A high-fat diet enriched with fish oil affects metabolic pathways in the hypothalamus of rats: a proteomic approach

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Research Article

Keywords: obesity, n-3 fatty acids; carbohydrates metabolism, TCA cycle, respiratory chain, pathway-enrichment analysis

Posted Date: April 19th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1348063/v2>

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Abstract

Background: Fatty acids of the n-3 series are recognized as a healthy type of fat but the effects of excess n-3 consumption have not been fully explored. The hypothalamus is a key site in the control of energy homeostasis and a better understanding of the effects of dietary challenges on this brain site is necessary.

Objective: To evaluate whether the long-term intake of a high-fat diet enriched fish-oil, rich in n-3 fat, modify the proteins content of the hypothalamus.

Methods: Male rats received for 8 weeks either a Control normolipidic diet or a hyperlipidic Fish oil diet. The hypothalami were dissected and processed for proteomic analysis, performed by LC-MS/MS. False Discovery Rate (FDR) correction for multiple testing was performed. Enriched pathways were determined using the Panther online platform.

Results: Twenty-nine proteins were significantly altered between the Control and the Fish groups, of which 15 were down-regulated and 14 were up-regulated in the Fish group. Pathway-enrichment analysis showed that 10 of those proteins were related to “Metabolism of Carbohydrate” and “TCA Cycle and Respiratory Electron Transport” pathways. Of these, 7 proteins were upregulated (Aldo-keto reductase family 1 member B1; Aspartate aminotransferase, mitochondrial; Glucose-6-phosphate isomerase; Malate dehydrogenase mitochondrial; L-lactate dehydrogenase A chain; Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial; Alpha-enolase) and 3 were downregulated (Phosphoglycerate mutase 1; NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial; NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial).

Conclusions: The chronic intake of a hyperlipidic diet rich in fish oil led to modifications on the expression of several hypothalamic proteins related to metabolic pathways. There are indications of possible pro-inflammatory activation and impairment of respiratory chain Complex 1 function, probably due to the excess fat content of the diet. Also, a protective role played by the type of fat was suggested by the findings indicating possible decrease of reactive oxygen species production, maintenance of NADH delivery to the respiratory chain, and production of anorexigenic signals. The results suggest that, although n-3 fat has recognized healthy properties, its consumption in excess affects the hypothalamus with deleterious effects. Further studies are necessary to verify these effects.

1. Background

Obesity prevalence has been pointed out as a pandemic health condition worldwide. The rise of body fat is known to increase the risk of development of comorbidities as cardiometabolic diseases, osteoarthritis, dementia, depression, and some types of cancer. The imbalance between food intake and energy expenditure is one of the causes of obesity and the excess ingestion of fatty acids favors this condition [1–2]. Polyunsaturated fatty acids (PUFA) induce more fat oxidation and energy expenditure than saturated fatty acids, indicating that the type of fat is relevant to the development of obesity [3].

PUFAs n-3 have been implicated as healthy fatty acids [4]. Our laboratory has previously evaluated the effects of a high-fat fish-oil diet in rats. We have shown that it prevented the activation of hypothalamic orexigenic nuclei and inflammatory mediators induced by a high-fat soy-oil diet [5–6]. It has also induced lower serum triglycerides levels and prevented the development of depressive-like behaviors, in comparison to a high-fat lard diet [7]. However, we have also found that the high-fat fish-oil diet increased serum glucose levels and impaired serotonin-induced hypophagia [8–9]. The above results indicate the need of further studies to better understand the role played by n-3 fatty acid in the development of the metabolic derangements of obesity. Since the hypothalamus is a key site in the control of energy homeostasis [10], a better understanding of the effects of dietary challenges on this brain site is necessary.

The non-target proteomic technique has been widely used to perform exploratory analysis of several conditions. It has been shown that the intake of a high-fat lard diet by mice modulated hypothalamic proteins involved in metabolic pathways [11]. We have previously demonstrated that the obesity induced by intrauterine growth restriction affected hypothalamic pathways related to glucose metabolism and mitochondrial function in adult male rats while glutamate metabolism and GABAergic synapse were affected in adult female rats [12–13]. We have not been able to find studies utilizing this approach to evaluate the effects of PUFA n-3-rich diets on the hypothalamus.

