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ORIGINAL ARTICLE

Hypothalamic gene expression in ω-3 PUFA-deficient male rats before, and following, development of hypertension

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Dietary deficiency of ω-3 fatty acids (ω-3 DEF) produces hypertension in later life. This study examined the effect of ω-3 DEF on blood pressure and hypothalamic gene expression in young rats, before the development of hypertension, and in older rats following the onset of hypertension. Animals were fed experimental diets that were deficient in ω-3 fatty acids, sufficient in short-chain ω-3 fatty acids or sufficient in short- and long-chain ω-3 fatty acids, from the prenatal period until 10 or 36 weeks-of-age. There was no difference in blood pressure between groups at 10 weeks-of-age; however, at 36 weeks-of-age ω-3 DEF animals were hypertensive in relation to sufficient groups. At 10 weeks, expression of angiotensin-II$_{1A}$ receptors and dopamine D$_3$ receptors were significantly increased in the hypothalamic tissue of ω-3 DEF animals. In contrast, at 36 weeks, α_{2a} and β_1 adrenergic receptor expression was significantly reduced in the ω-3 DEF group. Brain docosahexaenoic acid was significantly lower in ω-3 DEF group compared with sufficient groups. This study demonstrates that dietary ω-3 DEF causes changes both in the expression of key genes involved in central blood pressure regulation and in blood pressure. The data may indicate that hypertension resulting from ω-3 DEF is mediated by the central adrenergic system.
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INTRODUCTION

Consumption of a diet naturally rich in ω-3 fatty acids has been demonstrated to provide beneficial cardiovascular effects, including reducing blood pressure and plasma total cholesterol and triglyceride levels.[1] Supplementation with ω-3 fatty acids has also been demonstrated to reduce plasma triglycerides,[2] and lower blood pressure[3] and heart rate[4] in hypertensive patients, with mixed evidence relating to improved LDL:HDL cholesterol.[5,6] Some preliminary evidence suggests that prenatal ω-3 fatty acid supplementation may reduce diastolic blood pressure in human infants.[7]

The ω-3 fatty acids are involved in numerous, important physiological roles in the central and peripheral nervous system. They exert these effects on physiology via their metabolites (that is prostaglandins, leukotrienes, thromboxanes, resolvins)[8] and also by altering cell membrane composition.[9] Through these actions, dietary ω-3 fatty acids can change cell membrane fluidity,[10] reduce inflammation[11] and also alter expression of numerous genes[12] and proteins[13] in the brain. Decreased levels of the long-chain ω-3 fatty acid docosahexaenoic acid (DHA) in the brain alters brain function.[14] Dietary ω-3 fatty acid deficiency changes composition of cell membranes,[9] leading to alterations in membrane-associated proteins.[15] Numerous neurotransmitter systems are involved in the central control of blood pressure to varying degrees, including the adrenergic,[16] dopaminergic,[17] endothelin,[18] nitric oxide[19] and renin–angiotensin systems.[20]

The shortest chain ω-3 fatty acid, α-linolenic acid (ALA), is found commonly in vegetable oils, including canola and flaxseed oils. ALA can be converted, to a limited extent, to longer chain ω-3 fatty acids through a series of desaturation and elongation processes *in vivo*. Longer ω-3 fatty acids can also be obtained directly from diet, principally from consumption of fish and other marine foods. Prenatal[21,22] and life-long[23,24] deficiency of the essential dietary ω-3 fatty acid, ALA, can induce hypertension in the rat. However, studies of ω-3 fatty acid deficiency have not always produced hypertensive animals; this may be due to a number of contributing factors, including the use of different rat strains[25] and sex.[26] Recent data indicate that replication problems may relate to dietary interactions between ω-3 fatty acid-deficient diet and the dietary protein content.[23]

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In this study, possible central mechanisms of ω-3 fatty acid deficiency-induced hypertension were evaluated by determining the expression of genes related to hypertension in hypothalamic tissue. To examine possible programming influences of dietary ω-3 fatty acids, gene expression was examined before (10 weeks old), and following (36 weeks old), the development of hypertension.[23,24] The effects of dietary short-chain ω-3 fatty acids (SUF-S) and a combination of short- and long-chain ω-3 fatty acids (SUF-SL) were also examined to determine the effect of type of dietary ω-3 fatty acid on blood pressure and gene expression.

