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High-fat diets rich in soy or fish oil distinctly alter hypothalamic insulin signaling in rats☆

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Abstract

Hypothalamic insulin inhibits food intake, preventing obesity. High-fat feeding with polyunsaturated fats may be obesogenic, but their effect on insulin action has not been elucidated. The present study evaluated insulin hypophagia and hypothalamic signaling after central injection in rats fed either control diet (15% energy from fat) or high-fat diets (50% energy from fat) enriched with either soy or fish oil. Soy rats had increased fat pad weight and serum leptin with normal body weight, serum lipid profile and peripheral insulin sensitivity. Fish rats had decreased body and fat pad weight, low leptin and corticosterone levels, and improved serum lipid profile. A 20-mU dose of intracerebroventricular (ICV) insulin inhibited food intake in control and fish groups, but failed to do so in the soy group. Hypothalamic protein levels of IR, IRS-1, IRS-2, Akt, mTOR, p70S6K and AMPK were similar among groups. ICV insulin stimulated IR tyrosine phosphorylation in control (68%), soy (36%) and fish (34%) groups. Tyrosine phosphorylation of the pp185 band was significantly stimulated in control (78%) and soy (53%) rats, but not in fish rats. IRS-1 phosphorylation was stimulated only in control rats (94%). Akt serine phosphorylation was significantly stimulated only in control (90%) and fish (78%) rats. The results showed that, rather than the energy density, the fat type was a relevant aspect of high-fat feeding, since blockade of hypothalamic insulin signal transmission and insulin hypophagia was promoted only by the high-fat soy diet, while they were preserved in the rats fed with the high-fat fish diet.

Keywords: Polyunsaturated fatty acids; Hypothalamus; Insulin signaling; Obesity; Insulin hypophagia; Food intake; High-fat feeding

1. Introduction

High-fat intake has been shown to play a relevant part in the obesity epidemic and its associated comorbidities, such as insulin resistance and diabetes, with saturated and trans fats being the most implicated. However, some studies have indicated that excess consumption of polyunsaturated fatty acids (PUFAs) may also be of importance [1]. A crescent use of refined vegetable oils rich in n-6 PUFAs allied to decreased consumption of n-3 PUFA sources has been reported and these changes have been associated with increased risk for obesity-linked diseases [2]. In humans, high n-6 PUFA intake has been shown to positively correlate with obesity and insulin resistance [3,4], while an inverse relationship has been described between n-3 intake and body adiposity [5]. Some animal studies have corroborated these findings [6,7], while other studies have described increased body fat and insulin resistance after long-term intake of either n-6 or n-3 PUFAs [8,9].

Energy homeostasis is highly controlled by the central nervous system. The hypothalamus is the major site targeted by the adiposity signals leptin and insulin, which decrease food intake and increase energy expenditure by influencing the activity of multiple hypothalamic neurons, thus promoting inhibition of anabolic mediators and stimulation of catabolic effectors [10–13].

Few studies have focused on aspects of the hypothalamic control of energy homeostasis after high-PUFA intake. Mice and rats fed with either safflower (n-6 PUFAs) or fish (n-3 PUFAs) oil showed unchanged hypothalamic neuronal activation and expression of the anabolic mediators neuropeptide Y and agouti-related protein [9,14,15]. However, n-3 but not n-6 feeding reduced the expression of prepro-orexin and melanin-concentrating hormone, which are potent orexigenic factors [9]. We have recently reported that fish oil intake led to hypothalamic serotonergic impairment, while soy oil (n-6) diet induced a potentially obesogenic profile of hypothalamic nuclei activity [16,17]. These data indicated the existence of distinct effects of different PUFA oils on hypothalamic mediators of energy balance.

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Data on the effects of high-PUFA intake on hypothalamic insulin action are lacking. Insulin-induced hypophagia plays a relevant part in the maintenance of energy homeostasis, and impairment of insulin's hypothalamic action has been associated with obesity [10–13]. Insulin hypophagia has been shown to rely on the so-called phosphatidylinositol 3-kinase (PI3-K) pathway. The insulin receptor (IR) carries an intrinsic tyrosine kinase activity, and hormone binding induces receptor auto-phosphorylation and activates the enzyme. The receptor induces tyrosine phosphorylation of its substrates 1 (IRS-1) and 2 (IRS-2), among others. Tyrosine-phosphorylated IRSs associate with and activate PI3-K which, in turn, phosphorylates membrane phospholipids. This is followed by the activation of phosphatidylinositol-dependent protein kinases 1/2 and phosphorylation of serine residues of protein kinase B (Akt) [18–21]. High-fat saturated diets have been shown to affect these signaling steps and to induce central insulin resistance [22–24]. To the best of our knowledge, no studies have examined the effect of high-PUFA diets rich in soy or fish oil on hypothalamic insulin signaling.

