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Trabajo Original

Soybean oil prevents hypothalamic N3 fatty acid composition but does not prevent peripheral tissue fatty acid disturbance in rats

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RESUMEN

Los ácidos linoleico (LA) y alfa-linolénico (ALA) son los únicos ácidos grasos poliinsaturados (PUFA) N6 y N3 verdaderamente esenciales, y los precursores de los ácidos araquidónico y docosahexaenoico (DHA), los PUFAs más prevalentes en el cerebro de mamíferos. Las principales fuentes dietéticas de N6 son los aceites vegetales y la carne roja, y las principales fuentes de DHA incluyen el pescado. Esta limitación se hace evidente al considerar las dietas típicas occidentalizadas. Además, las fuentes marinas se encuentran actualmente amenazadas debido a la sobrepesca y la falta de sostenibilidad. En el presente estudio investigamos la composición de ácidos grasos (FA) de suero, hipotálamo, hígado y tejido adiposo blanco (WAT) de ratas alimentadas con dietas enriquecidas con aceite de pescado (FO) o aceite de soja (SO). Mientras que FO contiene abundante DHA, SO proporciona pequeñas cantidades de ALA, junto con su importante contenido de LA. Quince ratas Wistar de 35 días fueran alimentadas con una dieta control ó enriquecida con FO (FOD) o SO (SOD) durante 8 semanas. Las ratas fueran sacrificadas y se extrajo sangre, hipotálamo, hígado y WAT, y se analizó la composición de FA mediante cromatografía de gases. FOD aumentó el contenido de N3 y SOD aumentó el contenido de N6 en todos los tejidos. Sin embargo, la SOD aumentó significativamente el DHA en el hipotálamo y el suero, un resultado que no se observó en otros tejidos de la SOD. Mientras que las ratas SOD desarrollaron obesidad, las FOD no lo hicieron. Las ratas SOD desarrollaron obesidad y desequilibrio periférico de N6 / N3, pero su contenido hipotalámico de N3 aumentó. Estos resultados corroboran aún más la biomagnificación y la captación preferencial de FA por el cerebro. Se necesitan estudios adicionales para investigar cómo las dietas con desequilibrio de nutrientes afectan aún más el metabolismo cerebral.

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ABSTRACT

Linoleic (LA) and alpha-linolenic (ALA) acids are the only truly essential N6 and N3 polyunsaturated fatty acids (PUFAs), and the precursors of arachidonicand docosahexaenoic (DHA) acids, the most prevalent PUFAs in the mammalian brain. Whilst main dietary sources of N6 are plant oils and red meat, the main sources of DHA include seawater fish. This issue becomes apparent when considering typical westernised diets. Furthermore, marine sources are currently threatened due to overfishing and no sustainability. Here we investigated the serum, hypothalamus, liver and white adipose tissues (WAT) fatty acid (FA) composition of rats fed a diet enriched with either fish oil (FO) or soybean oil (SO). Whilst FO contains abundant DHA, SO provides small amounts of ALA, alongside its important LA content. Fifteen 35-day old Wistar rats were fed a control chow, or a diet enriched with FO (FOD) or SO (SOD) for 8 weeks. Rats were sacrificed, trunk blood collected, hypothalamus, liver and WAT dissected, and their FA composition analysed by gas chromatography. FOD increased N3 content and SOD increased N6 content in all tissues. However, SOD significantly increased DHA in hypothalamus and serum, a result not observed in other SOD tissues. Whilst the SOD rats developed obesity, the FOD did not. SOD rats developed obesity and imbalanced N6/N3 peripherally, but their hypothalamic N3 content was increased. Such results further corroborate biomagnification and the preferential FA uptake by the brain. Additional studies are necessary to investigate how nutrient-unbalanced diets further affect brain metabolism.

INTRODUCTION

Fatty acids (FA) constitute integral components of the cell structure, contribute to the modulation of the cell cycle and cell signalling, and in a way or another participate in virtually all homeostatic processes[1,2,3]. Linoleic acid (LA) and alpha-linolenic acid (ALA) are the only truly essential N6 and N3FAs, as human cells cannot insert double bonds at carbons beyond C9 in the fatty acid chain[4]. LA and ALA are the substrate for elongases and desaturases, enzymes that indiscriminately elongate and desaturate LA and ALA to their respective 20 carbons-product arachidonic acid (AA) and eicosapentaenoic acid (EPA)[5]. In a subsequent complex set of enzymatic reactions, AA and EPA are metabolised to N6-docosapentaenoic acid (N6-DPA) and docosahexaenoic acid (DHA)[5,6]. Even though LA and ALA are the only truly essential FAs, AA and DHA are considered as conditionally essential FAs because humans cannot synthetize them in enough amounts to attend metabolic demands[5,6,7,8].

Different areas of the human central nervous system show slightly different fatty acid composition, but in all areas, AA is the most abundant N6, whilst DHA is the most abundant N3. Makrides and colleagues found in the frontal cortex of breast-fed infants (aged 2 to 48 weeks) 10.9% AA and 8.5% DHA[9]. Fraser and colleagues found in the frontal cortex of aged non-demented individuals (aged 79.2 ± 9.6) 8.67% AA and 15.42% DHA[10]. Small variations may occur between individuals, attributed mostly to their long-term diets, but overall the brain is relatively protected during periods of nutritional deficiencies. We previously demonstrated that male Wistar rats fed a lard-rich diet for 8 weeks showed dramatic AA and DHA deficits in liver and white adipose tissue whilst their serum and hypothalamic AA and DHA levels remained similar to the ones of control rats[11].

The main dietary sources of N6 FA in the typical westernised diet are red meat and plant oils, mainly palm oil, sunflower oil, corn oil, rapeseed oil and soybean oil[12,13,14,15]. Those oils are major sources of LA, but soybean oil is the only one amongst those five oils featuring modest levels of ALA[16,17]. China, United States, Brazil and India are the four largest global consumers of soybean oil, with a combined estimated consumption of over 3.9 x 107 metric tons in 2019[18].

Interestingly, when considering the total population of those countries[19] and the calculated soybean oil consumption per capita, Brazilians are amongst largest consumers of soybean oil globally. Brazil is the second largest producer of soybean globally, and Argentina is the second largest producer within

Mercosur. Among the ten largest soybean producers globally, four are in South America: Brazil, Argentina, Paraguay and Bolivia[20]. Such finding is further added to the fact that soybean oil is the most affordable plant oil in Brazil[21], a factor of major significance for lower income social classes.

The main dietary sources of EPA and DHA are seafood and seawater fish, but it is widely accepted that westernised societies often do not consume enough fish[22,23,24]. Two issues of paramount importance complicate the increase in fish intake: fish is relatively more expensive than meat[25], a major factor for individuals within the lower income brackets. Furthermore, due to the total global demand, fish consumption is at its historical high levels, whilst fish stocks are at dramatic, unprecedented low levels[26]. Fishing is no longer a commercially sustainable activity[27,28,29].

Due to the many difficulties associated with fish intake, associated with a westernised culture and lifestyle that favours red meat consumption, an imbalanced N6/N3 ratio is unavoidable[30,31]. Low N3 diets are positively associated with increased risk of metabolic diseases such as cardiovascular disease, diabetes, and others[32,33,34]. Several clinical trials have been conducted recently on flaxseed oil[35,36] and walnut oil[37,38], important plant sources of ALA. One of the difficulties associated with walnut and flaxseed oils, however, refers to affordability, as those oils are far more expensive than soybean oil.