The present study thus used the proteomic technique to access the changes in this brain area after a chronic intake of a high-fat fish oil diet.

2. Materials And Methods

This study was approved by the Research Ethics Committee of the Universidade Federal de São Paulo – UNIFESP (CEUA N 1673/07) and animal protocols were conducted following the ethics procedures established by the Conselho Nacional de Experimentação Animal (CONCEA).

Two-month-old male Wistar rats were maintained (4–5 per cage) under controlled conditions of lighting (12:12h light-dark cycle, lights on at 6 am) and temperature ($24 \pm 1^\circ\text{C}$) and randomly assigned to receive either standard chow diet (Control group: 2.7 Kcal/g, 15% energy from fat) (Nuvilab CR1, Nuvital, PR, Brazil) or a high-fat diet enriched with fish oil (Fish group: 4.4Kcal/g, 45% energy from fat) ad libitum for 8 weeks. The fish diet contained (w/w) 50% of standard chow diet, 20% of fish oil (ROPUFA® '75'ω-3, Roche, DSM Nutritional Products, Brazil), 10% sucrose and 20% casein, to obtain a protein/energy ratio similar to that of the Control diet. Once a week, each rat was weighed, and a known amount of diet was introduced in the cage and weighed after 24 hours. Food intake was estimated by rat by dividing the cage value by the number of rats in the cage. Feed efficiency was calculated as (body weight gain/energy intake) x 100 [14].

After the 8 weeks of treatment, the animals were fasted overnight and euthanized. The hypothalamic were rapidly dissected and frozen in liquid nitrogen and kept at -80°C until further analysis.

2.1. Protein sample preparation

Proteomic analysis was performed as previously described, with minor modifications [12]. Because it is a complex structure, the entire hypothalamus was used. Each hypothalamus was homogenized in 1 mL of lysis buffer (8 M urea, 75 mM NaCl, 1 M Tris, and protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA)). After centrifugation (19,000 g for 30 minutes at 4°C) the protein concentration in the supernatants was determined (2-D Quant Kit GE Healthcare, Waukesha, WI, USA). Samples were pooled (3 samples/pool, 2 pools/group) and aliquots of 900 µg of protein were subjected to dialysis (Ultra-4 Centrifugal 3,000 NMWL filter devices, Merck Millipore) against 50 mM ammonium bicarbonate. Then, 200 µg of concentrated proteins was heated (15 min at 80°C) in the presence of 25 µL of 0.2% RapiGest SF (Waters, Milford, MA, USA), reduced with 100 mM DTT (30 min at 60°C), and alkylated with 300 mM iodoacetamide (30 min at room temperature in the dark). Proteins were digested (overnight at 37°C) with trypsin (Promega, Fitchburg, WI, USA) at a 1:100 (w:w) enzyme:protein ratio. The samples were then incubated with 5% trifluoroacetic acid (90 min at 37°C), centrifuged (19,000 g for 10 minutes at 4°C) and the supernatants transferred to the vials for MS analysis.

2.2. LC-MS/MS, data processing and database search

Proteomic analysis was performed by data-independent acquisition experiments in a nanoAcquity UPLC system coupled to a Synapt G2 HDMS Q-TOF mass spectrometer (Waters). Digested samples (2 pools per group, 3 technical replicates) were injected onto a trap column (nanoAcquity C18 trap column Symmetry 180 µm x 20 mm, Waters), and then transferred by an elution gradient (phase B gradient from 7–35% for 92 minutes at a 275 nL/min) to an analytical column (nanoAcquity C18 BEH 75 µm x 150 mm, 1.7 mm, Waters). The mobile phase A was 0.1% formic acid in water and the mobile phase B was 0.1% formic acid in acetonitrile. Data were acquired in HDMS^E mode, switching from low (4 eV) to high (ramped from 19 to 45 eV) collision energy. For external calibration, Glu-fibrinopeptide B solution (500 fmol/mL, in 0. % formic acid in 50% acetonitrile; Waters) was infused using a nanoLockSpray apparatus and scanned every 30 seconds [12].

