 were below detection limits. The combined composition of these fatty acids accounts for >62% of the total fatty acids of the brain.

2.4. Estimation of desaturase activity of brain

The product to precursor ratios of individual fatty acids in different regions of brain was calculated to estimate the activities of different desaturases as follows: 16:1n-7/16:0 for Δ9 desaturase, 20:3n-6/18:2n-6 for Δ6 desaturase, and 20:4n-6/20:3n-6 for Δ5 desaturase [9].

2.5. Biochemical measurements

Serum glucose concentration was determined using the glucose oxidase method with a Glucose Assay Kit 510-A (Sigma Diagnostics, St. Louis, MO, USA). Dilutions for serum were between 10- and 25-fold for lean and *fa*/*fa* Zucker rats. Absorbance was read at 450 nm (SPECTRAmax 340, Molecular Devices, Sunnyvale, CA, USA) and the concentration was adjusted for dilutions. Serum insulin concentration was determined using a rat specific radioimmunoassay kit (Linco Research, St. Charles, MO, USA). Lean Zucker rat serum was diluted by a factor of 5, while *fa*/*fa* Zucker rat serum was diluted by a factor of 100. All serum assays and standards were run in duplicate, and the coefficient of variation was <10%. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) with the formula: [fasting insulin (pmol/L)×fasting glucose (mmol/L)]/135 [39]. An adiposity index was computed for each rat as 100×(sum of fat pad weights)/(body weight) [40].

2.6. Data analysis

Results are expressed as the means±S.E.M. Data were analyzed using a factorial ANOVA and the general linear model procedure of SAS (version 9.1, Cary, NC, USA) as is suitable for the genotype (lean, *fa*/*fa*) by diet (FXO, MO and SO plus WM as required) factorial design. Significant differences (P<.05) among means of groups by dietary oil mixture or by regions of brain or any interaction effects were assessed using Bonferroni post hoc t tests. The relationships between desaturase indices of brain and insulin resistance and adiposity index were investigated using Pearson's correlation coefficient r to quantify the strength of the relationship.

3. Results

3.1. Experimental system

Due to genotype, *fa*/*fa* rats were significantly heavier than lean rats (Table 2). There were no differences in body weight among the

Table 2
Final body weight (g) of *fa/fa* and lean Zucker rats fed experimental diets for 9 weeks

Genotype	Diet				*P* value		
	FXO	MO	SO	WM	Diet	Geno	Diet×Geno
fa/fa	615±11^{X}	606±18^{X}	616±13^{X}	574±14^{X}	.060	<.001	.739
lean	385±4^{Y}	399±6^{Y}	394±10^{Y}	366±16^{Y}			

Values are means±S.E.M., n=8 per group.
Different capital superscripts ^{X} and ^{Y} indicate significant main effect of genotype (P<.05).
fa, *fa/fa* Zucker rat; ln, lean Zucker rat; Geno, genotype.

FXO, MO and SO groups; therefore, the WM group was not included in further analyses. Thus, any changes due to dietary intervention were not confounded by differences in body weight. The *fa/fa* rats consumed 41% more total feed than lean rats (Table 3). At 15 weeks of age, the body weight of *fa/fa* rats was 56% greater accompanied by 197% more visceral fat. Thus the adiposity index was significantly (P<.0001) higher than leans. In addition, the *fa/fa* rats were insulin resistant as indicated by the substantially greater (>18-fold, P<.0001) HOMA-IR value in *fa/fa* rats compared to lean rats. After 9 weeks SO diet consumption, both genotypes had lower (1693±92 pmol/L, P=.020) fasting serum insulin concentrations compared to rats fed with FXO (2569±159 pmol/L) and MO (2485±142 pmol/L).

3.2. Brain fatty acid composition

Although there was no significant difference in hippocampus weight between genotypes (P>.05, Table 3), the whole brains of *fa/fa* rats were 7% smaller than those of lean rats (P<.0001). The *fa/fa* brain weight was 0.31% of body weight, while the lean brain weight was 0.52% of body weight (P<.0001).

For SFA (16:0 and 18:0), no effect of diet, genotype or diet by genotype interaction was observed in all three studied brain regions (all P>.05, Table 4). For 16:1n-7 and 20:3n-6, there were similar genotype and diet main effects. Compared to lean rats, *fa/fa* rats had higher (all P≤.0002) 16:1 n-7 in all three brain regions and higher 20:3 n-6 in forebrain and cerebellum regardless of diet. Rats fed MO had higher and SO had lower (all P≤.006) proportion of 16:1 n-7 and 20:3 n-6 among the three diet groups regardless of genotype.