The present study thus evaluated whether the ability of insulin to inhibit food intake by stimulating its signaling pathway in the hypothalamus was affected in rats fed chronically with high-PUFA diets and whether the fat type was relevant to this effect.

Table 1
Fatty acid composition of the control, soy and fish diets

Fatty acid	% of total fatty acids		
	Control	Soy	Fish
C13:0			0.154
C14:0	0.114	0.168	0.175
C15:0	0.048	0.029	
C16:0	13.373	10.533	2.174
C17:0	0.091	0.080	
C18:0	2.945	3.200	2.443
C20:0	0.419	0.373	0.537
C21:0		0.032	
C22:0	0.042		0.886
C23:0	0.072	0.086	0.272
C24:0	0.336	0.257	
C14:1	0.025		
C16:1	0.206	0.089	0.273
C18:1n9	24.128	27.904	6.757
C18:1n11	1.189		1.412
C20:1n9		0.318	1.677
C20:1n11			0.627
C22:1n9			0.138
C24:1	0.037	0.002	0.364
C16:4n1	0.053	0.059	
C18:2n6c LA	49.453	51.261	5.927
C18:3n3 LNA	3.731	4.852	0.742
C18:3n4		0.252	
C18:3n6		0.030	0.117
C18:4n3	0.055	0.219	1.000
C20:2n6	0.049	0.034	0.304
C20:3n3			0.155
C20:3n6			0.420
C20:4n3	0.094		1.872
C20:4n6 AA	0.095		1.898
C20:5n3 EPA	1.971	0.086	38.850
C21:5n3	0.074	0.033	1.840
C22:2n6	0.041	0.031	0.045
C22:4n6			0.637
C22:5n3	0.219	0.003	4.601
C22:5n6		0.016	
C22:6n3 DHA	1.138	0.053	23.701
Total SFA	17.440	14.758	6.641
Total MUFA	25.585	28.313	11.248
Total PUFA	56.973	56.929	82.109
Total n6	49.638	51.372	9.348
Total n3	7.282	5.246	72.761
n6/n3	6.817	9.793	0.128

2. Methods

2.1. Animals and diets

All procedures were in compliance with the guidelines of the Committee on Research Ethics of the Federal University of São Paulo. Male Wistar rats were housed five per cage and maintained under controlled lighting (12-h light/dark cycle, lights on at 6 a.m.) and temperature (22±1°C) conditions, with free access to food and water.

Eight-week-old rats were randomly assigned to receive one of three *ad libitum* diet treatments for 8 weeks: standard chow (2.8 kcal/g, 15% kilocalories from fat, Nuvilab, Brazil) or high-fat diets (3.5 kcal/g, 50% kilocalories from fat) prepared by adding 20% (w/w) of either soy oil (Liza, Cargill Agrícola, Brazil) or fish oil (ROPUFA® '75' ω-3, Roche, DSM Nutritional Products, Brazil) to the standard chow. Casein was added to both high-fat diets (20%, w/w), to achieve the same protein content of the control chow, while butylated hydroxytoluene (0.02% of added oil) was the anti-oxidant agent. The fatty acid composition of the diets was determined as previously detailed [16,20] and is shown in Table 1. The data showed a high percentage of n-3 PUFAs in the fish diet, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas the soy and the control diets had high percentages of n-6 PUFAS, mainly linoleic acid.

2.2. Energy intake, body and fat pad weight, and serum parameters

Food intake and body weight were measured once a week. At the end of the 8 weeks of diet treatments, the rats were decapitated after an overnight fast. The retroperitoneal, epididymal and mesenteric adipose tissue pads were dissected and weighed. Trunk blood was collected and serum stored at −80°C. Glucose, total cholesterol, HDL-cholesterol and triacylglycerol levels were measured by enzymatic methods (Labtest Diagnóstica, Brazil). VLDL level was estimated from triacylglycerol levels (VLDL=triacylglycerols/5) and LDL-cholesterol level was calculated by the Friedewald equation (LDL-c=total cholesterol−(HDL-c)−VLDL) [25]. Serum levels of the peptide hormones insulin and leptin and of the steroid hormone corticosterone were determined using enzyme-linked immunosorbent assays, provided by Millipore Corp. (Bedford, MA, USA) and Assay Designs, Inc. (Ann Arbor, MI, USA, respectively). The homeostasis model assessment (HOMA) index was calculated by the formula: [Fasting serum insulin (ng/ml)×fasting serum glucose (mmol/L)]/(22.5×0.0417) [26].