Mass spectrometry raw data was processed with ProteinLynx Global Server software version 3.0.1 (Waters). Database search was performed using *Rattus norvegicus* sequences obtained in the UniProtKB/Swiss-Prot database (www.uniprot.org, including 9485 entries). The search parameters included: carbamidomethylation of cysteine as fixed modification, oxidation of methionine, N-terminal acetylation, glutamine and asparagine deamidation as variable modifications, up to 2 missed cleavage sites allowed for trypsin digestion, and automatic fragment and peptide mass tolerance. The protein identification criteria comprised: a minimum of 1 fragment ion per peptide, 5 fragment ions per protein and 2 peptides per protein, and the false discovery identification rate was set at 5%. Label-free quantitative assessments based on peptide intensities were performed for relative quantitation [15]. Results were exported into Excel files. Proteins identified in at least 2 technical replicates of each pool were included in the analysis. Normalization was performed in each sample according to the sum of protein intensities.

2.3. Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 21 Software (IBM, Armonk, NY, USA). The Shapiro-Wilk normality test was applied to all variables, with exception of proteome data. Comparisons between Control and Fish groups were performed by unpaired Student's "t" test for parametric variables or Wilcoxon test for nonparametric variables.

Proteome data were analyzed by Student's "t" test. False Discovery Rate (FDR) correction for multiple testing was performed on R software (<https://www.r-project.org/>). Statistical significance was set at FDR < 0.05.

2.4. Pathway enrichment analysis

Enriched pathways were determined using the online platform Panther (version 15.0, released 2020-07-28; <http://www.pantherdb.org/>) [16]. The overrepresentation test was carried out with proteins differentially expressed as the input list, whole *Rattus norvegicus* proteome was used as reference and Reactome Pathway (version 65, released 2020-11-17) was chosen as annotation data set. Enriched pathways were based on Fisher test followed by False Discovery Rate correction and significance was set to FDR < 0.05.

3. Results

3.1. Body weight and food intake

The cumulative caloric intake during the 8 weeks of diet treatment was significantly higher in the fish group and this led to a final body weight 4.5% higher than that of the control group (Table 1). Figure 1 shows that the caloric intake of the Fish group was higher at the 1st first and 5th weeks. The feed efficiency was higher in the Fish group during the 5th week of diet treatment. Together, these results indicate that, throughout diet treatment, the Fish group had light increases of cumulative caloric intake and feed efficiency, what was probably important to limit body weight gain in response to the hyper caloric diet.

Table 1
Body weight and cumulative food intake during chronic intake of control or fish-oil diet

	Control (n = 18)	Fish (n = 13)	P-value
Initial body weight (g)	284.7 (236.7–333.4)	273.3 (201.8–338.4)	0.737
Final body weight (g)	403.4 ± 5.6	421.5 ± 7.3	0.055
Body weight gain (g)	118.7 ± 9.9	148.2 ± 13.1	0.077
Cumulative caloric intake (Kcal)	122.3 (99.4–151.5)	141.3 (113.6–182.2)	0.007
Data presented as Mean ± SEM for variables with normal distribution and Median (minimum-maximum) for variables not normally distributed.			

3.2. Proteomics

The mean number of proteins detected in each hypothalamic replicate was 553. After application of inclusion criteria for relative quantitation (presence in at least two technical replicates of each pool), 339 proteins were compared between the groups (additional file 1). Twenty-nine proteins were significantly altered between fish-diet and control rats, of which 15 were down-regulated and 14 were up-regulated in the fish-diet group (Table 2).

Table 2
Hypothalamic proteins affected by fish-oil diet intake.