For forebrain 18:1 n-9, no effect of diet, genotype or diet by genotype interaction was observed (P>.05). The high n-6 diet (SO group) effectively elevated forebrain 18:2 n-6 in both *fa/fa* and lean rats (P=.001), while no effect of genotype or diet by genotype interaction was observed (all P>.05). The forebrain arachidonic acid (AA) was responsive to diet only, as rats from SO groups had significantly higher AA values than the rats from n-3 dietary groups (P=.0006). For the forebrain DHA, there was a diet by genotype interaction effect (P<.0001); high n-3 diets (FXO and MO groups) elevated DHA (P<.0001) in lean rats, but *fa/fa* rats fed FXO were unable to achieve higher DHA than rats fed high n-6 PUFA (SO diet). There was no genotype effect for forebrain DHA in rats fed SO diet or in rats fed MO diet.

For the cerebellum 18:1 n-9 and 18:2 n-6, no effect of diet, genotype or diet by genotype interaction was observed (all P>.05, Table 4). After 9 week of consuming the MO diet, the cerebellum had significantly lower AA (P=.02), but there were no differences in cerebellum AA between rats fed the FXO diet and those fed the SO diet. There was no genotype or diet by genotype interaction (P>.05). For cerebellum DHA, again, there was a diet effect only; rats fed high n-6 diet (SO group) had significantly lower cerebellum DHA than rats fed n-3 PUFA rich diets (FXO and MO groups, P=.004). Both lean and *fa/fa* rats from the FXO groups had similar cerebellum DHA as rats from MO groups (P>.05). There were no genotype and diet by genotype interactions observed.

For the hippocampus 18:2 n-6, no effect of diet, genotype or diet by genotype interaction was observed (all P>.05, Table 4). There was a diet effect for 18:1 n-9 as rats fed MO as well as FXO had significantly higher values than rats fed SO diet. Hippocampus AA and DHA responded to diet, as rats fed high n-6 diet (SO group) had significantly higher AA but with significantly lower DHA in comparison with rats fed high n-3 diets (FXO and MO groups, P<.0001). Feeding FXO and MO had equivalent effects in elevating the proportion of DHA in hippocampus of brain regardless of genotype (P>.05). There was no genotype and diet by genotype interaction effect (P>.05).

3.3. Estimated desaturase activity

There were genotype effects in all three estimated desaturase activities, as *fa/fa* rats had significantly higher Δ9 (all regions) and Δ6 (forebrain and cerebellum) but lower Δ5 (forebrain only) desaturase activities than lean rats (P≤.002, Table 5). There were also diet effects as rats fed with MO had the higher Δ9 (P≤.003) and Δ6 (P≤.01) but lower Δ5 (P≤.004) desaturase activities in all brain regions, while rats fed SO diet had the lower Δ9 and Δ6 but higher Δ5 (all P≤.01) desaturase activities in all brain regions. Rats fed FXO diet had intermediate Δ9, Δ6 and Δ5 desaturase activities across the brain regions compared with rats fed the other two diets.

3.4. Correlations between desaturase indices and insulin resistance and visceral fat

The Δ9 desaturase activity in all three brain regions was positively correlated with insulin resistance (P≤.022, Fig. 1). The Δ6 desaturase activity in both forebrain and cerebellum was positively (P≤.033) correlated with insulin resistance. In forebrain only the Δ5 desaturase activity was negatively (P=.013) correlated with insulin resistance regardless of genotype. The Δ9 desaturase activity in all three brain regions was positively correlated with adiposity index (P≤.0009, Fig. 2) regardless of genotype. In cerebellum only the Δ6 desaturase activity was positively (P=.019) correlated with adiposity index regardless of genotype. In forebrain only the Δ5 desaturase activity was negatively (P=.044) correlated with adiposity index regardless of genotype.