2.3. Intracerebroventricular insulin injection and food intake measurement

Animals were anesthetized with ketamine/xylazine (67/13 mg/kg) and stereotaxically [27] implanted with a 21-gauge cannula aimed at the left lateral ventricle (A −0.9, L +1.5 and V −3.0, from bregma). The cannula was secured to the skull with screws and dental cement, and the animals were individually caged thereafter, with *ad libitum* access to food and water. Cannula efficiency was tested 4 days after cannulation by evaluation of the drinking response elicited by intracerebroventricular (ICV) angiotensin II [28]. One week after the stereotaxic surgery, the rats were fasted for 6 h and received ICV injections of 2.0 μl of saline vehicle or 20 mU of regular insulin (human recombinant, Eli Lilly and Company, Indianapolis, IN, USA), as previously described [19–21]. The injections were performed in the animal room, immediately before lights out. The rats were then returned to their home cages and a known amount of their respective diets was introduced into the cages. The amount of food consumed was determined 12 and 24 h later by weighing the amount of food remaining in the cups and correcting for spillage.

2.4. Hypothalamic insulin signaling

The animals underwent ICV cannulation, as described above. One week after surgery, they were fasted for 6 h and were ICV injected with 2.0 μl of saline or 200 mU of regular insulin, as previously described [19,21]. Fifteen minutes later, they were decapitated, the hypothalami quickly removed and immediately homogenized in 1.0 ml of solubilization buffer at 4°C [1% Triton X-100, 100 mM Tris-HCl (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium

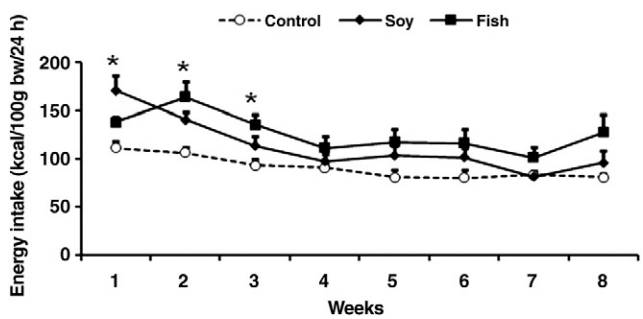

Fig. 1. Energy intake of rats fed with control (n=27), soy (n=24) or fish (n=29) diet for 8 weeks. *P<.05 vs. control.

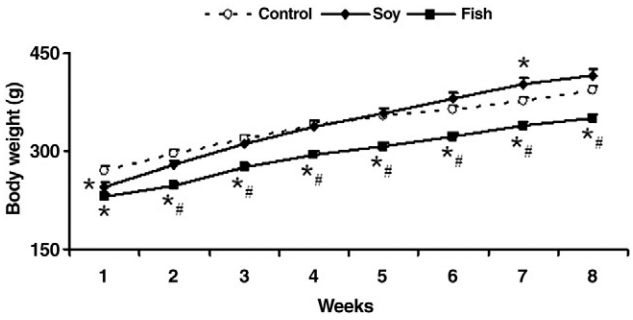

Fig. 2. Body weight of rats fed control (n=27), soy (n=24) or fish (n=29) diet for 8 weeks. *P<.05 vs. control, #P<.05 soy vs. fish.

orthovanadate, 2.0 mM phenylmethylsulfonyl fluoride and 0.1 mg aprotinin/ml]. Insoluble material was removed by centrifugation for 35 min at 12,000 rpm at 4°C. The protein concentration of the supernatants was determined by BCA assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The whole tissue extracts (75 μg) were denatured by boiling (5 min) in Laemmli sample buffer containing 100 mM DTT. Proteins were separated in 8% or 10% SDS-PAGE in miniature slab gel apparatus (Bio-Rad Laboratories).