Uniprot ID	Gene	Protein name	Fold change (Fish/Control)	FDR
Down-Regulated				
P62762	Vsnl1	Visinin-like protein 1	0.71	0.0421
P50408	Atp6v1f	V-type proton ATPase subunit F	0.74	0.0444
P11232	Txn	Thioredoxin	0.75	0.0185
Q5BK63	Ndufa9	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	0.76	0.0493
P19804	Nme2	Nucleoside diphosphate kinase B	0.76	0.0451
P20788	Uqcrcs1	Cytochrome b-c1 complex subunit Rieske, mitochondrial	0.78	0.0010
Q5XI73	Arhgdia	Rho GDP-dissociation inhibitor 1	0.78	0.0228
P26772	Hspe1	10 kDa heat shock protein, mitochondrial	0.79	0.0493
O35264	Pafah1b2	Platelet-activating factor acetylhydrolase IB subunit beta	0.80	0.0391
Q8VBU2	Ndrp2	Protein NDRG2 (Isoform 2 of Protein NDRG2)	0.80	0.0318
P34058	Hsp90ab1	Heat shock protein HSP 90-beta	0.82	0.0025
O35244	Prdx6	Peroxiredoxin-6	0.82	0.0391
P19234	Ndufv2	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	0.84	0.0318
P25113	Pgam1	Phosphoglycerate mutase 1	0.84	0.0254
P0DP29	Calm1	Calmodulin-1	0.87	0.0038
Up-regulated				
O35331	Pdxk	Pyridoxal kinase	1.62	0.0010
P07943	Akr1b1	Aldo-keto reductase family 1 member B1(Aldose reductase)	1.40	0.0451
P47942	Dpysl2	Dihydropyrimidinase-related protein 2	1.38	0.0000
Q5FVI6	Atp6v1c1	V-type proton ATPase subunit C 1	1.37	0.0318
Q68FY0	Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial	1.36	0.0010

FDR (false discovery rate) = P value corrected for multiple comparisons according to Benjamini-Hochberg test

Uniprot ID	Gene	Protein name	Fold change (Fish/Control)	FDR
P00507	Got2	Aspartate aminotransferase, mitochondrial	1.26	0.0185
P04642	Ldha	L-lactate dehydrogenase A chain	1.22	0.0318
P08461	Dlat	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	1.20	0.0340
Q6P502	Cct3	T-complex protein 1 subunit gamma	1.17	0.0318
Q6P6V0	Gpi	Glucose-6-phosphate isomerase	1.15	0.0340
P04764	Eno1	Alpha-enolase	1.13	0.0493
P32551	Uqcrc2	Cytochrome b-c1 complex subunit 2, mitochondrial	1.10	0.0451
P04636	Mdh2	Malate dehydrogenase, mitochondrial	1.10	0.0038
P15999	Atp5f1a	ATP synthase subunit alpha, mitochondrial	1.05	0.0340
FDR (false discovery rate) = P value corrected for multiple comparisons according to Benjamini-Hochberg test				

The pathway-enrichment analysis showed that 4 pathways were significantly affected by the intake of the fish-oil diet. In “Metabolism of carbohydrate” pathway, 6 proteins were affected, of which 5 were included in the “Glucose Metabolism” pathway. The “TCA cycle and respiratory electron transport” pathway had 5 proteins affected, of which 3 were included in the “Pyruvate metabolism and TCA cycle” pathway. The “Innate immune system” pathway had 10 proteins affected, of which 7 were included in the “Neutrophil degranulation” pathway. Five affected proteins were related to “Cellular response to stress” (Table 3).

Table 3
Pathways significantly affected by the fish-oil diet

Reactome Pathway	Uniprot ID	Protein	Fold change (Fish/Control)
Metabolism of carbohydrate (FDR = 4.26E-04)	P07943	Aldo-keto reductase family 1 member B1	1.40
	Glucose metabolism (FDR = 9.92E-05)	Aspartate aminotransferase, mitochondrial	1.26
		Glucose-6-phosphate isomerase	1.15
		Malate dehydrogenase, mitochondrial	1.10
		Phosphoglycerate mutase 1	0.84
		Alpha-enolase	1.13
TCA cycle and respiratory electron transport (FDR = 3.16E-04)	P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	0.84
	Q5BK63	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	0.76
	Pyruvate metabolism and TCA cycle (FDR = 1.06E-02)	L-lactate dehydrogenase A chain	1.22
		Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	1.20
		Malate dehydrogenase, mitochondrial	1.10
Innate Immune System (FDR = 1.29E-04)	P50408	V-type proton ATPase subunit F	0.74
	Q5FVI6	V-type proton ATPase subunit C 1	1.37
	P11232	Thioredoxin	0.75
	Neutrophil degranulation (FDR = 7.59E-04)	Peroxisredoxin-6	0.82
		Platelet-activating factor acetylhydrolase IB subunit beta	0.80
		Heat shock protein HSP 90-beta	0.82