Table 3
Characteristics of *fa/fa* and lean Zucker rats at 15 weeks of age

Measurement	*fa/fa*	lean	*P* value
Total feed intake (g/rat)	1790±22	1267±13	<.0001
Final body wt (g)	613.4±8.3	393.4±4.2	<.0001
Visceral fat pad wt (g)[a]	40.4±1.0	13.6±0.5	<.0001
Adiposity index	6.6±0.4	3.5±0.1	<.0001
Fasting insulin (pmol/L)	4253.9±418.3	243.5±33.4	<.0001
Fasting glucose (mmol/L)	7.2±0.2	6.9±0.3	.163
HOMA-IR	226.9±28.4	12.4±1.8	<.0001
Brain weight (g)	1.88±0.03	2.01±0.04	<.0001
Relative brain wt (g/100 g body wt)	0.31±0.01	0.52±0.01	<.0001
Hippocampus wt (mg)	77.5±2.7	77.0±6.0	.460
Relative hippocampus wt (mg/100 g body wt)	12.6±1.1	19.6±1.4	<.0001

Values are means±S.E.M., n=32 per genotype.
Adiposity index was computed as 100×(sum of fat pad weights)/(body weight).
HOMA-IR: [fasting insulin (pmol/L)×fasting glucose (mmol/L)]/135.
[a] Visceral fat=epididymal+peri-renal fat pads.

4. Discussion

DHA is the most abundant LC-PUFA in the brain, representing roughly 15% of total fatty acids [41]. Normal brain DHA can be

Table 4
Fatty acid profile in brain regions of *fa/fa* and lean Zucker rats fed experimental diets for 9 weeks

Fatty acids (g/100g)	fa+FXO	ln+FXO	fa+MO	ln+MO	fa+SO	ln+SO	*P* value		
							Diet	Geno	Diet×Geno
Forebrain									
16:0	17.9±0.6	17.2±1.6	18.7±0.6	19.2±0.2	19.4±0.4	18.6±0.2	.124	.580	.630
16:1n-7	0.6 ± 0.0^{BX}	0.4 ± 0.0^{BY}	0.6 ± 0.0^{AX}	0.5 ± 0.0^{AY}	0.6 ± 0.0^{BX}	0.4 ± 0.0^{BY}	.006	<.0001	.416
18:0	18.8±0.6	17.6±1.8	19.2±0.3	19.7±0.1	19.2±0.2	19.2±0.2	.216	.687	.514
18:1n-9	15.8±0.6	15.5±0.8	15.5±0.6	14.9±0.1	14.0±0.2	14.8±0.2	.063	.990	.375
18:2n-6	0.8 ± 0.0^{B}	0.7 ± 0.1^{B}	0.7 ± 0.0^{B}	0.7 ± 0.0^{B}	0.9 ± 0.0^{A}	0.8 ± 0.0^{A}	.001	.145	.335
20:3n-6	0.4 ± 0.0^{BX}	0.3 ± 0.0^{BY}	0.5 ± 0.0^{AX}	0.4 ± 0.0^{AY}	0.3 ± 0.0^{CX}	0.3 ± 0.0^{CY}	<.0001	.0002	.213
20:4n-6	8.4 ± 0.5^{B}	8.3 ± 0.8^{B}	8.1 ± 0.2^{B}	8.7 ± 0.1^{B}	10.1 ± 0.1^{A}	10.0 ± 0.2^{A}	.0006	.645	.637
22:6n-3	11.3 ± 0.4^{b}	13.6 ± 0.1^{a}	15.1 ± 0.7^{a}	15.1 ± 0.3^{a}	11.4 ± 0.3^{b}	11.1 ± 0.3^{b}	.0001	.011	.0001
Cerebellum									
16:0	16.7±1.6	15.2±0.3	14.3±0.8	15.2±0.5	14.3±0.6	14.0±0.6	.130	.655	.406
16:1n-7	0.5 ± 0.1^{AX}	0.3 ± 0.0^{AY}	0.4 ± 0.0^{AX}	0.4 ± 0.0^{AY}	0.4 ± 0.0^{BX}	0.3 ± 0.0^{BY}	.004	.0001	.125
18:0	17.3±1.3	17.2±0.3	15.7±0.5	17.0±0.7	16.0±0.4	15.7±0.4	.157	.626	.462
18:1n-9	16.2±0.9	17.5±0.3	18.0±0.7	17.1±0.3	16.5±0.5	16.9±0.2	.331	.654	.208
18:2n-6	0.8±0.0	0.9±0.1	0.8±0.1	0.8±0.0	0.9±0.1	0.9±0.1	.559	.296	.841
20:3n-6	0.5 ± 0.0^{BX}	0.4 ± 0.0^{BY}	0.5 ± 0.0^{AX}	0.4 ± 0.0^{AY}	0.4 ± 0.0^{CX}	0.3 ± 0.0^{CY}	.0006	<.0001	.717
20:4n-6	7.7 ± 1.0^{A}	6.8 ± 0.1^{A}	5.6 ± 0.2^{B}	6.2 ± 0.1^{B}	7.1 ± 0.3^{A}	6.8 ± 0.2^{A}	.016	.602	.334
22:6n-3	13.6 ± 0.6^{A}	11.0 ± 0.5^{A}	11.1 ± 1.3^{A}	12.2 ± 0.5^{A}	8.3 ± 0.9^{B}	7.9 ± 0.5^{B}	.004	.860	.512
Hippocampus									
16:0	19.1±0.2	18.7±0.1	18.5±0.1	19.0±0.2	19.2±0.2	18.9±0.2	.151	.693	.055
16:1n-7	0.5 ± 0.0^{AX}	0.4 ± 0.0^{AY}	0.5 ± 0.0^{AX}	0.4 ± 0.0^{AY}	0.4 ± 0.0^{BX}	0.3 ± 0.0^{BY}	.005	<.0001	.191
18:0	19.6±0.2	19.5±0.4	20.0±0.3	19.7±0.2	19.5±0.2	20.1±0.2	.550	.779	.226
18:1n-9	15.0 ± 0.2^{A}	15.3 ± 0.2^{A}	15.0 ± 0.3^{A}	15.1 ± 0.3^{A}	13.7 ± 0.1^{B}	13.9 ± 0.2^{B}	<.0001	.368	.837
18:2n-6	0.6±0.0	0.7±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	.259	.988	.228
20:3n-6	0.4 ± 0.0^{A}	0.4 ± 0.0^{A}	0.4 ± 0.1^{A}	0.4 ± 0.0^{A}	0.3 ± 0.0^{B}	0.2 ± 0.0^{B}	<.0001	.165	.721
20:4n-6	10.8 ± 0.1^{B}	10.7 ± 0.2^{B}	10.4 ± 0.5^{B}	10.2 ± 0.2^{B}	12.1 ± 0.1^{A}	12.2 ± 0.3^{A}	<.0001	.941	.907
22:6n-3	13.3 ± 0.1^{A}	12.3 ± 0.1^{A}	14.0 ± 0.1^{A}	13.8 ± 0.2^{A}	10.3 ± 0.1^{B}	10.1 ± 0.3^{B}	<.0001	.394	.216