METHODS

Animals and diets

Sprague–Dawley adult breeder rats (18 female, 9 male) were purchased from Animal Resource Center (Canning Vale, Perth, WA, Australia). These animals were provided one of three semisynthetic diets, which were identical apart from the source of dietary fat (see Table 1). The ω-3 fatty acid-deficient (ω-3 DEF) diet was deficient in ω-3 fatty acids (10% safflower oil), the SUF-S diet was sufficient in short-chain ω-3 fatty acids (7% safflower oil+3% flaxseed oil) and the SUF-SL was sufficient in short- and long-chain ω-3 fatty acids (7% safflower oil + 2.9% flaxseed oil + 0.1% tuna oil). Female rats received the diets from 1 week before mating until weaning of the offspring. After weaning (3 weeks), the male pups (n=20 per diet) were housed in pairs. The pups were continued on the same diets as their mothers for the duration of the experiment. Room temperature was maintained at 22 ± 2 °C, and food and water were available *ad libitum*. Following blood pressure measurements (at 10 or 36 weeks-of-age), animals were killed by i.p. Lethabarb (1 ml kg^{-1}), the hypothalamic block was removed and stored at −80 °C until use. The study was approved by the Animal Ethics committee of La Trobe University (approval AEC0506P).

Tail-cuff plethysmography

Animals were acclimated to the system (Rat Blood Pressure System, IITC, Woodland Hills, CA, USA) for 2 days before measurements; rats were placed in restrainers in the heated (27 ± 2 °C) system. Following the two acclimation days, testing was performed over two further days. Three recordings were taken per animal per day; the average of the six measurements was calculated for systolic blood pressure and diastolic blood pressure. This procedure was performed at 10 weeks-of-age, and then in the older animals at 36 weeks-of-age.

RNA isolation

RNA was purified from 76 to 100 mg of tissue from each hypothalamic block. Samples were homogenized and total RNA was extracted with RNeasy Tissue Mini Kit (Qiagen, Alameda, CA, USA) according to the manufacturer's instructions. RNA quantity was confirmed with spectrophotometry by using the Nanodrop instrument (Nanodrop, Wilmington, DE, USA).

QRT-PCR

Total RNA was used for quantitative real-time polymerase chain reaction (QRT-PCR) analysis as described earlier.[27] Briefly, 2 μg of total RNA from each sample were reverse transcribed in the presence of random primers in a total volume of 20 μl. After dilution with 20 μl of water, 1 μl of the diluted reaction mix was used as template in QRT-PCR. The 20 μl reaction volume contained 0.2 mM of dNTP, 1×PCR reaction buffer (ABGene, Epsom, UK), 6 mM of each primer, 4 mM of $MgCl_2$, 1×SYBR Green I (Molecular Probes, Eugene, OR, USA) at final concentration and 0.5 U of thermostart *Taq* DNA polymerase (ABGene). Amplification was carried out with the following cycling parameters: 600 s heat start at 95 °C, 45 cycles of denaturation at 95 °C for 25 s, annealing at 60 °C for 25 s and fluorescence detection at 72 °C for 15 s. A total of 45 cycles were run. All the PCRs were performed in triplicate. After amplification, a melting curve was created to verify the specificity of the PCR reactions. Relative expression ratios were normalized to glyceraldehyde 3-phosphate dehydrogenase and hypoxanthine-guanine phosphoribosyl transferase, as widely used housekeeping genes.[28] The PCR primers used in this study are listed in Table 2.

Table 1 Composition of the experimental diets

	DEF	*SUF-S*	*SUF-SL*
Component			
Sucrose	10.69	10.69	10.69
Casein	20.00	20.00	20.00
Cellulose	5.00	5.00	5.00
Starch	38.70	38.70	38.70
Dextrose monohydrate	7.00	7.00	7.00
Kaolin	3.00	3.00	3.00
AIN 93 trace minerals	0.14	0.14	0.14
Sodium chloride	0.26	0.26	0.26
Potassium Di-H+ phosphate	1.62	1.62	1.62
Potassium sulfate	0.06	0.06	0.06
Potassium citrate	0.83	0.83	0.83
AIN 93 vitamin mix	2.70	2.70	2.70
Fat source			
Safflower oil	10.00	7.00	7.00
Flax seed oil	0.00	3.00	2.90
Fish oil	0.00	0.00	0.10

Abbreviations: DEF, ω-3 fatty acid deficient animals; SUF-S, short-chain ω-3 fatty acids; SUF-SL, short- and long-chain ω-3 fatty acids.

Fatty acid analysis

Fatty acid composition of the prefrontal cortex phospholipids was determined compared against calibrated standard fatty acid methyl esters. Lipids were extracted from tissue using chloroform–methanol and the phospholipids isolated from the total lipids by thin layer chromatography.[22] An internal standard fatty acid (C23:0, Nu-Chek-Prep, Elysian, MN, USA) was added to each phospholipid fraction from the thin layer chromatography plate before methylation to produce the phospholipid fatty acid methyl esters; these were separated by capillary GLC using a BPX-70 column (SGE, Melbourne, Victoria, Australia).