Electrotransfer of proteins from the gel to nitrocellulose membranes was performed in a semi-dry transfer apparatus (Bio-Rad Laboratories). Nonspecific protein binding to the membrane was reduced by pre-incubation for 2 h at 22°C in blocking buffer (5% nonfat dry milk, 10 mM Tris, 150 mM, NaCl and 0.02% Tween 20). The nitrocellulose membranes were incubated overnight at 4°C with primary antibody against phosphotyrosine (pTyr) for detection of phosphorylation of the pp185 band, which contains IRS-1 and IRS-2. For detection of tyrosine phosphorylation of IR and IRS-1 and serine phosphorylation of Akt proteins, anti-phosphotyrosine-IR, anti-phosphotyrosine-IRS-1 and anti-phosphoserine-Akt primary antibodies were used, respectively. Protein levels of IR, IRS-1, IRS-2, Akt, mTOR, p70S6K and AMPK were evaluated with the respective primary antibodies.

The blots were subsequently incubated with peroxidase-conjugated secondary antibodies. Specific bands were detected by chemiluminescence, and visualization/capture was performed by exposure of the membranes to Amersham Hyperfilm ECL (GE Healthcare Life Sciences, USA). Band intensities were quantified by optical densitometry of developed autoradiographs (Scion Image software, Scion Corporation, Frederick, MD, USA). The results are expressed in arbitrary densitometric units. For evaluation of protein loading, all membranes were stripped and re-blotted with anti-alpha-tubulin primary antibody.

The antibodies against IR, IRS-1, pTyr, p-IR, AMPK, p70S6K and alpha-tubulin were obtained from Santa Cruz Biotechnology (Santa Cruz Biotechnology, CA, USA), and the antibodies against pIRS-1, IRS-2, Akt, pAkt and mTOR were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.5. Statistical analysis

The statistical analysis was performed using the GraphPad Prism statistics software package version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). The data are expressed as means±S.E.M. Comparisons among the groups (control, soy and fish) were performed by one-way ANOVA followed by Tukey's test for multiple comparisons. Food intake measurements after ICV insulin or vehicle were analyzed by Student's t test. A value of P<.05 was considered statistically significant.

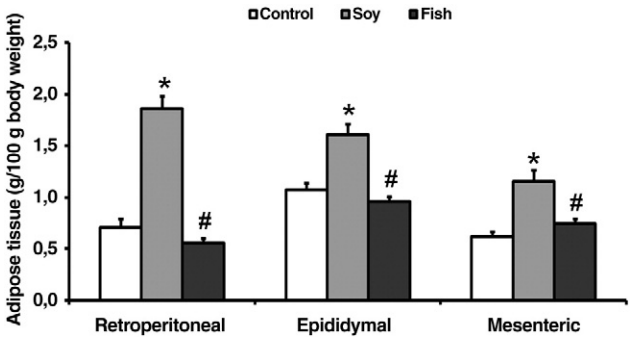

Fig. 3. Adipose depot weights of rats fed control (n=15), soy (n=11) or fish diet (n=12) for 8 weeks. *P<.05 vs. control, #P<.05 soy vs. fish.

Table 2
Serum parameters of the control, soy and fish groups

	Control	Soy	Fish
Glucose (mg/dl)	117.7±3.6 (9)	104.3±3.2[a] (7)	101.3±3.3[a] (8)
Triacylglycerols (mg/dl)	112.9±3.2 (10)	101.8±3.3 (9)	94.5±4.6[a] (9)
Total cholesterol (mg/dl)	120.6±10.3 (10)	98.5±8.8 (12)	77.6±2.1[a] (8)
HDL-c (mg/dl)	57.9±4.7 (10)	56.2±5.9 (12)	52.7±6.0 (9)
LDL-c (mg/dl)	40.1±10.2 (10)	24.7±4.5 (9)	17.1±3.5 (8)
VLDL (mg/dl)	22.5±0.6 (10)	20.3±0.6 (9)	18.9±0.9 (9)
Insulin (ng/ml)	2.1±0.5 (9)	2.5±0.6 (7)	1.9±0.4 (8)
Leptin (ng/ml)	1.5±0.3 (9)	7.2±1.1[a] (7)	2.0±0.2[b] (8)
Corticosterone (ng/ml)	138.5±29.2 (9)	139.4±17.2 (7)	56.1±10.7[a,b] (8)
HOMA	14.8±3.9 (9)	15.2±4.0 (7)	11.3±2.5 (8)

Values in parentheses indicate the number of animals.
[a] P<.05 vs. control.
[b] P<.05 soy vs. fish.