Values are means±S.E.M., $n=8$ per group.
Differences among groups ($P<.05$) are identified by different superscripts: capital superscripts $^{A>B>C}$ for main effect of diet, $^{X>Y}$ for genotype effect, and the low-case superscripts $^{a>b}$ for diet and genotype interaction.

maintained in adult rats by the liver supply of DHA through elongation and desaturation of ALA when dietary DHA is absent but ALA is sufficient (≥4.6 g/ kg diet) [19]. However, reducing ALA at or below 1 g/kg diet results in at least 25% lower brain DHA in many species including fish [42], rats [43] and guinea pigs [44] despite elevated expression and activities of Δ5 and Δ6 desaturases in response to n-3 PUFA deprivation [45]. In the present study providing 0.9 g/kg ALA resulted in ~20% lower brain DHA compared to ALA at 35.5 g/kg, despite the higher Δ5 desaturase activity on the basis of n-6 PUFA synthesis in the SO group.

Conversely, desaturase enzymes can be down-regulated in conditions such as liver disease, type 1 diabetes, ageing and possibly insulin resistance resulting in brain DHA deficiency [19]. Indeed in the present model of insulin resistance, lower forebrain DHA was observed in the *fa/fa* rats fed a diet with more than ample ALA (35.5 g/kg diet) for 9 weeks. Synthesis of DHA was likely the limiting factor rather than deposition of DHA since *fa/fa* rats fed a diet with preformed DHA had forebrain values not different from lean controls. This contrasts data from healthy animal models where lack of dietary DHA in healthy monkeys, piglets, and mice did not decrease brain DHA [46] when sufficient quantities of ALA were in the diet [47]. Even in our lean rats, those fed high ALA compared to preformed DHA had similar brain DHA. These results thus suggest that ALA (flaxseed oil in the present study) was not effective as a precursor for synthesizing DHA within young adult forebrain when accompanied by insulin resistance.