Reactome Pathway	Uniprot ID	Protein	Fold change (Fish/Control)
	Q6P6V0	Glucose-6-phosphate isomerase	1.15
	O35331	Pyridoxal kinase	1.62
	P19804	Nucleoside diphosphate kinase B	0.76
	P25113	Phosphoglycerate mutase 1	0.84
Cellular responses to stress (FDR = 2.31E-02)	P11232	Thioredoxin	0.75
	O35244	Peroxiredoxin-6	0.82
	P34058	Heat shock protein HSP 90-beta	0.82
	P50408	V-type proton ATPase subunit F	0.74
	Q5FVI6	V-type proton ATPase subunit C 1	1.37

Reactome Pathway determined with the online platform Panther. Enriched pathways were based on Fisher test followed by False Discovery Rate correction and significance was set to FDR < 0.05.

4. Discussion

The polyunsaturated n-3 fatty acid has been implicated as a health fatty acid [4]. The present study showed that 8 weeks of intake of the high-fat fish oil diet, rich in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), caused only a small non-significant increase of body weight in comparison to the control diet, along with a light increase of caloric intake and feed efficiency. These results agree with previous findings of our laboratory [5–8] and indicate that the fish oil diet was protective against the potentially deleterious effects of high-fat intake. Accordingly, fish oil intake has been found to reduce the adverse effect of overfeeding in early life in rats [17].

The hypothalamus receives inputs from gastrointestinal-borne molecules, including the nutrients themselves and factors induced by nutrients, and this sensing affects the hypothalamic control of energy homeostasis [18]. Hypothalamic neuroinflammation induced by high-fat intake of saturated or n-6 PUFAs has been shown to impair this homeostatic role of the hypothalamus [19]. Contrarily, the chronic high-fat intake of fish oil diet decreased hypothalamic levels of IL-6 and TNF- α [5].

To better understand the effects of high-fat fish oil diet intake, we performed a proteomic analysis of the hypothalamus. We found that the 29 proteins differentially expressed in the Fish group belonged to 4

different main pathways: Metabolism of Carbohydrate, TCA cycle and Respiratory Electron Transport, Innate Immune System, and Cellular Response to Stress.

Included in the Metabolism of Carbohydrate pathway, the Glucose Metabolism pathway had 5 affected proteins. Phosphoglycerate mutase 1 was downregulated by 16% while the other 4 proteins were upregulated: aspartate aminotransferase, mitochondrial (26%), Glucose-6-phosphate isomerase (15%), Malate dehydrogenase, mitochondrial (10%), and Alpha-enolase (13%).

Glucose-6-phosphate isomerase, Phosphoglycerate mutase 1, Alpha-enolase participate in the glycolytic pathway, but they do not play a regulatory role in the pathway. Glucose-6-phosphate isomerase catalyzes the isomerization of glucose-6-phosphate to fructose-6-phosphate. A 20% decrease of this protein in the cerebral cortex of diabetic rats after carotid occlusion has been shown to exacerbate the ischemia-induced damage [20]. This enzyme is also known as neuroleukin and is involved in neuronal growth, differentiation, and motility, besides its function in glucose metabolism. Inhibition of this protein in PC12 cells increased the susceptibility to apoptosis [21]. In the present work, its increased expression in the Fish group could represent a protective mechanism.