Statistical analysis

Differences between groups were analyzed using two-way analysis of variance, followed by Fisher's protected least-significant difference test (STATISTICA 7.0, Tulsa, OK, USA). $P<0.05$ was considered as a statistically significant difference.

RESULTS

Blood pressure

There were no differences in systolic (ω-3 DEF: 123.5 ± 1.9 mm Hg; SUF-S: 120.1 ± 1.9 mm Hg; SUF-SL: 124.2 ± 2.2 mm Hg) or diastolic (ω-3 DEF: 82.5 ± 1.6 mm Hg; SUF-S: 81.3 ± 1.2 mm Hg; SUF-SL: 82.1 ± 1.5 mm Hg) blood pressure between the groups at 10 weeks-of-age. However, by 36 weeks-of-age the ω-3 DEF group had significantly elevated systolic blood pressure (149.4 ± 3.7 mm Hg) compared with the SUF-S (133.1 ± 3.6 mm Hg) and the SUF-SL (132.2 ± 2.0 mm Hg) groups ($P<0.05$). Diastolic blood pressure was not significantly higher at 36 weeks in ω-3 DEF animals (87.9 ± 2.7 mm Hg) compared with the SUF-S (83.3 ± 2.1 mm Hg) and SUF-SL (84.5 ± 1.6 mm Hg) animals. There were no significant effects between SUF-S and SUF-SL groups, and no differences in heart rate (data not shown). Blood pressure results are displayed in Figure 1.

Hypothalamic gene expression

At 10 weeks-of-age, there was a significant upregulation of angiotensin II receptor type 1 (AT_{1A}) gene expression in ω-3 DEF animals compared with the SUF-S and SUF-SL groups ($P<0.05$). Similarly, there was increased expression of the dopamine receptor D_3 in ω-3