The observation that the *fa/fa* rats did not have lower DHA in cerebellum or hippocampus regardless of diet suggests that the

Table 5
Desaturase indices in brain regions of *fa/fa* and lean Zucker rats fed experimental diets for 9 weeks

Desaturase indices	fa+FXO	ln+FXO	fa+MO	ln+MO	fa+SO	ln+SO	*P* value		
							Diet	Geno	Diet×Geno
Forebrain									
Δ9 16:1/16:0×100	3.4 ± 0.1^{BX}	2.3 ± 0.1^{BY}	3.5 ± 0.1^{AX}	2.7 ± 0.1^{AY}	3.0 ± 0.0^{CX}	2.0 ± 0.1^{CY}	<.0001	<.0001	.225
Δ6 20:3/18:2	0.58 ± 0.03^{BX}	0.46 ± 0.01^{BY}	0.65 ± 0.03^{AX}	0.54 ± 0.03^{AY}	0.34 ± 0.02^{CX}	0.33 ± 0.02^{CY}	<.0001	.0009	.065
Δ5 20:4/20:3	19.3 ± 1.7^{BY}	25.7 ± 1.7^{BX}	17.4 ± 0.9^{CY}	21.9 ± 0.6^{CX}	32.3 ± 1.6^{AY}	36.5 ± 0.4^{AX}	<.0001	<.0001	.676
Cerebellum									
Δ9 16:1/16:0×100	3.2 ± 0.2^{AX}	2.2 ± 0.0^{AY}	3.1 ± 0.1^{AX}	2.5 ± 0.2^{AY}	2.6 ± 0.1^{BX}	1.8 ± 0.1^{BY}	<.0001	<.0001	.214
Δ6 20:3/18:2	0.60 ± 0.04^{BX}	0.46 ± 0.04^{BY}	0.66 ± 0.05^{AX}	0.50 ± 0.03^{AY}	0.48 ± 0.05^{BX}	0.40 ± 0.05^{BY}	.0147	.002	.646
Δ5 20:4/20:3	17.0 ± 3.2^{A}	17.7 ± 0.6^{A}	10.9 ± 0.5^{B}	14.8 ± 0.4^{B}	17.7 ± 1.4^{A}	20.8 ± 2.5^{A}	.0041	.086	.623
Hippocampus									
Δ9 16:1/16:0×100	2.7 ± 0.0^{AX}	2.1 ± 0.0^{AY}	2.6 ± 0.2^{AX}	2.2 ± 0.1^{AY}	2.2 ± 0.1^{BX}	1.7 ± 0.0^{BY}	.0033	.0002	.406
Δ6 20:3/18:2	0.65 ± 0.03^{B}	0.57 ± 0.00^{B}	0.71 ± 0.08^{A}	0.73 ± 0.05^{A}	0.47 ± 0.02^{C}	0.39 ± 0.02^{C}	<.0001	.242	.513
Δ5 20:4/20:3	27.4 ± 0.9^{B}	29.1 ± 1.0^{B}	26.3 ± 5.5^{B}	24.7 ± 1.8^{B}	41.2 ± 0.9^{A}	50.8 ± 0.6^{A}	<.0001	.192	.172

Values are means±S.E.M., $n=8$ per group.
Differences among groups ($P<.05$) are identified by different superscripts: $^{A>B>C}$ for main effect of diet, $^{X>Y}$ for genotype effect.
Δ9 (16:1n-7/16:0×100), Δ6 (20:3n-6/18:2n-6) and Δ5 (20:4n-6/20:3n-6).