3. Results

3.1. Energy intake, body and fat pad weight, and serum parameters

In comparison to the control group, daily energy intake was higher in the first week of diet treatment in the soy group and in the second and third weeks in the fish group (Fig. 1).

Body weight was lower in the fish group than in both the control and the soy groups throughout the diet treatment period. The soy group also showed higher body weight than the control group in the seventh week (Fig. 2).

The soy group had higher retroperitoneal, epididymal and mesenteric adipose tissue pads than both the control (162%, 49% and 85%, respectively) and the fish (70%, 40% and 35%, respectively) groups (Fig. 3).

Both hyperlipidic groups had significantly lower fasting glucose levels than the control group, while insulin and HOMA index were similar among the groups. The fish group had significantly lower triacylglycerol and total cholesterol levels than the control group. The fish group had lower corticosterone levels than both the control and the soy groups. The soy group had higher leptin levels than the control and the fish groups (Table 2).

3.2. Food intake after ICV insulin

In the control group, the ICV injection of insulin significantly decreased intake of the control diet by 21% in the first 12-h post-injection period and by 19.8% during the entire 24-h period, in comparison with the injection of vehicle. In the soy group, insulin-induced inhibition of food intake failed to reach statistical significance. The observed reductions were 24% in the first 12-h period (P=.11) and 25% in the entire 24-h period (P=.08) after the ICV

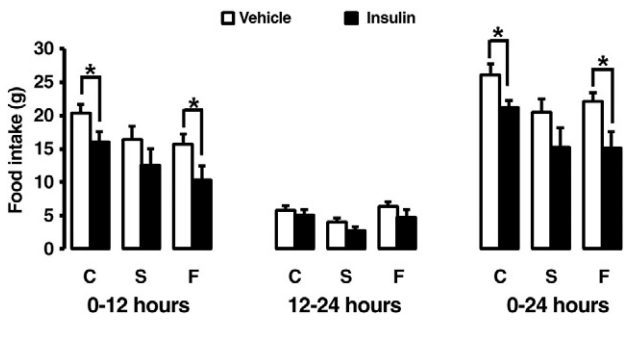

Fig. 4. Food intake of control (n=16), soy (n=11) or fish (n=10) rats during the first and the second 12-h periods and during the entire 24-h period after the ICV injection of saline or 20 mU insulin. Values are means±S.E.M. *P<.05.

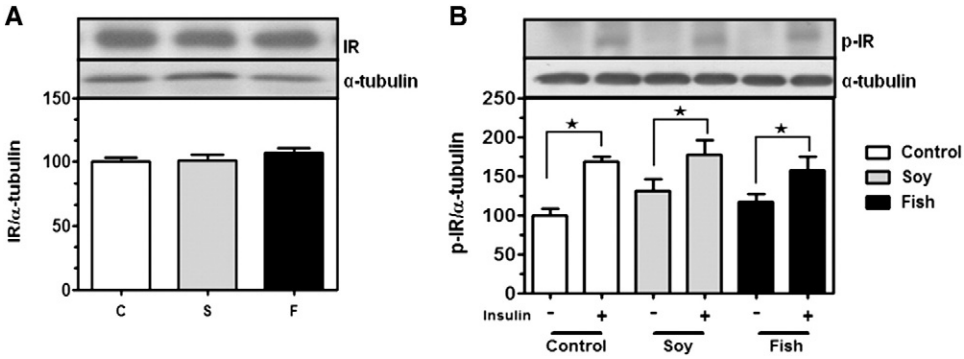

Fig. 5. Protein levels of the insulin receptor (IR) (A) and IR tyrosine phosphorylation (B) in the hypothalamus of control, soy or fish rats treated with an ICV injection of saline (n=8) or 200 mU of insulin (n=12). Values are means±S.E.M. *P<.05.

injection. In the fish group, insulin significantly reduced food intake, by 34% in the first 12-h postinjection period and by 32% during the entire 24-h period (Fig. 4).

3.3. Hypothalamic insulin signaling pathway

IR protein levels were similar among groups (Fig. 5A). Insulin significantly stimulated IR tyrosine phosphorylation in comparison with the respective basal level (vehicle treatment) in control (68%), soy (36%) and fish groups (34%) (Fig. 5B).