Table 2 Primer sequences

Gene	*Accession number*	*Forward primer*	*Reverse primer*
5-HT$_B$ receptor	NM_017250	5′-ACAAAACATGGAATTCGAAATGG-3′	5′-TGGTTGAACTTCGGAGCCTTA-3′
Adrenergic receptor kinase, β_1	NM_012776	5′-CGAGGTGGCAAGCAGTTTGT-3′	5′-GCAGCTCTTTTTTCCATTGCA-3′
Adrenergic receptor kinase, β_2	NM_012897	5′-GCCCCCAAGTTCCTCAACA-3′	5′-CAGCGGTGGCTTGGAGAA-3′
Adrenergic receptor, α_{1a}	NM_017191	5′-AGTCTGTGAATGGAAGTTTTTCTCTTC-3′	5′-ATTGGTCCTTTGGCACTGTAATC-3′
Adrenergic receptor, α1b	NM_016991	5′-ACTTTTAGGGTCCCTTTTCATCCT-3′	5′-GCCCCTACGATGGTGTTCTC-3′
Adrenergic receptor, α_{1d}	NM_024483	5′-CCATCCGGCAAGTTTTGGT-3′	5′-CAGAGCGGAAGAGCAACAGAT-3′
Adrenergic receptor, α_{2a}	NM_012739	5′-GGTGGTGATCGGCGTGTT-3′	5′-CGACCGCTATGAGCGTGTAG-3′
Adrenergic receptor, α_{2b}	NM_138505	5′-CAGGACCACCCGGTGGTA-3′	5′-AAGTGGGCCCCAGAGAAATG-3′
Adrenergic receptor, α_{2c}	NM_138506	5′-CTGCCAGAACCGCTCTTTAAGT-3′	5′-CCGGGTTGAGCGAACTGT-3′
Adrenergic receptor, β_1	NM_012701	5′-TTCGTGTACCTCCGGGTGTT-3′	5′-CGCTCGCAGCTGTCGAT-3′
Adrenergic receptor, β_2	NM_012492	5′-GGACCTTTCTGCTGTGAATGTG-3′	5′-CCTCAAATCCCTGCCTACAACA-3′
Adrenergic receptor, β_3	NM_013108	5′-CGTTGTCTAGGATCCACCTTGAA-3′	5′-GCACGGGTGGCCTGATATC-3′
Angiotensin 1-converting enzyme 1	NM_012544	5′-GGAGTACACCTGGACACCAAACA-3′	5′-GAAGTTGACGCGACTGGACTCT-3′
Angiotensin II receptor-associated protein	NM_001007654	5′-GCCGCAGGTAAAGGAGTCTTC-3′	5′-TTTCGCAAGTCGGTTCCAA-3′
Angiotensin II receptor, type 1	NM_030985	5′-CAGGAGCTGGATGGATTGGT-3′	5′-GGGAGACTGATGAGATTGCATTT-3′
Angiotensin II receptor, type 2	NM_012494	5′-TTGTGTTGGCATTCATCATTTG-3′	5′-ATACCCATCCAGGTCAGAGCAT-3′
Angiotensin receptor 2; SPT	NM_030656	5′-GGCTACAGCACATCTGCACAA-3′	5′-CCGGGTCCTTCACAAAGAAC-3′
Angiotensin/vasopressin receptor	M85183	5′-CCGGACACTCGGAGGTTAGTC-3′	5′-GGCCCAGAAAGGGAGGTATT-3′
Angiotensinogen	NM_134432	5′-CCCTGGCTTCCCGTCACT-3′	5′-AGTTAGCGCCATCTCCAAGGA-3′
Apoptotic protease-activating factor 1; APAF1	AF320222	5′-ACACACTAGACAAGCCCTCTACCA-3′	5′-GGGAATATCTAATCTGAAATCCACCTA-3′
Bradykinin receptor B1	NM_030851	5′-TCGCCAACTTCTTTGCCTTT-3′	5′-CGGCCTGCGAAGACATAAA-3′
Caveolin	NM_031556	5′-CAGACGAGGTGAATGAGAAGCA-3′	5′-TCGCGGTTGACCAGATCAA-3′
Dopamine β-hydroxylase	NM_013158	5′-GCCGGCCCCTTCGTT-3′	5′-CGAGGAGAGGCTGAAGAACAA-3′
Dopamine receptor D_{1A}	NM_012546	5′-CCGATAGCTGGGCTCATCA-3′	5′-CCTTAAGCAGCCGACTTGGT-3′
Dopamine receptor D_2	NM_012547	5′-TGCCTGTGCCGGATCAG-3′	5′-CGTGTTCCCTGCTTTCCTATG-3′
Dopamine receptor D_4	NM_012944	5′-GCTATGTCAACAGTGCCCTCAA-3′	5′-TTGCGGAAGACACTTCGAAA-3′
Dopamine receptor D_5	NM_012768	5′-CCGGAGTCGTGGAGCCTAT-3′	5′-ACCTTGGTCTCCTTCTTGATGGA-3′
Dopamine receptor D_3	NM_017140	5′-TGCTGGCTGCCCTTCTTC-3′	5′-GGGACACGTGGCATGCTT-3′
Dual endothelin 1, angiotensin II receptor	NM_001004448	GCGGGTGCTGCATCCA-3′	5′-ATCTCCTTGCTCCCCAAAGAG-3′
Endothelin 1	NM_012548	5′-CGAGCCCTATGGCCAACTC-3′	5′-GCCGGACAGATGTTCTTGCT-3′
Endothelin 2	NM_012549	5′-CTGATGGGTTCAGCACAAGCT-3′	5′-AGGGTAGCCCTGGCAGTGA-3′
Endothelin 3	XM_345480	5′-TTCCAGAAAGTTCCCAGTCTTCTC-3′	5′-TGCGCAGGCCTTGTCAT-3′
Endothelin-converting enzyme 1	NM_053596	5′-TTTGAGAAGGGCAGAATTAGGAA-3′	5′-GGAAACTCCGGGCTTGAGA-3′
Endothelin receptor type B	NM_017333	5′-AACCGTGCTTGCGGAAAG-3′	5′-TGGAAAGTTAGAACGGTTAAAAAACA-3′
Erythropoietin receptor	NM_017002	5′-CTGGTATTGGATGAATGGTTGCT-3′	5′-CCCAGGCCCAGAGAGGTT-3′
Nitric oxide synthase 2, inducible	NM_012611	5′-CGGGATGTGGCTACCACTTT-3′	5′-TCAACCTGCTCCTCACTCAAGTT-3′
Nitric oxide synthase 3, endothelial	NM_021838	5′-TCATTAGGTTGACCAAGGCAAA-3′	5′-GGCAGCCTCGCAACTGA-3′

DEF animals relative to either of the ω-3 fatty acid-sufficient groups ($P<0.05$). Three genes, *adrenergic receptor α_{2B}*, *dopamine β-hydroxylase* and *dopamine receptor D_2*, had reduced expression in ω-3 DEF animals compared with the SUF-S ($P<0.05$), but not the SUF-SL, group.

At 36 weeks-of-age, neither AT_{1A} nor D_3 receptor expression remained upregulated in ω-3 DEF animals; however, adrenergic receptors α_{2A} and β_1 were significantly downregulated in the ω-3 DEF group compared with both ω-3 fatty acid-sufficient groups ($P<0.05$). ω-3 DEF animals had significantly reduced expression of adrenergic receptor kinase β_1 compared with animals on the SUF-S diet ($P<0.05$), and endothelin receptor type B compared with the SUF-SL group ($P<0.05$). The gene expression data for all genes analyzed is presented in Table 3.