As soybean oil is a highly consumed plant oil, and it presents the best ALA content amongst the most affordable plant oils, it becomes imperative to further explore and investigate the effects of soybean oil supplementation upon tissue fatty acid metabolism. The aim of the present study was to investigate the effects of an energy dense, soybean oil-enriched diet for eight weeks upon the fatty acid profile of hypothalamus, serum, liver, epididymal, retroperitoneal and mesenteric white adipose tissue of male Wistar rats.

METHODS

Animal model

This study received ethical approval from the Research Ethics Committee of the Universida de Federal de Sao Paulo, Brazil. Institutional guidelines for the care and use of our animals have been strictly followed throughout. The principles of this study have been guided by the 'Three R's' of Animal Welfare in Research: 'Replacement, Reduction and Refinement'[39]. We have used the smallest number of rats possible to obtain enough reliable data to observe an effect that could be attributed to the diet, therefore answering the research question.

Fifteen male Wistar rats, weaned on the 21st day of life, were obtained from CEDEME (Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia, Brazil), and housed in the animal house of the Division of Nutrition Physiology, Sao Paulo Federal University, under controlled lighting (12 hours light/dark cycle, lights on at 6:00) and temperature (22 ± 1°C), with ad libitum access to standard rat chow (Nuvilab CR-1, Nuvital Nutrientes SA, Colombo, PR, Brazil) and water. After two weeks acclimatization, the rats were randomised into three groups of five rats each, and fed the standard chow (Ctrl) or a diet enriched with fish oil (FOD) (Campestre Óleos Vegetais, São Bernardo do Campo, Brazil) or soybean oil (SOD) (Campestre Óleos Vegetais, São Bernardo do Campo, Brazil) ad libitum for 8 weeks, as detailed below.

Diets

The diets were prepared following method previously described by our group[11]. Briefly, standard rat chow was ground and enriched with 20% fish oil or soybean oil (w/w), 10% sucrose (w/w) (União Refinado, São Paulo, Brazil), 20% casein (w/w) (Labsynth, Diadema, Brazil) and 0.02% (w/w) butylated hydroxytoluene (BHT) (Merck, Brazil). The mixture was mechanically homogenised with the addition of lukewarm water and passed through a milling machine to produce pellets, which were dried in a forced ventilation oven at 60°C for 24h. The standard rat chow energy content was 2.8 kcal/g, whilst the oil enriched diets were 4.1 kcal/g. The fatty acid composition of the fish oil and soybean oil utilized in this study, as well as the oil-enriched diets, were determined by gas chromatography following protocol described below. Oil and diet fatty acid results are presented in Table 1.

Tissue analyses

At the end of the 8th week dietary intervention period, all rats were sacrificed by decapitation under mild sedation in ethyl ether after overnight fast. Trunk blood was collected and serum obtained by centrifugation. Retroperitoneal (RET), epididymal (EPI) and mesenteric (MES) fat pads, hypothalamus and liver were dissected and weighted. A well-trained researcher performed the hypothalamic dissection to ensure accuracy and precision. All samples were stored at -80°C until analysis.

Total lipids were extracted from the Ctrl diet, FOD diet, SOD diet, as well as from serum, hypothalamus, liver, RET, EPI and MES, as described previously[11]. Briefly, samples were homogenized in hexane / isopropanol (3:2 v/v) (Sigma, St Louis, MO) containing 0.01% BHT, and washed with chloroform / methanol / water (2:1:1 v/v/v) (Sigma, St Louis, MO). The samples were centrifuged, and the organic layer obtained was evaporated under oxygen-free nitrogen (OFN). Total lipids were partitioned again in chloroform / methanol / water solution (8/4/3 v/v/v). The resulting total lipid extracts were dried under OFN and kept in air-tight tubes under OFN until analysis.

FA analysis of the lipid extracts, as well as of the fish oil and the soybean oil used in this study, was performed as standardized previously[40]. Briefly, FA methyl esters (FAME) were obtained by heating the samples with 15% acetyl chloride in anhydrous 99.8% methanol (Sigma-Aldrich, Germany) in a sealed glass tube at 70°C for 3 hours under OFN. The reaction was stopped by adding 5% NaCl (Fisher Scientific, England) solution at room temperature. FAMEs were extracted with petroleum ether containing 0.01% BHT, any remaining acid neutralized with 2%

potassium bicarbonate and any remaining water removed with anhydrous sodium sulphate. FAMEs were separated by gas chromatography with flame ionization detector (Fisons Instruments, Milan, Italy) fitted with a capillary column (BPX70, 60 m x 0.32 mm x 0.25 µm, Thames Restek, UK).

Table 1. Fatty acids standards used for determination of retention time and identification of fatty acid methyl esters.

Analyte	
F.A.M.E. Mix, C4-C24	
C4:0	Methyl butyrate 4 wt. %
C6:0	Methyl hexanoate 4 wt. %
C8:0	Methyl octanoate 4 wt. %
C10:0	Methyl decanoate 4 wt. %
C11:0	Methyl undecanoate 2 wt. %
C12:0	Methyl dodecanoate 4 wt. %
C13:0	Methyl tridecanoate 2 wt. %
C14:0	Methyl myristate 4 wt. %
C14:1n7	Methyl myristoleate 2 wt. %
C15:0	Methyl pentadecanoate 2 wt. %
C15:1	Methyl cis-10-pentadecenoate 2 wt. %
C16:0	Methyl palmitate 6 wt. %
C16:1n7	Methyl palmitoleate 2 wt. %
C17:0	Methyl heptadecanoate 2 wt. %
C17:1	Methyl cis-10-heptadecenoate 2 wt. %
C18:0	Methyl stearate 4 wt. %
C18:1n9 trans	Methyl elaidate 2 wt. %
C18:1n9	Methyl oleate 4 wt. %
C18:2n6t	Methyl linolelaidate 2 wt. %
C18:2n6	Methyl linoleate 2 wt. %
C18:3n6	Methyl γ-linolenate 2 wt. %
C18:3n3	Methyl linolenate 2 wt. %
C20:0	Methyl arachidate 4 wt. %
C20:1n9	Methyl cis-11-eicosenoate 2 wt. %
C21:0	Methyl heneicosanoate 2 wt. %
C20:2n6	cis-11,14-Eicosadienoic acid methyl ester 2 wt. %
C20:3n6	cis-8,11,14-Eicosatrienoic acid methyl ester 2 wt. %
C20:4n6	Methyl arachidonate 2 wt. %
C20:3n3	cis-11,14,17-Eicosatrienoic acid methyl ester 2 wt. %
C22:0	Methyl behenate 4 wt. %
C22:1n9	Methyl cis-13-docosenoate 2 wt. %
C20:5n3	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester 2 wt. %
C23:0	Methyl tricosanoate 2 wt. %
C22:2n6	cis-13,16-Docosadienoic acid methyl ester 2 wt. %
C24:0	Methyl tetracosanoate 4 wt. %
C24:1n9	Methyl cis-15-tetracosenoate 2 wt. %
C22:6n3	cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester 2 wt. %
C16:1n7	palmitoleic acid / cis-9-Hexadecenoic acid *
C17:0	Heptadecanoic acid *
C20:1n9	cis-11-Eicosenoic acid *
C20:2n6	cis-11,14-Eicosadienoic acid methyl ester
C20:3n6	cis-8,11,14-Eicosatrienoic acid methyl ester
C20:4n6	Methyl arachidonate
C22:0	Docosanoic acid / Behenic acid *
C22:1n9	cis-13-Docosenoic acid / Erucic acid *
C20:5n3	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester
C22:2n6	cis-13,16-Docosadienoic acid methyl ester
C24:1n9	cis-15-Tetracosenoic acid / Nervonic acid *
C22:6n3	cis-4,7,10,13,16,19-Docosahexaenoic acid *
DMA 16:0	16:0 dimethylacetal
DMA 18:0	18:0 dimethylacetal
DMA 18:1	18:1 dimethylacetal
C18:1n7 trans	trans- Vaccenic acid / 11-trans-Octadecenoic acid *
C18:1n7 Cis	cis-vaccenic acid / cis-11-Octadecenoic acid *
C22:4n6	cis-7,10,13,16-Docosatetraenoic acid *
C22:5n3	cis-7,10,13,16,19-Docosapentaenoic methyl ester