Phosphoglycerate mutase 1 acts on the conversion of 3-phosphoglycerate to 2-phosphoglycerate in glycolysis. Its levels have been found to increase in human glioma tissue, proportionally to tumor grade, as well as in C6 glioma cells, and its knockdown inhibited cell proliferation and promoted apoptosis in U87 glioma cells [22–23]. The increased expression of this protein in cancer cells is consistent with the increase of glycolysis as main source of energy supply to the tumor [24]. On the other hand, its super-expression in mouse hippocampus facilitated phosphorylation of cAMP response element-binding protein (CREB) and increased cellular proliferation and neuroblast differentiation, responses indicative of a possible role of this protein in promoting tissue regeneration [25]. In the HT22 hippocampal cell line, phosphoglycerate mutase 1 activation attenuated ischemia-induced cell damage and decreased reactive oxygen species levels. In rodents, super-expression of this protein decreased the damage of the CA1 hippocampal area, after 4 days of brain ischemia [26]. The above data indicated that, although deleterious when expressed in cancer cells, phosphoglycerate mutase 1 had beneficial effects in the response of hippocampal cells to injury. We could not find papers on the hypothalamic status of this protein in response to challenges potentially affecting the hypothalamus. Importantly, in rat liver, six weeks of a high-fat saturated diet induced steatosis and increased phosphoglycerate mutase 1 by 57%, while the combination of Fuzhuan brick tea restored enzyme levels to those of control animals and ameliorated the hepatic fat accumulation [27].

Alpha-enolase is one of the sub-units of the enzyme enolase, responsible for the interconversion of 2-phosphoglycerate and phosphoenolpyruvate in glycolysis. In human brain, alpha-enolase has been located mainly in the glia [28]. A proteomic study has found increase of alpha-enolase in the hippocampus and cingulate gyrus of Alzheimer's disease patients [29]. On the other hand, analyzing oxidized proteins in the cortex Alzheimer's disease patients another proteomic study found increased levels. The authors suggested that oxidation could lead to loss of protein function and impair glycolysis

[30]. This is in agreement with data showing glucose hypometabolism in this pathology [31]. Interestingly, a prospective study found that the intake of n-3 fatty acids-rich seafoods by elderly subjects protected from the development of Alzheimer`s Disease, a finding that was associated with DHA intake [32].

Two mitochondrial enzymes of the malate-aspartate shuttle (MAS), aspartate aminotransferase and malate dehydrogenase, showed increased levels in the Fish group. MAS transfers to mitochondria the electrons of the NADH generated in the citosol during glycolysis. Decreased levels of mitochondrial MAS proteins have been found in a proteomic analysis of postmortem brain tissue (putamen, thalamus and parietal lobe) of ischemic stroke victims [33]. In microglial Bv2 cells, the inhibition of aspartate aminotransferase decreased intracellular ATP levels and caused activation of apoptosis [34].

In the present study, aldo-keto reductase family 1 member B1, an enzyme important in detoxification processes, showed increased levels in the Fish group. In rats, increased levels of this enzyme were found in astrocytes after spinal cord injury, and associated with increased proliferation of astrocytes through stimulation of Akt pathways and glucose utilization [35]. On the other hand, it has also been attributed a pro-inflammatory role through its involvement in prostaglandins metabolism [36]. Increased levels have been reported in diabetes, asthma and sepsis and its inhibition decreased oxidative stress in these pathologies [37].

Additionally, malate dehydrogenase is the last enzyme of the TCA cycle, converging malate to oxaloacetate with the production of 1 NADH. In synaptosomes, high levels of oxidative stress impaired NADH production by blocking aconitase and alpha-ketoglutarate dehydrogenase enzymes [38]. In HT22 hippocampal cells, the induction of oxidative stress up regulated mitochondrial malate dehydrogenase mRNA levels by 22% [39]. Since high-fat intake may induce oxidative stress [40], the increase of malate dehydrogenase seen in the present study could represent an attempt to maintain NADH levels in TCA.

The TCA cycle and respiratory electron transport pathway had other 4 affected proteins. Two proteins were upregulated: L-lactate dehydrogenase A chain (22%) and dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial (20%). NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial, was downregulated by 16% and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial, was downregulated by 24%

L-lactate dehydrogenase A chain is part of the lactate dehydrogenase enzyme, responsible for the conversion of glucose-derived pyruvate into lactate. In the brain, this enzyme is present mainly in astrocytes [41]. Astrocytes form lactate and release it to neurons, where it is transformed in pyruvate [42]. Lactate has been shown to have anorexigenic properties and L-lactate dehydrogenase is important to this function. The intracerebroventricular (i.c.v.) injection of pyruvate or lactate decreased food intake and body weight gain, effects abolished by the suppression of L-lactate dehydrogenase [43]. Blocking L-lactate dehydrogenase centrally has been shown to also suppress the hypophagia induced by intraperitoneal lactate [44]. The increased of L-lactate dehydrogenase found in the present study could explain, at least in part, the absence of excess caloric intake of the Fish group.