Fatty acid analysis

The proportion of DHA in the frontal cortex was significantly lower in the ω-3 DEF group compared with the SUF-S and SUF-SL groups at both 10 and 36 weeks ($P<0.05$). The levels of both 22:4 ω-6 and 22:5 ω-6 were significantly elevated in rats fed the ω-3 DEF diet at both 10 and 36 weeks compared with the SUF-S and SUF-SL ($P<0.05$). There were no differences in brain fatty acids between the groups receiving dietary ω-3 fatty acids (SUF-S or SUF-SL). The results of the brain fatty acid analysis are presented in Table 4.

DISCUSSION

This study examined the effects of ω-3 fatty acid deficiency on expression of genes in the hypothalamic area related to hypertension. This was assessed in animals either before (10 weeks old), or following (36 weeks old), development of hypertension. Hypertension was observed only in the systolic blood pressure of the 36 week-old rats. In 10-week-old animals, before the development of hypertension, ω-3 fatty acid deficiency resulted in the significant upregulation of AT_{1A} and D_3 receptor gene expression. In the hypertensive ω-3 fatty acid-deficient animals (36 weeks old), neither AT_{1A} nor D_3 receptor gene expression was altered. However, adrenergic receptors α_{2A} and β_1 were

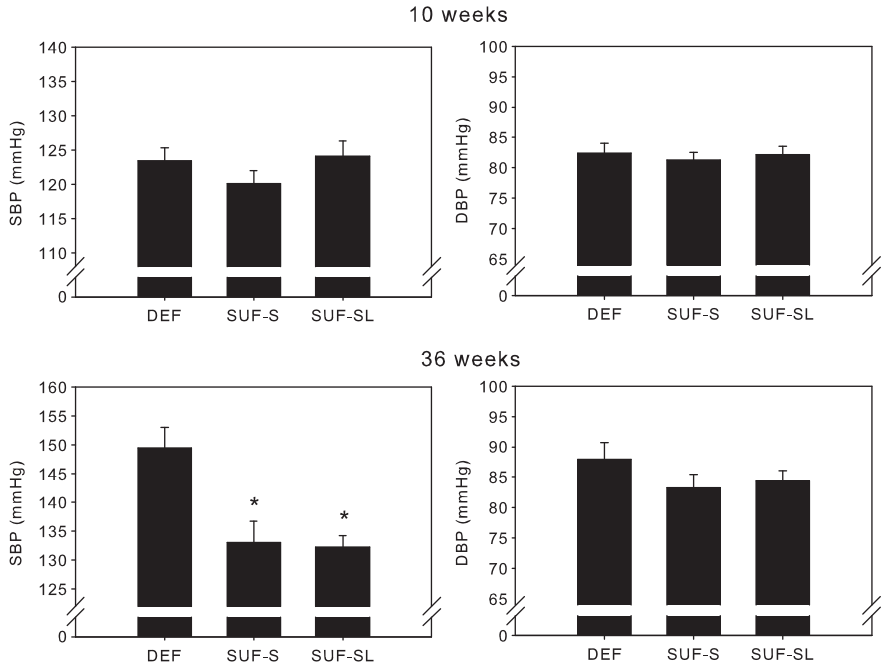

Figure 1 Systolic and diastolic blood pressure of rats at 10 and 36 weeks-of-age. Values are expressed as mean ± s.e.m. $*P<0.05$ *vs.* ω-3 DEF.

both downregulated compared with animals provided diets sufficient in dietary short-chain or short- and long-chain ω-3 fatty acids.

The hypertension present at 36, but not 10, weeks-of-age is consistent with previous reports that have demonstrated that ω-3 deficiency-induced hypertension is not present at 12[23,24] or 18[23] weeks-of-age, but is present after 24 weeks. In contrast to previous data from our group,[23,24] only systolic blood pressure was elevated in ω-3 fatty acid-deficient animals; in this study, diastolic blood pressure was not significantly different compared with the SUF-S or SUF-SL groups.

Diets containing safflower oil have been used in several studies to produce an ω-3 fatty acid deficiency model of hypertension, due to the low ALA levels in safflower oil (<0.5% total fatty acids).[21–23,29] Previously, it has been established that replacing 30% of the dietary safflower oil with flaxseed oil (rich in ALA) prevented the development of hypertension in later life.[23,24] In this study, we established that the addition of a small amount of tuna oil (at 1% of dietary fat, containing 30% DHA) did not produce any additional benefit to blood pressure. In human terms, this would be roughly equivalent to an adult human taking a 1 g fish oil capsule per day based on the 65 g recommended daily intake for dietary fat. Supplementation with fish oil has previously been demonstrated to produce antihypertensive effects in animal models of hypertension, including spontaneously hypertensive rats (SHR)[30] and TGR(mRen-2)27 rats.[29] Fish oil has also been demonstrated to reduce blood pressure in hypertensive patients.[3] When compared with the previous research, the current study contained a relatively low long-chain ω-3 fatty acid dose and this may explain why there was no difference between the SUF-S and SUF-SL groups. This also indicates that short-chain fatty acids alone are sufficient to prevent hypertension in male Sprague–Dawley rat model.