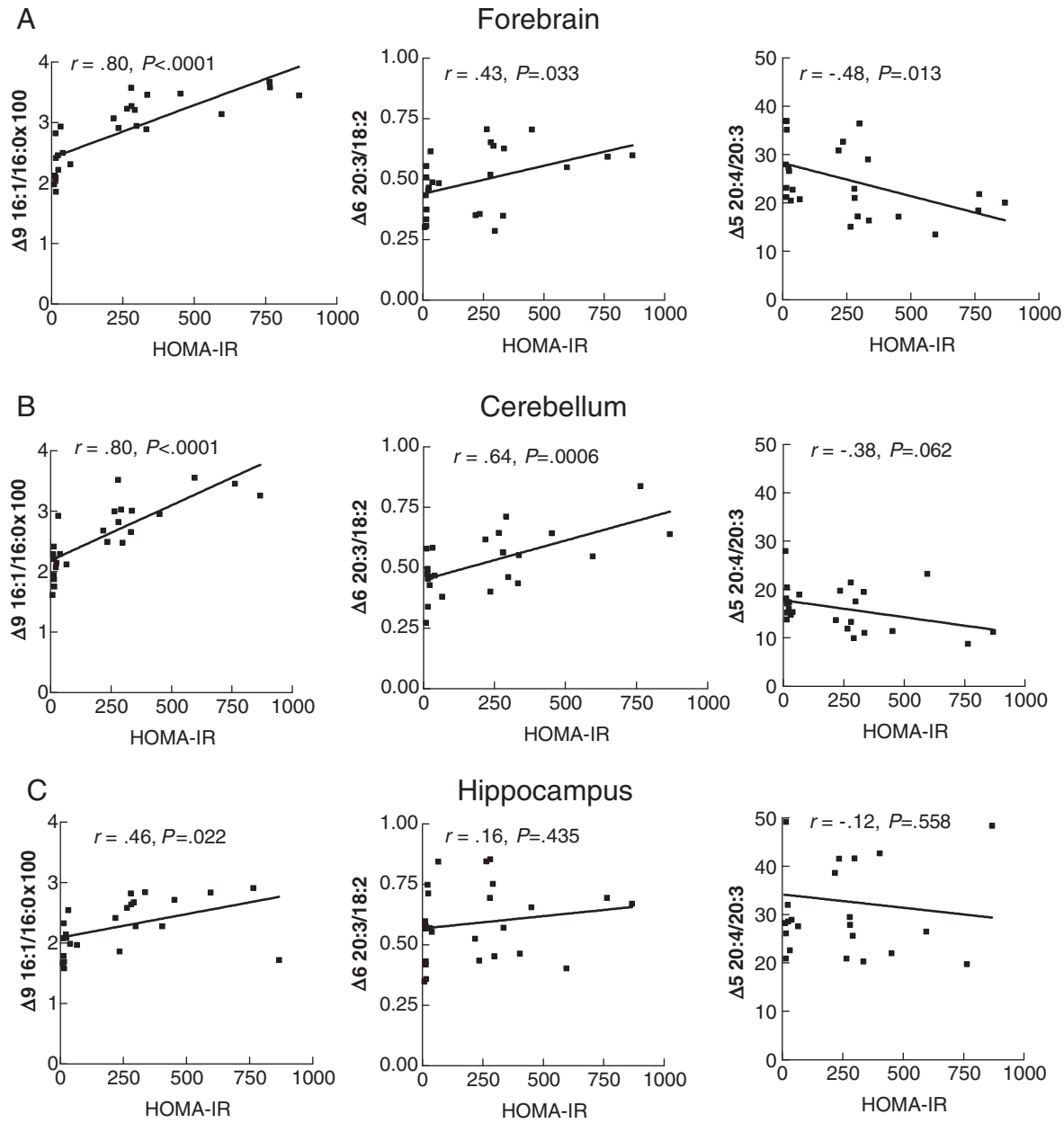

Fig. 1. The relationships between HOMA-IR and Δ9, Δ6 and Δ5 desaturase indices in forebrain (A), cerebellum (B) and hippocampus (C) of *fa/fa* and lean Zucker rats fed experimental diets for 9 weeks. HOMA-IR=fasting insulin (pmol/L)×fasting glucose (mmol/L)/135. Δ9 desaturase:16:1n-7/16:0; Δ6 desaturase: 20:3n-6/18:2n-6; Δ5 desaturase : 20:4n-6/20:3n-6.

forebrain desaturase or elongase activity is vulnerable to hyperinsulinema. This supposition is supported by the lower Δ5 desaturase activity in *fa/fa* rat forebrain but not the other regions. Lower forebrain DHA is associated with alterations in neurotransmission pathways [48] and has also been observed in some pathological conditions associated with neurobehavioral changes, such as Alzheimer disease [49] and schizophrenia [50]. The present study adds insulin resistance as one more factor that specifically affects forebrain DHA status in a model of obese youth. Obesity is also characterized by hypercortisolemia [51] that inhibits transcription of desaturase enzymes [26]. Future studies should examine relationships among brain desaturase activity, DHA, hyperinsulinemia and corticosteroids in the obese *fa/fa* Zucker rat.