IRS-1 and IRS-2 protein levels were similar among groups (Fig. 6A and B). Tyrosine phosphorylation of the pp185 band, which contains IRS-1 and IRS-2 proteins, was significantly stimulated by ICV insulin in the hypothalamus of control (78%) and soy (53%), but not of fish rats (11%, P=.29) (Fig. 6C). Tyrosine phosphorylation of IRS-1 was significantly stimulated by insulin in the hypothalamus of control rats (94%), while it was completely absent in the hyperlipidic groups (Fig. 6D).

Protein levels of Akt were similar among groups (Fig. 7A). Akt serine phosphorylation was significantly stimulated by insulin in the hypothalamus of control (90%) and fish rats (78%), but not in the soy rats (43%, P=.06) (Fig. 7B).

Protein levels of mTOR, p70S6K and AMPK were similar among the groups (Fig. 8A–C).

4. Discussion

High dietary intake of polyunsaturated fats of the n-6 series, such as soy oil, has been shown to induce alterations present in human obesity, while protective effects have been attributed to high intake of n-3 PUFA [2–8]. We have previously demonstrated that hyperlipidic isocaloric diets rich in either soy oil or fish oil had differential consequences on aspects of the hypothalamic control of food intake and energy homeostasis in rats, impairing feeding-induced neuronal activation or serotonin hypophagia, respectively [12,13,16,17]. Since the anorexigenic effect of hypothalamic insulin plays a pivotal part

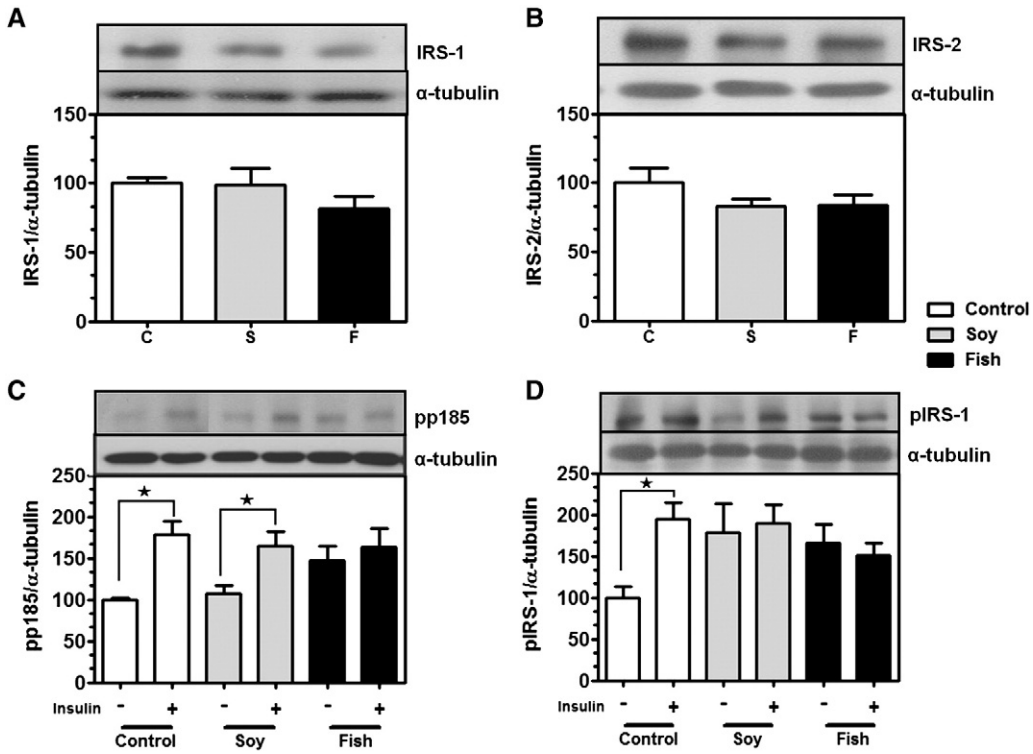

Fig. 6. Protein levels of insulin receptor substrates 1 (IRS-1) and 2 (IRS-2) (A and B), and tyrosine phosphorylation of the pp185 band and IRS-1 (C and D) in the hypothalamus of control, soy or fish rats treated with an ICV injection of saline (n=9) or 200 mU of insulin (n=12). Values are means±S.E.M. *P<.05.