The gene expression changes found in early adulthood in ω-3 DEF animals included upregulation of AT_{1A} receptor expression, a receptor that is known to play a significant role in the transmission of central blood pressure signals. ω-3 DEF animals had higher expression compared with both ω-3 fatty acid-sufficient groups. Activity of ANG II-sensitive neurons in the anterior hypothalamic area is enhanced in the SHR model,[31] and central infusion of ANG II results in sodium-dependent hypertension;[32] this is eliminated when animals are treated with the AT_{1A} receptor antagonist losartan.[33] There is evidence that ω-3 fatty acids affect hypertension through antagonism of the RAS.[34,35] Indeed, ω-3 fatty acids are known to affect vascular responses to infusion of exogenous ANG II.[36–38] However, this change in expression of AT_{1A} did not correspond with a change in blood pressure, and later, when blood pressure was increased in the ω-3 fatty acid-deficient male rats, there was no longer a difference in AT_{1A} receptor expression between ω-3 DEF and either of the SUF groups. This may indicate that the expression change is part of the developmental programming that is known to occur with ω-3 fatty acid deficiency,[21,22,29] or it may indicate that AT_{1A} receptor expression and the central RAS are not involved in hypertension induced by ω-3 fatty acid deficiency.

There was also significant upregulation of dopamine receptor D_3 gene expression in the ω-3 DEF group relative to both ω-3 fatty acid-sufficient groups at 10 weeks-of-age. The D_3 receptor is known to be involved in the development of hypertension; indeed, $D_3-/-$ mice are renin-dependent hypertensive.[39] However, effects of D_3 receptors in hypertension appear to be predominantly peripherally mediated, specifically at the renal level.[40] Consistent with the current study, dietary ω-3 fatty acid deficiency has been associated with central overexpression of dopamine receptor genes in rat pups,[41] albeit different subtypes of dopamine receptors (D_1, D_2). Given that hypertension was not present at the time of this upregulation, and central D_3 receptors do not appear to play a significant role in the mediation of hypertension, it seems unlikely that this is a mechanism in ω-3 fatty acid deficiency hypertension.

In hypertensive ω-3 fatty acid-deficient rats at 36 weeks, there was a significant down regulation of α_{2A} *adrenergic receptor* and β_1 *adrenergic*

Table 3 mRNA expression of 37 genes related to hypertension by QRT-PCR in the hypothalamus of rats fed a diet sufficient in SUF-S or sufficient in both SUF-SL relative to DEF-fed animals