FAME peaks were identified by comparison with the retention times of previously injected authentic standards. All fatty acid standards were obtained from Sigma-Aldrich (Merck, Germany) and are presented as supplementary material. Peak areas were quantified (EZChrom Chromatography Data System, Scientific Software Inc., San Ramon, CA, USA) and the values presented as % of total fatty acids.

Statistical analysis

Shapiro-Wilk test was performed in order to confirm the normal distribution of body weight gain (BW), tissue mass and tissue FA composition. Data are expressed as mean ± standard deviation of the mean. One-way ANOVA followed by Tukey's HSDpost hoc test was employed to identify differences amongst individual groups. All tests were performed with SPSS Version 26 for $p < 0.05$(IBM, Chicago, IL, USA).

RESULTS

SOD and FOD show substantially different fatty acid profiles

The FA profiles of the fish oil and the soybean oil used in this study are shown in Table I, alongside the FA composition of the Ctrl, FOD and SOD diets. Nearly 75% of the fish oil FA comprised EPA and DHA, featuring also nearly identical amounts of LA (0.4%) and ALA (0.3%). The fish oil contained under 8% of ∑MUFAs, with 4.5% oleic acid (OA), and the ∑SFA was under 3%. The only N3FA detected in soybean oil was ALA, with 5.3% of the total FA content. The soybean oil also contained 52.2% of LA, 10.7% of palmitic acid (PA) and 26.2% OA (Table I).

The Ctrl diet FA profile resembles the profile of the soybean oil, with 4.1% ALA, 54.9% LA, 24.6% OA and 12.5% PA (Table I). The FOD contained 0.8% ALA, 39.9% EPA, 22.1% DHA, 7.5% LA, 7% OA and 2.2% PA. The SOD contained 5.7% ALA, 51.3% LA, 25.6% OA and 11.2% PA. Whilst the FOD N6/N3 ratio was 0.15, the SOD ratio was 9.02 (Table I).

Body and tissue weight were distinctly affected in SOD and FOD

The BW gain (calculated as BW on the final day of dietary intervention minus BW on the first day of dietary intervention) of the SOD rats at the end of dietary intervention was significantly higher than the FOD rats (Table II), however no differences were observed between SOD and Ctrl groups. The liver relative weight was significantly higher in FOD, and significantly lower in SOD, as compared to Ctrl. The RET relative weight was significantly lower in FOD and bigger in SOD as compared to Ctrl. The EPI relative weight was significantly higher in SOD as compared to FOD and Ctrl. The MES relative weight was significantly higher in SOD as compared to Ctrl. No differences were detected in hypothalamus relative weight (Table II).

Table I. Fatty acid composition (% of total fatty acids) of the fish oil, soybean oil, control diet (Ctrl), fish oil-enriched diet (FOD) and soybean oil-enriched diet (SOD).

	Abbreviation	Fish oil	Soybean oil	Ctrl	FOD	SOD
c16:0	PA	0.5	10.7	12.3	2.2	11.2
c18:0	SA	2.2	2.8	2.4	2.2	3
ΣSFA		2.8	13.7	14.8	4.5	14.3
c16:1n-7	POA	0.2	0.1	0	0.2	0.1
c18:1n-9	OA	4.5	26.2	24.6	7	25.6
c18:1n-7	VA	1.5	1.5	1.1	1.3	1.5
c20:1		1.4	0.2	0.2	1.2	0.2
c24:1		0.3			0.3	
ΣMUFA		7.8	28	25.9	10	27.4
c18:2n-6	LA	0.4	52.2	54.9	7.5	51.3
c18:3n-6	GLA	0.1	0	0		0.1
c20:3n-6	DHGLA	0.3			0.2	
c20:4n-6	AA	2.1			1.8	
c22:4n-6	N6-DTA	0.2			0.1	
c22:5n-6	N6-DPA	0.5			0.5	
ΣN6PUFA		3.5	52.2	54.9	10.2	51.4
c18:3n-3	ALA	0.3	5.3	4.1	0.8	5.7
c20:5n-3	EPA	47.3			39.9	
c22:5n-3	N3-DPA	4.9			4.5	
c22:6n-3	DHA	25.6			22.1	
ΣN3PUFA		78.1	5.3	4.1	67.2	5.7
N6/N3		0.04	9.85	13.39	0.15	9.02

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: dihomo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid

Table II. Body weight (BW) gain (g) (BW at the end of dietary intervention minus first study day), relative tissue weight (g tissue / 100 g BW) of retroperitoneal (RET), epididymal (EPI) and mesenteric (MES) adipose tissue, liver, and hypothalamus.

	Ctrl				FOD					SOD			
	Mean		SD		Mean		SD			Mean		SD	
BW gain (g)	179.8	±	27.2		153.9	±	35.2			216.5	±	30.8	*
Liver	3.30	±	0.22		4.09	±	0.12	#		3.14	±	0.20	*
RET	1.11	±	0.14		0.76	±	0.12	#		1.93	±	0.26	#*
EPI	1.29	±	0.16		1.46	±	0.30			1.88	±	0.23	#*
MES	1.06	±	0.18		1.49	±	0.17			1.70	±	0.40	#
Hypothalamus	0.011	±	0.001		0.011	±	0.001			0.011	±	0.000	

Data presented as means ± SD. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet. # p< 0.05 vs Ctrl. * p< 0.05 vs. FOD.

Liver fatty acid profile shows increased N6 in SOD rats

The liver FA profile is presented in Table III. The SOD group showed significantly lower amounts of stearic (SA), and lower ∑SFA, in relation to FOD. OA was higher, vaccenic acid (VA) was lower, and the ∑MUFA content was higher in SOD as compared to FOD. N6-DPA was the only N6 FA not significantly higher in SOD as compared to FOD; all the other N6FAs were significantly higher in SOD. Except for ALA, N3 FAs were significantly decreased in SOD as compared to FOD. The N6/N3 ratio was significantly higher in SOD as compared to FOD. In comparison to Ctrl, the SOD rats showed overall decreased SFA, increased MUFA and N6PUFA, and unchanged N3PUFA (Table III).