Brain fatty acids have only been studied in female Zucker *fa/fa* rats [27,38]. In the present study, all three estimated desaturase activities of forebrain were altered in male *fa/fa* rats in comparison with lean rats. The altered desaturase indices of forebrain were significantly correlated with insulin resistance (HOMA-IR). While the present study cannot clearly distinguish between reduced liver and brain conversion of ALA to DHA in the aetiology of low forebrain DHA, muscle and adipose DHA was previously reported as similar between our *fa/fa* and lean rats [31], suggesting liver synthesis is adequate in the *fa/fa* model of insulin resistance. Thus the lower brain DHA observed herein is likely ascribed to pathophysiology localized in forebrain. However, some, but not all [52], *in vitro* studies show increased Δ9 [53], Δ6 [38] and decreased Δ5 [53] desaturase activities in liver microsomes of *fa/fa* Zucker rats as compared with lean rats. Further studies are warranted.

The underlying mechanism for this regionally selective modification in DHA composition is consistent with previous studies. Mice [54] and rats [55,56] fed n-3 PUFA deficient diets have reduced DHA, particularly in forebrain. DHA accretion [55,56] and uptake

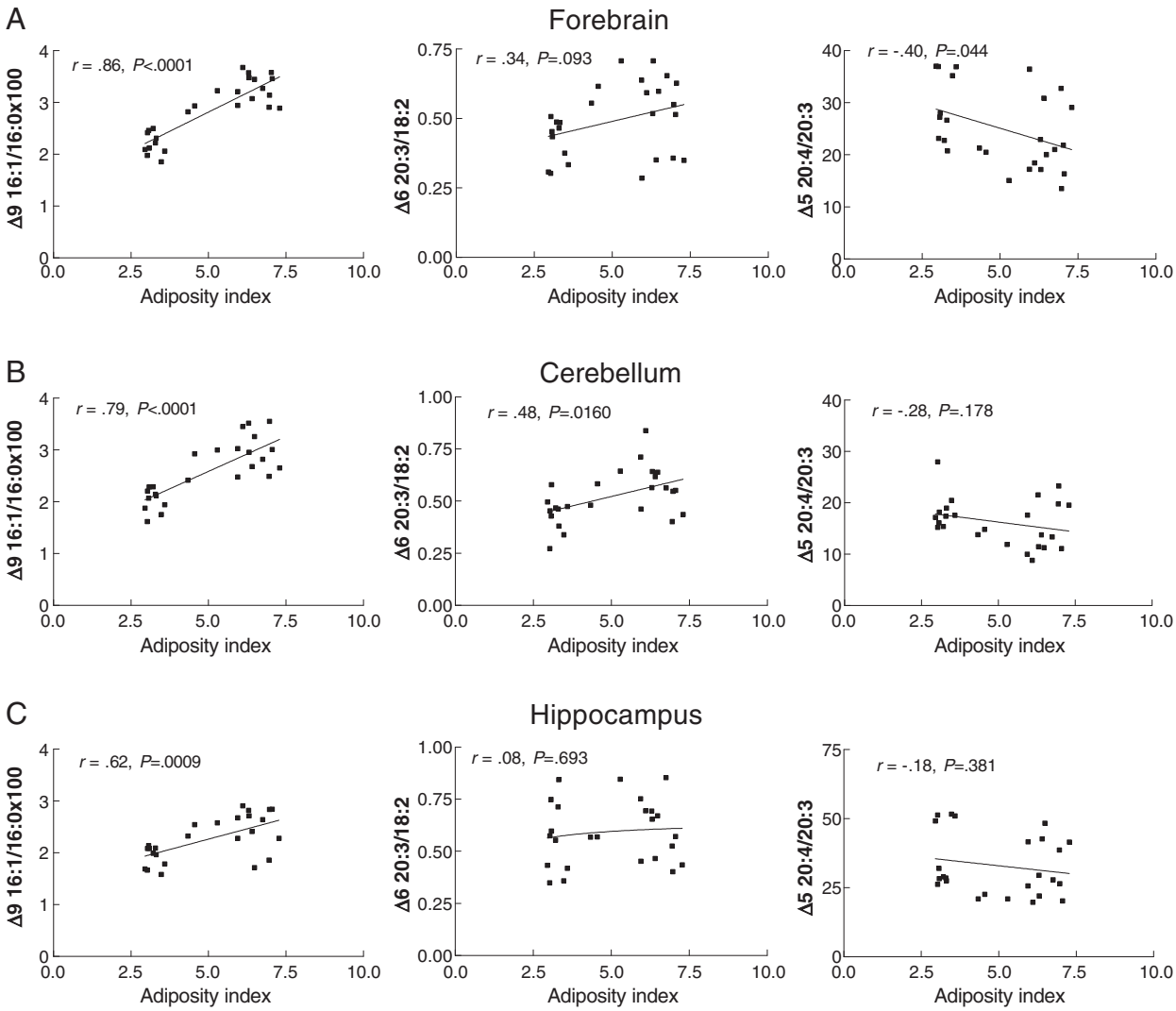

Fig. 2. The relationships between adiposity index and Δ9, Δ6 and Δ5 desaturase indices in forebrain (A), cerebellum (B) and hippocampus (C) of *fa/fa* and lean Zucker rats fed experimental diets for 9 weeks. Adiposity index=100×(sum of fat pad weights)/(body weight).

coefficients [57] are different among specific brain regions under normal physiological conditions. In our hyperinsulinemic model, the indices of desaturase activities between *fa/fa* and lean rats fed FXO were similar in cerebellum and hippocampus, but lower in forebrain resulting in lower accretion of DHA in forebrain and higher accretion in both cerebellum and hippocampus (*t* test, $P<.005$). We speculate that hyperinsulinemia is able to stimulate synthesis of DHA in cerebellum and hippocampus, but not forebrain. Indeed insulin infused into forebrain does not alter energy metabolism whereas in hippocampus phosphocreatinine was enhanced [58], suggesting insulin might have a greater influence in hippocampus and possibly cerebellum. Cerebellum glucose is tightly regulated and non-responsive to both hyper- and hypoglycaemia in men with type 1 diabetes whereas forebrain glucose readily reflects blood values [59]. Future work should aim to characterise insulin metabolism in each brain region under normal and hyperinsulinemic states to confirm if desaturase enzymes are also differentially affected.