Gene	SUF-S	± s.d.	SUF-SL	± s.d.	SUF-S	± s.d.	SUF-SL	± s.d.
	10 weeks-old				36 weeks-old			
5-Hydroxytryptamine receptor 2B	1.49	0.46	1.23	0.48	1.24	0.04	1.25	0.05
Adrenergic receptor kinase, β_1	−1.18	0.37	−1.04	0.33	2.00[a]	0.02	−1.55	0.03
Adrenergic receptor kinase, β_2	1.39	0.4	−1.02	0.26	1.01	0.04	1.07	0.04
Adrenergic receptor, α_{1a}	1.7	0.1	−1.09	0.19	1.21	0.04	1.19	0.04
Adrenergic receptor, α_{1b}	−1.19	0.55	−1.5	0.56	−1	0.04	−1.02	0.04
Adrenergic receptor, α_{1d}	1.06	0.08	−1.89	0.12	−1.07	0.03	−1.2	0.03
Adrenergic receptor, α_{2a}	1.34	0.36	1.13	0.1	1.99[a]	0.09	2.21[a]	0.06
Adrenergic receptor, α_{2b}	2.11[a]	0.38	−1.03	0.11	1.1	0.04	1.16	0.04
Adrenergic receptor, α_{2c}	−1.59	0.7	−1.55	0.37	−1.13	0.03	1.21	0.05
Adrenergic receptor, β_1	1.09	0.18	1.06	0.3	2.25[a]	0.08	2.15[a]	0.08
Adrenergic receptor, β_2	1.15	0.26	−1.34	0.11	1.41	0.05	1.34	0.05
Adrenergic receptor, β_3	−1.09	0.65	1.27	0.68	1.08	0.04	1.37	0.05
Angiotensin 1-converting enzyme 1	−1.4	0.42	−1.57	0.2	1.26	0.05	1.35	0.05
Angiotensin II receptor-associated protein	1.12	0.08	−1.3	0.1	1.09	0.04	1.28	0.05
Angiotensin II receptor, type 1	−3.26[a]	0.26	−2.23[a]	0.29	−1.1	0.03	−1.03	0.04
Angiotensin II receptor, type 2	1.34	0.53	1.16	0.06	1.03	0.04	1.37	0.05
Angiotensin receptor 2; serine:pyruvate aminotransferase SPT	1.55	0.35	−1.43	0.22	1.3	0.05	1.12	0.04
Angiotensin/vasopressin receptor	1.55	0.13	−1.06	0.24	1.17	0.04	1.08	0.04
Angiotensinogen	1.08	0.14	1.68	0.19	1.56	0.06	1.68	0.06
Apoptotic protease-activating factor 1; APAF1	1.2	0.27	1.17	0.05	−1.28	0.03	1.14	0.04
Bradykinin receptor B_1	−1.4	0.27	−1.69	0.35	1.51	0.05	1.32	0.05
Caveolin	1.15	0.15	1.25	0.11	1.03	0.04	−1.05	0.04
Dopamine β hydroxylase	2.11[a]	0.39	−1.08	0.1	1.03	0.04	1.12	0.04
Dopamine receptor D_{1A}	1.45	0.38	1.42	0.2	−1.35	0.03	1.2	0.04
Dopamine receptor D_2	2.01[a]	0.36	−1.45	0.08	1	0.04	1.13	0.04
Dopamine receptor D_4	1.04	0.25	−1.39	0.21	1.08	0.04	1.12	0.04
Dopamine receptor D_5	1.36	0.45	1.04	0.19	1.15	0.04	1.04	0.04
Dopamine receptor D_3	−2.79[a]	0.72	−2.52[a]	0.54	−1.08	0.03	−1.14	0.03
Dual endothelin 1, angiotensin II receptor	1.52	0.33	1.13	0.32	1.94	0.07	1.51	0.06
Endothelin 1	1.22	0.45	−1.01	0.17	1.06	0.04	−1.03	0.04
Endothelin 2	1.32	0.28	1.04	0.19	−1.09	0.03	−1.12	0.03
Endothelin 3	1.39	0.27	−1.01	0.14	1.57	0.06	1.05	0.04
Endothelin-converting enzyme 1	1.26	0.16	−1.09	0.04	1.23	0.04	1.37	0.05
Endothelin receptor type B	1.4	0.35	1.42	0.4	1.87	1.2	2.19[a]	0.91
Erythropoietin receptor	1.3	0.25	−1.49	0.12	1.09	0.04	−1.05	0.04
Nitric oxide synthase 2, inducible	1.27	0.19	−1.21	0.18	1.33	0.05	1.29	0.05
Nitric oxide synthase 3, endothelial cell	1.32	0.2	−1.17	0.15	−1.01	0.04	−1.01	0.04

Abbreviations: DEF, ω-3 fatty acid deficient animals; QRT-PCR, quantitative real-time-polymerase chain reaction; SUF-S, short-chain ω-3 fatty acids; SUF-SL, short- and long-chain ω-3 fatty acids.
[a]$P<0.05$ *vs.* DEF. Data are expressed as mean ± s.d. and are presented as expression relative to ω-3 DEF animals, which are equal to 1.

receptor genes compared with both sufficient groups. Importantly, the central pre-synaptic α_{2A} adrenergic receptor agonists are known to produce hypotension,[42] and α_{2A} adrenergic receptor−/− mice are hypertensive,[43] and unresponsive to the action of α_2 agonists.[44] Conversely, central administration of a β adrenergic receptor antagonist produces a potent hypotensive effect.[45] Although this appears to be a paradox with regard to the α_{2A} expression data, this increase may be a response to a reduction in β adrenergic activity as a result of pre-synaptic α_{2A} negative feedback on noradrenalin release.[46] When viewed together, these data provide the first evidence that the hypertension as a result of ω-3 fatty acid deficiency may be caused by overactivity of the adrenergic system.

A number of genes were significantly upregulated in only one of the sufficient groups. For example, at 10 weeks-of-age, the *adrenergic receptor α_{2A}*, *dopamine β-hydroxylase* and *dopamine receptor D_2* were all upregulated, but only in the SUF-S group compared with deficient animals. The D_2 upregulation is consistent with the previously mentioned studies in deficient rat pups.[41] Although central α_{2A} receptors,[47] dopamine β-hydroxylase[48] and D_2 receptors[49] are all involved in blood pressure regulation, given that there was no difference in blood pressure at this time, and the inconsistency between sufficient groups, it seems unlikely that these factors are involved in ω-3 fatty acid deficiency-mediated hypertension.