Table III. Fatty acid composition of liver total lipid extract obtained from male Wistar rats fed a standard rodent chow (Ctrl) (2.7kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (SOD) (4.1 kcal/g) for 8 weeks.

		Ctrl				FOD					SOD			
	Abbreviation	M		SD		M		SD			M		SD	
C16:0	PA	17.61	±	1.08		12.87	±	1.16	#		14.22	±	0.61	#
C18:0	SA	14.66	±	0.98		16.71	±	0.97			10.95	±	2.47	#*
ΣSFA		33.0	±	1.1		30.0	±	0.6	#		25.6	±	2.4	#*
C16:1n-7	POA	0.74	±	0.35		0.40	±	0.08			0.18	±	0.02	#
C18:1n-9	OA	7.34	±	0.56		8.45	±	1.12			13.71	±	1.88	#*
C18:1n-7	VA	3.27	±	0.38		2.27	±	0.23	#		1.65	±	0.07	#*
ΣMUFA		11.7	±	1.0		11.7	±	1.0			15.8	±	1.8	#*
C18:2n-6	LA	18.04	±	1.66		10.44	±	0.83	#		28.32	±	3.22	#*
C18:3n-6	GLA	0.26	±	0.01		0.05	±	0.02	#		0.61	±	0.08	#*
C20:3n-6	DHGLA	0.55	±	0.04		0.57	±	0.05			0.90	±	0.14	#*
C20:4n-6	AA	25.07	±	0.88		12.35	±	1.40	#		17.19	±	2.90	#*
C22:4n-6	N6-DTA	0.86	±	0.06		0.03	±	0.01	#		1.33	±	0.34	#*
C22:5n-6	N6-DPA	0.23	±	0.11		0.15	±	0.02			0.27	±	0.16	
ΣN6PUFA		45.0	±	1.6		23.6	±	0.7	#		48.6	±	1.2	#*
C18:3n-3	ALA	0.35	±	0.07		0.48	±	0.12			0.59	±	0.52	
C20:5n-3	EPA	0.31	±	0.05		13.79	±	0.72	#		0.41	±	0.11	*
C22:5n-3	N3-DPA	1.48	±	0.25		3.63	±	0.49	#		1.09	±	0.16	*
C22:6n-3	DHA	4.98	±	1.09		12.31	±	1.05	#		5.19	±	0.76	*
ΣN3PUFA		7.10	±	0.83		30.21	±	1.13	#		7.29	±	0.79	*
N6/N3		6.4	±	0.8		0.8	±	0.0	#		6.8	±	0.9	*

Data presented as mean ± SD of the % of total fatty acids. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: di homo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid. # $p < 0.05$ vs Ctrl. * $p < 0.05$ vs. FOD.

Serum fatty acid profile shows improved N3 in SOD

The serum FA profile is presented in Table IV. There were no differences in SFA content between SOD and FOD. Palmitoleic acid (POA) was significantly decreased in SOD, and so was the ∑MUFA content. Except for N6-DPA, all N6 FAs were significantly higher in SOD as compared to FOD. ALA was significantly higher in SOD as compared to FOD, but the other N3 FAs were higher in FOD. Same as what was observed in liver, the N6/N3 ratio was significantly higher in SOD as compared to FOD. Interestingly, the DHA content was significantly higher in SOD as compared to Ctrl (Table IV).

Table IV. Fatty acid composition of serum total lipid extract obtained from male Wistar rats fed a standard rodent chow (Ctrl) (2.7 kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (SOD) (4.1 kcal/g) for 8 weeks.

		Ctrl				FOD					SOD			
	Abbreviation	**M**		**SD**		**M**		**SD**			**M**		**SD**	
C16:0	PA	20.35	±	0.91		20.18	±	2.70			17.49	±	0.62	#
C18:0	SA	11.99	±	0.58		15.94	±	2.03	#		15.42	±	1.28	#
ΣSFA		32.9	±	1.0		36.7	±	4.7			33.3	±	1.6	
C16:1n-7	POA	0.81	±	0.26		0.53	±	0.06	#		0.25	±	0.10	#*
C18:1n-9	OA	9.53	±	0.98		8.58	±	0.21			7.14	±	1.18	#
C18:1n-7	VA	2.01	±	0.30		1.35	±	0.08	#		1.09	±	0.10	#
ΣMUFA		12.6	±	1.1		11.2	±	0.3			8.8	±	1.4	#*
C18:2n-6	LA	20.52	±	0.82		6.38	±	1.26	#		16.90	±	0.96	#*
C18:3n-6	GLA	0.23	±	0.03		0.06	±	0.02	#		0.18	±	0.10	*
C20:3n-6	DHGLA	0.35	±	0.08		0.25	±	0.05			0.38	±	0.07	*
C20:4n-6	AA	26.11	±	0.90		10.90	±	0.86	#		33.07	±	0.69	#*
C22:4n-6	N6-DTA	0.57	±	0.13		0.06	±	0.05	#		0.38	±	0.12	#*
C22:5n-6	N6-DPA	0.13	±	0.07		0.16	±	0.04			0.14	±	0.07	
ΣN6PUFA		47.9	±	1.5		17.8	±	2.1	#		51.1	±	0.6	#*
C18:3n-3	ALA	0.44	±	0.05		0.25	±	0.03	#		0.36	±	0.06	#*
C20:5n-3	EPA	0.45	±	0.11		21.04	±	3.65	#		0.23	±	0.11	*
C22:5n-3	N3-DPA	0.92	±	0.11		1.81	±	0.24	#		0.51	±	0.09	#*
C22:6n-3	DHA	2.52	±	0.50		8.44	±	1.08	#		3.86	±	0.44	#*
ΣN3PUFA		4.35	±	0.40		31.54	±	4.94	#		4.97	±	0.52	*
N6/N3		11.1	±	1.1		0.6	±	0.1	#		10.4	±	1.2	*

Data presented as mean ± SD of the % of total fatty acids. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: dihomo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid. # $p< 0.05$ vs Ctrl. * $p< 0.05$ vs. FOD.

Hypothalamus fatty acid profile shows increased DHA in SOD compared to Ctrl

The hypothalamus FA profile is presented in Table V. No significant differences in SFA content were found between SOD and FOD. POA and OA were significantly lower in SOD as compared to FOD, contributing to lower ∑MUFA content. The amounts of the less unsaturated and shorter N6 FAsLA, gamma-linolenic (GLA) and dihomo-gamma-linolenic (DHGLA) acids were the same between SOD and FOD, but the longer and more unsaturated AA, N6 docosatetraenoic (N6-DTA) and N6-DPA were significantly higher in SOD, which contributed to higher ∑N6PUFA content. The amount of EPA was lower in SOD, but no other statistically significant differences were found in N3 fatty acids between SOD and FOD. The SOD group was the only to show a hypothalamic N6/N3 ratio of 1.0, which was statistically lower than Ctrl and statistically higher than FOD. Same as in serum, the DHA content was significantly higher in SOD as compared to Ctrl (Table V), a result that was not found for SOD rats in their other peripheral tissues.