The protein dihydrolipoyllysine-residue acetyltransferase is a component of the E2 enzyme of the pyruvate dehydrogenase complex, responsible for formation of acetyl-CoA from glycolysis-derived pyruvate. This enzyme has been attributed a protective role in situations of metabolic impairment. After expose of rats to chronic mild stress, the up-regulation of its hippocampal levels protected against the development of depressive- like symptoms [45]. Mild brain injury led to increased levels of the 3 enzymes of pyruvate dehydrogenase complex while severe injury decreased their levels. The authors indicated that the increased levels in mild brain injury was to protect the metabolism during a transiently malfunction of mitochondria [46].

The proteins NADH dehydrogenase [ubiquinone] flavoprotein 2 and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, downregulated in the Fish group, are subunits of Complex I of the respiratory chain. Complex 1 has been found to generate more reactive oxygen species (ROS) than Complex III [47]. Low levels of NADH dehydrogenase [ubiquinone] flavoprotein 2 has been associated lower ROS production and prolonged longevity [48]. On the other hand, the knocking out of NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9 in HEK293T cells impaired complex I assembly and the cells were unable to utilize galactose as a carbon source, indicating that oxidative phosphorylation was disrupted [49]. Also, decreased levels of this enzyme have been found in the brain of Alzheimer's Disease patients [50].

5. Conclusions

In conclusion, the chronic intake of a hiperlipidic diet rich in fish oil led to modifications on the expression of several hypothalamic proteins related to metabolic pathways. As shown in Fig. 2, there are indications of possible pro-inflammatory activation and impairment of respiratory chain Complex 1 function, probably due to the excess fat content of the diet. Also, a protective role played by the type of fat was suggested by the findings indicating possible decrease of ROS production, maintenance of NADH delivery to the respiratory chain, and production of anorexigenic signals. Further studies are necessary to verify these effects.

Abbreviations

CREB
cAMP response element-binding protein
DHA
docosahexaenoic acid
EPA
eicosapentaenoic acid
FDR
false discovery rate
i.c.v
intracerebroventricular

MAS
malate-aspartate shuttle
PUFA
Polyunsaturated fatty acids
ROS
reactive oxygen species
TCA
tricarboxylic acid cycle.

Declarations

Ethics approval and consent to participate: “Research Ethics Committee of the Universidade Federal de São Paulo – UNIFESP (CEUA N 1673/07)”

Consent for publication: “Not applicable”.

Availability of data and materials: “Not applicable”.

Competing interests: “The authors declare that they have no competing interests”.

Funding: “This research was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, grant number 07/05360-4”.

Author Contributions: “Conceptualization, A.P.P., M.C.C.A and E.B.R.; formal analysis, A.P.S. and A.P.P.; investigation, A.P.S., A.P.P. V.T.B., M.M.T., L.M.O and A.K.T.; writing—original draft preparation, A.P.S, A.P.P. and E.B.R.; writing—review and editing, A.P.S. and E.B.R.; supervision, E.B.R.; All authors have read and agreed to the published version of the manuscript.”

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Figures

Figure 1

Caloric intake and feed efficiency during intake of control or fish-oil diet

Caloric intake was an estimate of ingestion per rat in cage; N = 4 cages for Control Group; N = 3 cages for Fish Group. Feed efficiency N = 18 rats for Control Group; N = 13 rats for Fish Group. * p < 0.05 vs. control.

Figure 2

Proteins related to metabolic pathways differentially expressed in the Fish-group.

Red proteins: upregulated; blue proteins: downregulated. TCA: tricarboxylic acid cycle; MAS: malate-aspartate shuttle; ROS: reactive oxygen species; ?: uncertain effect

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