At 36 weeks, the adrenergic receptor kinase β_1 was upregulated in the SUF-S group compared with deficient animals. This receptor is involved in the desensitization of the noradrenergic system.[50] Conversely, endothelin receptor type B was only upregulated in the SUF-SL group. Endothelin-1 is known to produce biphasic hypertensive than hypotensive effects;[51] however, these appear to be mediated through ETA receptors.[52] The effect of these late expression changes in single sufficient groups remains to be resolved, but appears to be unrelated to hypertension caused by ω-3 fatty acid deficiency.

Table 4 Brain phospholipid fatty acid composition of male rats fed DEF, SUF-S or SUF-SL diets

	10 weeks old			*36 weeks old*		
Fatty acid	*DEF*	*SUF-S*	*SUF-SL*	*DEF*	*SUF-S*	*SUF-SL*
16:0	16.7±1.3	17.8±1.0	18.9±0.6	16.3±0.6	16.3±0.4	16.0±1.0
18:0	17.9±0.9	17.4±0.3	19.7±1.5	19.5±0.5	19.4±0.5	18.9±0.7
20:0	1.1±1.1	1.0±0.1	1.0±0.1	0.6±0.1	0.6±0.1	0.6±0.2
18:1	19.7±1.4	21.2±0.3	21.4±1.6	14.7±0.8	15.3±0.4	15.8±0.5
20:1	3.2±0.3	2.9±0.2	2.9±0.5	1.5±0.2	1.6±0.1	1.9±0.8
24:1	4.8±0.4	4.8±0.5	3.6±0.7	3.8±0.5	3.6±0.4	3.7±0.6
18:2ω6	0.9±0.1	1.0±0.1	1.1±0.1	0.8±0.1	0.8±0.1	0.8±0.1
20:4ω6	8.0±0.4	7.2±0.3	7.8±0.1	9.6±0.4	8.9±0.4	8.7±0.7
22:4ω6	3.4±0.2	2.4±0.3*	2.6±0.1*	3.7±0.2	3.1±0.1*	2.9±0.2*
22:5ω6	6.3±0.3	0.1±0.1*	0.1±0.1*	7.2±0.4	0.3±0.1*	0.3±0.1*
22:6ω3	5.3±0.9	11.4±0.6*	12.4±0.1*	5.3±0.3	12.8±0.2*	12.9±0.9*

Abbreviations: DEF, ω-3 fatty acid deficient animals; SUF-S, short-chain ω-3 fatty acids; SUF-SL, short- and long-chain ω-3 fatty acids.
Results are displayed as % of total phospholipid fatty acids and are means±s.e.m. (n=10 per group).
*P<0.05 *vs.* DEF.

The prefrontal cortex proportions of DHA, the predominant ω-3 fatty acid in the brain, were related to diet. Despite the addition of fish oil (containing DHA) to the SUF-SL diet, there was no difference in DHA level in the prefrontal cortex between SUF-S and SUF-SL animals. This indicates that this dose of fish oil was too low to increase brain DHA content; alternatively, given that rats convert ALA to DHA efficiently,[53] unlike humans,[54] this may be indicative of 'normal' brain DHA content in both sufficient groups. Conversely, the levels of 22:4 ω-6 and 22:5 ω-6 were both elevated in rats in the DEF group. This finding is similar to previous ω-3 fatty acid deficiency studies[9] and is indicative of production of ω-3 fatty acid deficiency in the ω-3 DEF group.

The current study has limitations and raises a number of questions to be answered by future research. In this experiment, peripheral tissues such as renal, cardiac and plasma tissues were not examined for fatty acid composition or gene expression. These are tissues of importance in the development of hypertension and a peripheral mechanism for the changes observed in blood pressure following ω-3 DEF cannot be excluded. Peripheral tissues were not examined because the focus of this study was examining a possible early programming role of the central nervous system in hypertension following ω-3 DEF. Obviously, this does not rule out involvement of peripheral factors; indeed, the central and peripheral nervous systems must interact in the production of hypertension in ω-3 DEF animals.

Overall, the results of the current study indicate that hypertension caused by ω-3 fatty acid deficiency may be mediated by changes in the central adrenergic system. Future research is required to determine if this is the mechanism possibly by direct central inhibition of adrenergic receptors in ω-3 DEF animals. Furthermore, examining the effects of ω-3 DEF in the peripheral system will greatly improve our understanding of the etiology of ω-3 fatty acid deficiency-mediated hypertension.

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