Table V. Fatty acid composition of hypothalamus total lipid extract obtained from male Wistar rats fed a standard rodent chow (Ctrl) (2.7 kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (SOD) (4.1 kcal/g) for 8 weeks.

		Ctrl				FOD					SOD			
	Abbreviation	M		SD		M		SD			M		SD	
C16:0	PA	19.87	±	0.78		18.85	±	1.11			18.44	±	0.89	
C18:0	SA	18.12	±	0.44		17.95	±	0.24			17.89	±	0.54	
ΣSFA		38.4	±	1.0		37.2	±	1.1			36.8	±	0.6	
C16:1n-7	POA	0.48	±	0.05		0.50	±	0.04			0.41	±	0.02	*
C18:1n-9	OA	20.90	±	0.50		21.72	±	0.56			20.19	±	0.44	*
C18:1n-7	VA	3.95	±	0.28		3.44	±	0.28	#		3.56	±	0.07	
ΣMUFA		26.5	±	0.8		27.5	±	0.8			25.3	±	0.4	*
C18:2n-6	LA	1.35	±	0.95		0.40	±	0.07	#		0.90	±	0.14	
C18:3n-6	GLA	0.04	±	0.01		0.05	±	0.01			0.05	±	0.05	
C20:3n-6	DHGLA	0.22	±	0.02		0.21	±	0.04			0.23	±	0.06	
C20:4n-6	AA	10.68	±	0.97		9.01	±	0.30	#		11.28	±	0.17	*
C22:4n-6	N6-DTA	3.43	±	0.16		2.42	±	0.10	#		3.73	±	0.18	#*
C22:5n-6	N6-DPA	0.39	±	0.02		0.14	±	0.02	#		0.52	±	0.12	*
ΣN6PUFA		16.1	±	0.3		12.2	±	0.4	#		16.7	±	0.3	#*
C18:3n-3	ALA	0.05	±	0.04		0.03	±	0.02			0.06	±	0.03	
C20:5n-3	EPA	0.07	±	0.03		0.49	±	0.09	#		0.15	±	0.02	*
C22:5n-3	N3-DPA	1.08	±	0.70		1.07	±	0.08			0.92	±	0.16	
C22:6n-3	DHA	12.97	±	1.74		17.18	±	1.46	#		16.03	±	0.84	#
ΣN3PUFA		14.16	±	2.00		18.76	±	1.53	#		17.15	±	0.81	#
N6/N3		1.2	±	0.2		0.7	±	0.1	#		1.0	±	0.0	#*

Data presented as mean ± SD of the % of total fatty acids. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: dihomo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid. # $p< 0.05$ vs Ctrl. * $p< 0.05$ vs. FOD.

Epidydimal white adipose tissue fatty acid profile is negatively affected in SOD

The EPIFA profile is presented in Table VI. The amounts of PA and SA were slightly lower in SOD as compared to FOD, but their individual differences did not reach statistical significance. When added together however, their lower concentration contributed to significantly lower ∑SFA content in SOD as compared to FOD. The SOD group showed significantly lower amounts of POA, but significantly higher amounts of OA, which led to a significantly higher ∑MUFA content in SOD compared to FOD. The EPI N6 FA profile appears to be more complex than in the previously described tissues. LA and N6-DTA were significantly higher in SOD as compared to FOD. Whilst GLA and DHGLA were similar, AA and N6-DPA were significantly lower in SOD. The ∑N6PUFA was significantly higher in SOD as compared to FOD. ALA was significantly higher, but the other N3 FAs were significantly lower, in SOD as compared to FOD, leading to lower ∑N3PUFA content in SOD. The N6/N3 ratio was nearly twenty times higher in SOD as compared to FOD (Table VI).

Table VI. Fatty acid composition of epidydimal adipose tissue total lipid extract obtained from male Wistar rats fed a standard rodent chow (Ctrl) (2.7 kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (FOD) (4.1 kcal/g) for 8 weeks.

		Ctrl				FOD					SOD			
	Abbreviation	**M**		**SD**		**M**		**SD**			**M**		**SD**	
C16:0	PA	20.35	±	0.70		17.51	±	1.75	#		15.63	±	0.96	#
C18:0	SA	2.74	±	0.32		3.22	±	0.42			3.12	±	0.30	
ΣSFA		23.9	±	0.8		21.9	±	1.7			19.3	±	1.2	#*
C16:1n-7	POA	2.78	±	1.12		3.71	±	1.02			1.34	±	0.36	*
C18:1n-9	OA	24.24	±	0.45		16.99	±	0.97	#		26.01	±	0.39	#*
C18:1n-7	VA	2.66	±	0.27		2.14	±	0.15	#		1.88	±	0.14	#
ΣMUFA		29.9	±	1.5		23.3	±	1.7	#		29.4	±	0.5	*
C18:2n-6	LA	39.06	±	1.52		17.98	±	1.52	#		44.38	±	1.03	#*
C18:3n-6	GLA	0.11	±	0.01		0.07	±	0.02	#		0.08	±	0.01	#
C20:3n-6	DHGLA	0.20	±	0.04		0.22	±	0.04			0.20	±	0.03	
C20:4n-6	AA	1.61	±	0.27		1.67	±	0.18			1.07	±	0.23	#*
C22:4n-6	N6-DTA	0.40	±	0.08		0.11	±	0.04	#		0.23	±	0.06	#*
C22:5n-6	N6-DPA	0.09	±	0.03		0.18	±	0.05	#		0.06	±	0.03	*
ΣN6PUFA		41.5	±	1.5		20.2	±	1.4	#		46.0	±	1.1	#*
C18:3n-3	ALA	2.43	±	0.14		1.31	±	0.08	#		3.23	±	0.20	#*
C20:5n-3	EPA	0.09	±	0.03		16.16	±	1.64	#		0.10	±	0.03	*
C22:5n-3	N3-DPA	0.32	±	0.09		3.31	±	0.47	#		0.23	±	0.08	*
C22:6n-3	DHA	0.33	±	0.07		10.21	±	1.58	#		0.29	±	0.10	*
ΣN3PUFA		3.18	±	0.24		30.98	±	3.40	#		3.84	±	0.26	*
N6/N3		13.1	±	0.8		0.7	±	0.1	#		12.0	±	0.8	#*

Data presented as mean ± SD of the % of total fatty acids. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: dihomo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid. # $p < 0.05$ vs Ctrl. * $p < 0.05$ vs. FOD.

Retroperitoneal white adipose tissue fatty acid profile is negatively affected in SOD

The RET FA profile is presented in Table VII. The amounts of PA and SA were significantly lower in SOD as compared to FOD, contributing to significantly lower ∑SFA content. The SOD group showed significantly lower amounts of POA and VA, but significantly higher amounts of OA, which led to a significantly higher ∑MUFA content in SOD compared to FOD. The RET N6 profile was nearly identical to the one found in EPI: LA was significantly higher in SOD as compared to FOD. N6-DTA was also higher, but the difference between SOD and FOD did not reach statistical significance. GLA and DHGLA were similar, whilst AA and N6-DPA acids were significantly lower, in SOD as compared to FOD. The ∑N6PUFA was significantly higher in SOD as compared to FOD. The RET N3 profile was identical to the one found in EPI: ALA was significantly higher, whilst the other N3 FAs and the ∑N3PUFA content were lower, in SOD as compared to FOD. Same as for EPI, the RET N6/N3 ratio was nearly twenty times higher in SOD as compared to FOD (Table VII).