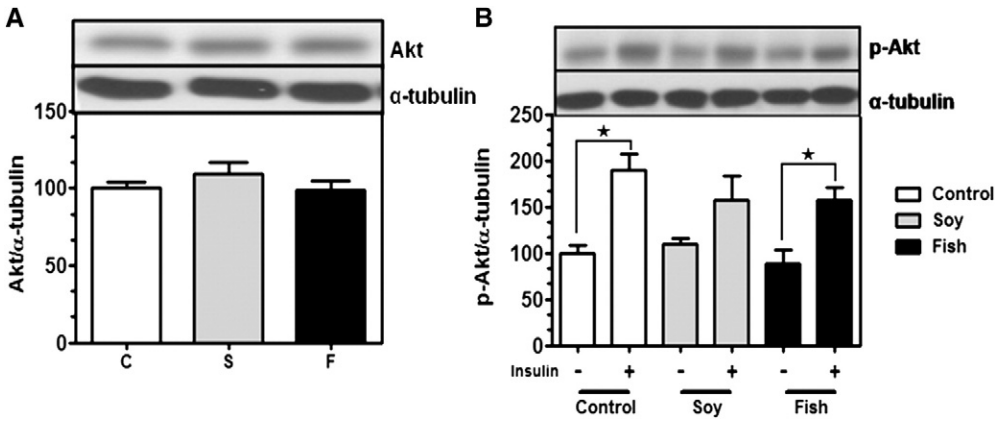

Fig. 7. Protein levels of Akt (A) and Akt serine phosphorylation (B) in the hypothalamus of control, soy or fish rats treated with an ICV injection of saline (n=8) or 200 mU of insulin (n=16). Values are means±S.E.M. *P<.05.

in the maintenance of energy balance [10–13], we herein examined the feeding inhibition induced by centrally injected insulin in rats fed soy oil or fish oil-enriched diets.

We found that the rats fed with the soy diet had only a non-significant hypophagic response to ICV insulin. Examination of the insulin's signaling cascade in the hypothalamus revealed that the initial steps, namely, IR and pp185 phosphorylation, were significantly activated. Since IRS-1 phosphorylation was not stimulated, it is likely that IRS-2 activation was responsible for the observed pp185 band activation [29]. IRS-2 has been shown to be highly expressed in rat hypothalamus and to be an important target of the activated insulin receptor in the mediation of insulin-induced hypophagia, and its stimulation has been suggested to compensate for IRS-1 deficiency [19,30]. Activation of IRS-1 and IRS-2 in the central nervous system has been shown to play an important role in appetite regulation, participating in the signal transmission of the insulin receptor and also influencing leptin receptor signaling. Impairment of IRS activation has been demonstrated to lead to hyperphagia and obesity [10–12,19,20].

However, in the present experiments, Akt serine phosphorylation was absent in the soy group, indicating that a defective transmission of the insulin signal distal to IRSs was responsible for the observed attenuated hypophagic response to the ICV dose of the hormone.

On the other hand, the fish group showed evident Akt activation, consistent with the significant feeding inhibition induced by insulin in this group. It is intriguing that we failed to demonstrate IR substrate activation in the fish group, as indicated by the absence of both pp185 and IRS-1 phosphorylation. It is possible that other IR substrates were involved. The rat brain has been shown to express IRS-4. Although the involvement of this protein in insulin signaling during feeding inhibition has not been demonstrated, the substrate has been shown to bind PI 3-kinase [31–33]. It is reasonable to suggest a role of IRS-4 in insulin signal transmission to Akt in the fish group.

The present findings thus showed divergent responses to ICV insulin between the soy and the fish groups, since only in the soy rats were insulin hypophagia and Akt activation defective. Multiple mechanisms may have contributed to these differences between diet effects. Recently, the mammalian target of rapamycin (mTOR) and p70 ribosomal S6 kinase (p70S6K) proteins have been implicated in insulin resistance. Stimulation of the mTOR/p70S6K pathway has been shown to induce serine phosphorylation of IRS-1/2 which, unlike their tyrosine phosphorylation, blockades the transmission of the insulin signal [34–36]. A role for the mTOR/p70S6K pathway in rats fed PUFA diets has not been defined. In the present study, we failed to find altered mTOR and p70S6K protein levels in the hypothalamus of the high-fat groups. However, these findings may not be taken as completely ruling out an involvement of these proteins on the observed soy diet effects, since the phosphorylation of p70S6K, a key step in pathway activation, has not been examined in the present experiments.