This is the first study regarding the effect of n-6 or n-3 LC-PUFA on brain desaturase activities, however, controversy has surrounded the roles of n-6 vs. n-3 fatty acids with regard to insulin resistance [60,61]. Even though the SO diet did not support brain DHA, the lower insulin in this group suggests that diets with n-6:n-3 ratios above 1 may improve insulin resistance and thereby also support endogenous synthesis of fatty acids (i.e. insulin stimulates synthesis). However, high amounts of n-6 PUFA but not n-3 PUFA result in the opposite as demonstrated by Mohan et al. [62] using the same Zucker model (10 weeks old and feeding duration of 10 weeks) where serum insulin values in safflower oil (high in LA) fed obese rats were twice those of the menhaden or coconut oil fed *fa/fa* rats. Low plasma LA is a common feature in individuals with insulin resistance, and insulin sensitivity is associated with a high proportion of LA [63]. In the population-based ($n=895$) Finnish study, men with a high proportions of LA in plasma fatty acids, indicating a high intake of dietary LA, had a lower risk of developing diabetes and showed lower increases in serum insulin and blood glucose a 4 year follow-up [64]. Thus low LA is likely a manifestation of insulin resistance and thus further research is required to optimize dietary LA recommendations.

The brain is an organ generally well-protected against external blood-borne influences, but the cerebral fatty acid composition can be extensively modulated by dietary lipids [65]. It could be argued that the cerebral fatty acid composition can also be modulated by insulin resistance. Since all three brain regions were responsive to modification by dietary LC-PUFA, it is possible that dietary DHA supplementation can be used as a preventive or treatment strategy to minimize the DHA deficiency in the brain of insulin-resistant

individuals. The possible compromised cognitive function and suboptimal learning ability would greatly affect the potential of youth at school, work, social, and family situations [66]. The possible combined burden of the obesity epidemic and insulin resistance in youth is thus concerning beyond the standard chronic disease concerns [67]. Cognitive impairment has long been thought to be irremediable and terminal; however, increasing understanding of its associations with common and modifiable conditions such as insulin resistance and DHA status will challenge these assumptions.

Acknowledgments

We would like to acknowledge the assistance of Shirley C. Fitzpatrick-Wong and Marilyn Latta for fatty acid analysis and Amy Noto on data management. This study was supported by grants from the Flax Council of Canada, Canada-Manitoba Agri-Food Research and Development Initiative and Natural Sciences and Engineering Research Council of Canada.

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