Table VII. Fatty acid composition of retroperitoneal adipose tissue total lipid extract obtained from male Wistar rats fed a standard rodent chow (Ctrl) (2.7 kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (SOD) (4.1 kcal/g) for 8 weeks.

		Ctrl				FOD					SOD			
	Abbreviation	M		SD		M		SD			M		SD	
C16:0	PA	20.76	±	0.52		20.16	±	1.19			15.49	±	1.46	#*
C18:0	SA	2.97	±	0.09		4.24	±	0.32	#		3.25	±	0.18	*
ΣSFA		24.6	±	0.5		25.7	±	1.4			19.2	±	1.7	#*
C16:1n-7	POA	2.06	±	0.18		2.37	±	0.57			0.86	±	0.36	#*
C18:1n-9	OA	24.96	±	0.67		19.66	±	1.11	#		26.55	±	0.71	#*
C18:1n-7	VA	2.72	±	0.33		2.51	±	0.21			1.84	±	0.04	#*
ΣMUFA		30.0	±	0.9		25.3	±	0.9	#		29.5	±	0.4	*
C18:2n-6	LA	39.70	±	0.99		18.10	±	1.63	#		45.42	±	2.13	#*
C18:3n-6	GLA	0.08	±	0.02		0.05	±	0.02	#		0.07	±	0.01	
C20:3n-6	DHGLA	0.15	±	0.03		0.22	±	0.03	#		0.18	±	0.06	
C20:4n-6	AA	0.98	±	0.19		1.29	±	0.13	#		0.78	±	0.20	*
C22:4n-6	N6-DTA	0.27	±	0.07		0.14	±	0.05	#		0.21	±	0.05	
C22:5n-6	N6-DPA	0.06	±	0.01		0.26	±	0.06	#		0.03	±	0.02	*
ΣN6PUFA		41.3	±	1.0		20.1	±	1.7	#		46.7	±	1.9	#*
C18:3n-3	ALA	2.27	±	0.18		1.04	±	0.08	#		2.95	±	0.18	#*
C20:5n-3	EPA	0.06	±	0.01		10.51	±	2.71	#		0.08	±	0.01	*
C22:5n-3	N3-DPA	0.21	±	0.04		3.66	±	0.40	#		0.16	±	0.02	#*
C22:6n-3	DHA	0.16	±	0.04		10.56	±	0.91	#		0.18	±	0.06	*
ΣN3PUFA		2.70	±	0.22		25.78	±	1.93	#		3.37	±	0.22	*
N6/N3		15.3	±	1.1		0.8	±	0.1	#		13.9	±	1.5	*

Data presented as mean ± SD of the % of total fatty acids. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: dihomo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid. # p< 0.05 vs Ctrl. * p< 0.05 vs. FOD.

Mesenteric white adipose tissue fatty acid profile is negatively affected in SOD

The MES FA profile is presented in Table VIII. The amount of SA was significantly lower in SOD as compared to FOD, but as opposed to EPI and RET, the ∑SFA content in MES was similar between SOD and FOD. The MUFA, N6 and N3 PUFA profile observed in MES was identical to the one observed in RET.

Table VIII. Fatty acid composition of mesenteric adipose tissue total lipid extract obtained from male Wistar rats fed a standard rodent chow (Ctrl) (2.7 kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (SOD) (4.1 kcal/g) for 8 weeks.

		Ctrl				FO					SO			
	Abbreviation	M		SD		M		SD			M		SD	
C16:0	PA	19.27	±	0.88		13.49	±	1.07	#		14.22	±	0.80	#
C18:0	SA	3.22	±	0.21		4.49	±	0.25	#		3.53	±	0.24	*
ΣSFA		23.2	±	1.0		18.9	±	1.2	#		18.1	±	0.9	#
C16:1n-7	POA	1.27	±	0.27		1.24	±	0.35			0.53	±	0.19	#*
C18:1n-9	OA	25.92	±	0.68		16.83	±	0.38	#		27.82	±	0.39	#*
C18:1n-7	VA	2.72	±	0.22		2.77	±	0.08			1.92	±	0.07	#*
ΣMUFA		30.1	±	1.0		21.4	±	0.6	#		30.5	±	0.4	*
C18:2n-6	LA	41.50	±	1.36		16.98	±	1.19	#		46.20	±	1.19	#*
C18:3n-6	GLA	0.07	±	0.01		0.08	±	0.03			0.05	±	0.01	
C20:3n-6	DHGLA	0.11	±	0.02		0.11	±	0.13			0.12	±	0.03	
C20:4n-6	AA	0.80	±	0.07		1.83	±	0.10	#		0.57	±	0.10	#*
C22:4n-6	N6-DTA	0.26	±	0.04		0.12	±	0.01	#		0.14	±	0.05	#
C22:5n-6	N6-DPA	0.05	±	0.02		0.27	±	0.03	#		0.03	±	0.01	*
ΣN6PUFA		42.8	±	1.4		19.4	±	1.1	#		47.1	±	1.0	#*
C18:3n-3	ALA	1.98	±	0.24		1.12	±	0.06	#		2.61	±	0.21	#*
C20:5n-3	EPA	0.05	±	0.01		15.92	±	0.82	#		0.03	±	0.01	*
C22:5n-3	N3-DPA	0.19	±	0.04		4.29	±	0.18	#		0.11	±	0.03	*
C22:6n-3	DHA	0.15	±	0.02		13.23	±	1.35	#		0.11	±	0.04	*
ΣN3PUFA		2.37	±	0.23		34.56	±	1.96	#		2.86	±	0.21	*
N6/N3		18.2	±	1.3		0.6	±	0.0	#		16.5	±	1.2	*

Data presented as mean ± SD of the % of total fatty acids. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; PA: palmitic acid; SA: Stearic acid; POA: Palmitoleic acid; OA: oleic acid; VA: cis-vaccenic acid; LA: Linoleic acid; GLA: gamma-linolenic acid; DHGLA: dihomo-gamma-linolenic acid; AA: Arachidonic acid; N6-DTA: N6-docosatetraenoic acid; N6-DPA: N6-docosapentaenoic acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; N3-DPA: N3-docosapentaenoic acid; DHA: Docosahexaenoic acid. # p< 0.05 vs Ctrl. * p< 0.05 vs. FOD.

LA/AA and ALA/DHA ratios are unbalanced in SOD compared to Ctrl and FOD

In the liver, the LA/AA ratio was significantly higher in SOD as compared to both FOD and Ctrl. Although not statistically significant, the ALA/DHA ratio was three times higher in SOD as compared to FOD, and nearly doubled in SOD as compared to Ctrl. In serum, the ratio LA/AA was similar between SOD and FOD, and both were significantly lower than Ctrl. The ALA/DHA was significantly lower in FOD as compared to SOD, which was significantly lower as compared to Ctrl. There were no statistically significant differences in hypothalamic LA/AA or ALA/DHA ratios, although it appears the ratios reduce from Ctrl to SOD and from SOD to FOD.

In EPI, the LA/AA ratio was significantly higher in SOD as compared to Ctrl, which in turn was significantly higher as compared to FOD. The ALA/DHA ratio was significantly higher in SOD as compared to FOD, but SOD was not significantly higher than Ctrl. In RET, both LA/AA and ALA/DHA ratios were significantly higher in SOD as compared to FOD, but the SOD ratios were not significantly higher than Ctrl. In MES, both LA/AA and ALA/DHA ratios were significantly higher in SOD as compared to Ctrl, which were significantly higher as compared to FOD.

DISCUSSION

Obesity and other metabolic conditions characterized by mild chronic inflammatory backgrounds, including metabolic syndrome and type 2 diabetes[41], cardiovascular disease[42], dyslipidaemia[43] and dementia[44] are major Public Health issues. Combined, their mortality rates constitute by far the most important cause of death worldwide[45,46].

High fat diets are long known for their role in the development of obesity[47] and inflammation[48]. Not only the amount of fat but also the imbalanced proportion of N6 to N3FAs are well known to exacerbate chronic conditions[49,50].

A typical westernised diet features abundance of red meat and derivatives[51], which are often cooked with plant oils. The five most consumed plant oils in the Western world are palm oil, sunflower oil, corn oil, rapeseed oil and soybean oil[12,15]. The first four aforementioned oils are important sources of not only OA but also LA[16,17,52]. However, amongst the five oils mentioned above, soybean oil is the one with the highest ALA content. Whilst Alves found a concentration of 2.7% ALA in soybean oil(17), Kim found 7.8%[16],and in our study the ALA concentration found was 5.3%. Although it is worth noting the different protocols employed across the three studies, the overall ALA concentrations agree with the range found in other soybean oil FA studies.

Seawaterfish is the most important source of long chain N3PUFAs, particularly EPA and DHA, but marine mammals – a food source for some cultures such as the Inuit – also show higher N3 content as compared to humans[53]. There is currently no government-set specific recommendation for the N6/N3 ratio[54], but there is a suggestion that it would be appropriate to maintain it below 5/1[55]. It has been described that Western populations show a N6/N3ratio of 15 to 16/1[30], reaching levels as high as 20/1[31].

A simplistic approach to resolve the high N6/N3 ratio would be to increase fish intake. However, two paramount issues are associated with increasing fish consumption. Firstly, fish is more expensive than meat[25], a factor of significant importance when considering food choices for low-income families. Secondly, due to the total global demand, fish consumption is at its historical high levels, leading to a catastrophic impact upon marine environment. Fishing is no longer a commercially sustainable activity[27,28,29]. Another simplistic approach to resolve the high N6/N3 ratio would be to decrease N6 intake, which works only up to a certain extent, as it does not excuse the essentiality of N3 FAs. An urgent alternative for fish-derived N3 FAs is required to provide the nutritional demands for humans.

Some plant oils are under intense scrutiny due to their important ALA content, for example walnut oil[56]. Several studies have investigated the effects of walnut oil intake upon the secretory function of the adipose tissue[57], biomarkers of cardiovascular function[58], type 2 diabetes[37] and obesity[59], amongst others. In the present study, we chose to investigate the effects of a soybean oil-enriched diet upon tissue fatty acid composition, comparing the results to the well-known effects of diets enriched with fish oil. Soybean oil is significantly more affordable than walnut oil.

In the present study, rats were kept on one of the three diets for 8 weeks ad libitum. The body weight gain accrued along the period of dietary intervention was statistically higher in SOD as compared to FOD, while Ctrl showed a body weight gain that was intermediary and statistically similar between both SOD and FOD (Table II).The RET and EPI relative weight (g/100 g BW) was significantly higher in SOD as compared to Ctrl and FOD, whilst MES was significantly higher in SOD as compared to Ctrl only. The liver relative weight was significantly lower in SOD as compared to FOD, and similar to Ctrl (Table II). We have not measured carcass total lipid content, but the combined findings of higher adipose tissue relative weight associated with lower liver relative weight in SOD rats suggest that SOD are more obese than Ctrl and FOD. This suggestion is further corroborated by the significant findings of higher body weight in SOD when compared to FOD. Furthermore, although not statistically different, SOD group showed an increment of 17% in body weight gain compared to Ctrl group, which suggests that a longer experimentation period would have made the SOD rats also significantly more obese than Ctrl.

The SOD liver fatty acid profile is dramatically different from FOD (Table III). ∑SFA and ∑N3PUFA were significantly lower in SOD, whilst ∑MUFA and ∑N6PUFA were significantly higher. The N6/N3 ratio was 8.5 times higher in SOD as compared to FOD. Relevant differences were also found between SOD and Ctrl: whilst ∑SFA was significantly decreased, ∑MUFA and ∑N6PUFA were significantly increased, with no changes in ∑N3PUFA. Interestingly however, the N6/N3 ratio was nearly identical between SOD and Ctrl (Table III).

The SOD serum FA profile showed important differences as compared to FOD. Furthermore, the serum results (Table IV) are surprisingly different from the liver results (Table III). Serum ∑SFA is similar between SOD and FOD, and in the opposite direction of the liver profile, ∑MUFA is lower in SOD as compared to FOD. ∑N6PUFA was significantly higher, and ∑N3PUFA significantly lower, in SOD as compared to FOD. The N6/N3 ratio was17 times higher in SO as compared to FO (Table IV).

An unexpected finding in serum SO was its N3 FA profile compared to Ctrl. Whilst ALA and N6-DPA were significantly lower, DHA was significantly higher, in SOD as compared to Ctrl (Table IV). Such finds suggest enhanced uptake of ALA by SOD peripheral tissues followed by bioconversion to its longer forms and exportation, hence the higher ALA available in their diet, but combined with lower ALA and higher DHA in serum (Table IV).

The hypothalamus was also subjected to FA changes following the dietary intervention (Table V). Whilst ∑SFA and ∑N3PUFA was similar between SOD and FOD, ∑MUFA was lower and ∑N6PUFA was higher in SOD as compared to FOD. The N6/N3 ratio was significantly higher in SOD as compared to FOD. Important differences were found between SOD and Ctrl. ∑N6PUFA and ∑N3PUFA were significantly higher in SOD. The ∑N3PUFA differences are attributed mainly to significantly increased DHA in SOD. Despite not reaching statistical

significance, the EPA level in SOD was doubled in relation to Ctrl. The hypothalamic N6/N3 ratio was significantly lower in SOD as compared to Ctrl.

It has been suggested that the overall N6/N3 ratio in the healthy human brain is close to 1/1(31 and others). Interestingly, the SOD group was the only to show a hypothalamic N6/N3 ratio of 1.0 exactly (Table V). However, a direct comparison cannot be made due to obvious species differences. Some small differences can be expected even between rodents. For example, Carrié and colleagues found in 4-months old mice fed a 6% fat-content diet, whose FA profile contained 3.6% ALA, that the hypothalamic AA content was 8.8% ± 0.2 and the DHA was 15.8% ± 0.6[60], compared to our findings of AA at 10.7% ± 0.9 and DHA at 12.9% ± 1.74.

The FA composition of EPI (Table VI), RET (Table VII) and MES (Table VIII) showed a similar pattern following the dietary intervention across all three groups. When comparing SOD versus FOD, SOD∑SFA was significantly lower in EPI and RET. ∑MUFA and ∑N6PUFA were higher, and ∑N3PUFA was lower, in SOD EPI, RET and MES. The N6/N3 ratio was also significantly higher in SOD as compared to FOD. When comparing SOD versus Ctrl, the results follow identical patterns across the three tissues: lower ∑SFA, higher ∑N6PUFA, and unchanged ∑MUFA and ∑N3PUFA.The N6/N3 ratio was lower in SOD as compared to Ctrl, but the only tissue in which this difference reached statistical significance was EPI.

Overall, the soybean oil diet generally decreased SFA and increased N6PUFAs in liver and white adipose tissues, as compared to Ctrl, a result that can only be expected from a diet with high N6PUFA content. However, a small amount of ALA present in soybean oil was apparently enough to increase the amount of DHA in serum and hypothalamus without reducing its concentration in liver and white adipose tissues. Such hypothesis is corroborated by our N6 findings: in comparison to Ctrl, the SOD liver and adipose tissues showed increased amounts of LA, the precursor of AA; however, the AA content in those tissues was decreased. In the opposite direction, we found lower LA and higher AA in SOD serum, as compared to Ctrl. We speculate that peripheral tissues such as the liver and adipose tissue uptake LA and ALA, elongate and desaturate them to their products AA and DHA, which are subsequently exported via blood to the brain.

It is widely accepted that the conversion of ALA to DHA in humans is less than 1%[61]. Lin and colleagues[62] demonstrated in infants that only a very small amount of administered ALA was effectively converted to DHA. Furthermore, a systematic review published in 2014 showed that the supplementation with ALA-rich nuts and seeds was not sufficient to promote significant differences in DHA content[63]. On the other hand, however, it has been shown in experimental conditions that cyclooxygenases 1 and 2 reach much more slowly towards N3 as compared to N6 FAs, and the subsequent cascade of intracellular events is relatively less intense in the presence of abundant N3, as compared to abundant N6[64]. The latter findings could further explain why we have found higher levels of DHA in SOD serum and hypothalamus as compared to Ctrl, whilst DHA remained unchanged in liver and white adipose tissue.

In order to further explore our results, we compared and contrasted the LA/AA and ALA/DHA ratios in the six tissues analysed. A more direct comparison would be to investigate the ALA/EPA ratio instead of DHA, as the enzymes that elongate and desaturate LA to AA and ALA to EPA do not discriminate between substrates[65]. However, as AA and DHA are the two most abundant N6 and N3 FAs in the hypothalamus of rats[11,66] and in the human brain[10], the ALA/DHA ratio, rather than ALA/EPA, would provide a more valuable insight.

The SOD liver, EPI, RET and MES showed increased LA/AA and ALA/DHA ratio in comparison to FOD and Ctrl in all instances, reaching statistically significant increases in eleven out of sixteen possible instances (Table IX). No differences were found in hypothalamic LA/AA and ALA/DHA ratios across the three groups. However, serum LA/AA and ALA/DHA were significantly lower in SOD as compared to Ctrl, whilst the serum ALA/DHA was higher in SOD as compared to FOD.

We have not measured elongase and desaturase activity in our study; however, as a higher ratio denotes proportionally more substrate than product, we speculate two non-self-excluding hypotheses: firstly, in the SOD group the conversion of shorter and less unsaturated fatty acids onto their longer and more unsaturated products took place peripherally in a less effective manner as compared to FOD and Ctrl, as evidenced by the ratio findings in liver and adipose tissue. Our second hypothesis is that the elongation / desaturation processes in SOD are as effective as in FOD and Ctrl, but in SOD the products are exported from peripheral tissues for preferential uptake by the brain. If any of our two hypotheses is correct, it further solidifies the evidence that despite providing sufficient amounts of essential fatty acids to the brain, a soybean oil-based diet is a deficient diet. In the longer term, such diet will only exacerbate chronic conditions with a mild pro-inflammatory background. Biomagnification of essential fatty acids has been well documented across the placenta in humans, aiming at protecting the foetus[67,68,69], and it is reasonable to suggest that biomagnification continues postpartum, aiming at protecting the brain.

In conclusion, the present study showed that the intake of a high fat diet enriched with soybean oil for 8 weeks increasedN3 FA content in the hypothalamus of rats, a result mainly attributed to the ALA content of soybean oil. However, such apparently promising finding was followed by increased markers of obesity, associated with profound disturbances in peripheral tissue FA profile. Future studies are essential to investigate the impact of nutrient-deficient diets on overall metabolism, but similarly importantly, there is a pressing need for the identification of affordable and sustainable food sources.

Table IX. Ratios of LA/AA and ALA/DHA in liver, serum, hypothalamus, epididymal, retroperitoneal and mesenteric white adipose tissues of male Wistar rats fed a standard rodent chow (Ctrl) (2.7kcal/g), or a diet enriched with fish oil (FOD) or soybean oil (SOD) (4.1kcal/g) for 8 weeks.

	Ctrl				FOD					SOD			
Liver	**M**		**SD**		**M**		**SD**			**M**		**SD**	
LA/AA	0.72	±	0.07		0.86	±	0.16			1.71	±	0.49	#*
ALA/DHA	0.07	±	0.03		0.04	±	0.01			0.12	±	0.11	
Serum	**M**		**SD**		**M**		**SD**			**M**		**SD**	
LA/AA	0.79	±	0.03		0.58	±	0.08	#		0.51	±	0.04	#
ALA/DHA	0.18	±	0.04		0.03	±	0.00	#		0.10	±	0.02	#*
Hypothalamus	**M**		**SD**		**M**		**SD**			**M**		**SD**	
LA/AA	0.13	±	0.11		0.04	±	0.01			0.08	±	0.01	
ALA/DHA	0.004	±	0.004		0.001	±	0.001			0.004	±	0.002	
Epidydimal	**M**		**SD**		**M**		**SD**			**M**		**SD**	
LA/AA	24.9	±	4.3		10.9	±	2.0	#		43.2	±	9.1	#*
ALA/DHA	7.49	±	1.54		0.13	±	0.03	#		12.56	±	5.38	*
Retroperitoneal	**M**		**SD**		**M**		**SD**			**M**		**SD**	
LA/AA	41.5	±	7.4		14.1	±	2.3	#		62.7	±	20.5	*
ALA/DHA	14.90	±	4.62		0.10	±	0.01	#		17.84	±	4.87	*
Mesenteric	**M**		**SD**		**M**		**SD**			**M**		**SD**	
LA/AA	52.4	±	4.6		9.3	±	1.0	#		83.5	±	17.0	#*
ALA/DHA	13.71	±	3.18		0.09	±	0.01	#		25.48	±	10.03	#*

Data presented as means ± SD. N=5 for each group. FOD: fish oil-enriched diet; SOD: soybean oil-enriched diet; LA/AA: % of linoleic acid over % of arachidonic acid; ALA/DHA: % of alpha-linolenic acid over % of docosahexaenoic acid. # p< 0.05 vs Ctrl. * p< 0.05 vs. FOD.

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Authors contributions:
RLHW, AAB and EBR conceptualized the study. All authors have substantially contributed to the acquisition, analysis and interpretation of results. All authors contributed to manuscript write-up. All authors have approved the version to be published and are equally accountable for all aspects of this work.

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