Although numerous recent studies have shown that hypothalamic inflammation induced by saturated diets is a key factor leading to central insulin resistance [11,22–24,37,38], this relationship has not yet been defined for PUFA diets. We have recently found increased hypothalamic levels of inflammatory mediators, such as TRAF6 and NFκBp65, after consumption of soy-rich diet, in agreement with the attenuation of insulin signaling. On the other hand, the fish diet has been found to decrease the levels of the inflammatory proteins TRAF6,

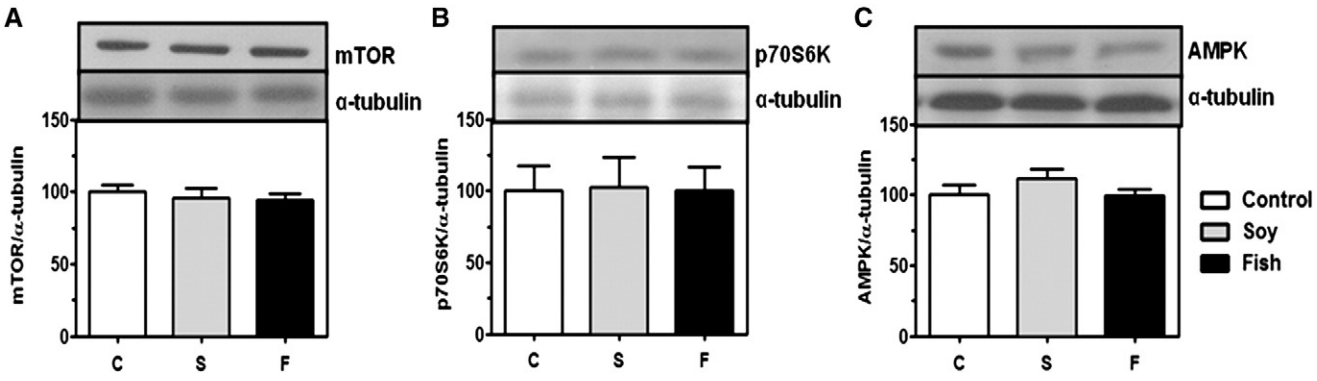

Fig. 8. Protein levels of the mammalian target of rapamycin (mTOR, n=8) (A), p70 ribosomal S6 kinase (p70S6K, n=4) (B) and adenosine monophosphate-activated protein kinase (AMPK, n=8) (C) in the hypothalamus of control, soy or fish rats. Values are means±S.E.M.

TNF-α and IL-6, while the receptor levels of the anti-inflammatory cytokine IL-10 were increased [39].

We have additionally determined the hypothalamic levels of AMP-activated protein kinase (AMPK), an orexigenic factor whose hypothalamic overexpression reportedly led to obesity. However, AMPK levels were not altered by either PUFA diet, similarly to findings in mice with obesity induced by saturated diet [40,41].

The peripheral parameters evaluated in the present study have also pointed to dissimilar effects of the soy and fish diets. Soy rats developed high body adiposity, as evidenced by increased fat pad weight and serum leptin. This suggests leptin resistance, in accordance with studies in obese animals and humans [42,43]. Hyperleptinemia and leptin resistance are frequently associated with central insulin resistance, as we have shown in genetically obese rats, in adult obese rats submitted to intrauterine undernutrition and in rats with obesity induced by high-fat saturated diet [1,19,21,22,24]. Since a positive cross-talk between insulin and leptin signaling pathways has been demonstrated, a likely mechanism contributing to impairment of insulin action in leptin-resistant states is the lack of this interaction [44].

Contrasting with these findings in the soy group, the fish group showed normal fat pad weight and leptin levels and improved blood lipid profile, agreeing with data from human and rodent studies [5–7,45–47]. Additionally, the fish group presented reduced levels of corticosterone, a feature likely to favor insulin sensitivity, since glucocorticoids are known to oppose insulin actions [48]. It is noteworthy that we have previously found high body fat in rats fed fish-oil diet since weaning, indicating that an earlier start on high-fat intake may lead to less favorable outcomes [17].

The fish diet is a rich source of n-3 PUFAs, as we and other authors have demonstrated [12–17] and as confirmed in the present work. Here we showed that, although α-linolenic acid was present in the control and soy diets, the fish diet contained more than 10-fold the amount of n-3 PUFAs, mainly as EPA and DHA, compared to the soy diet. These considerations support the suggestion that the high n-3 density of the fish diet was operant in the determination of the observed effects.

Among the possible mechanisms underlying the fish-diet effects is the reported adipokine modulation, with increased adiponectin and decreased leptin expression [47]. Importantly, n-3 PUFAs have recently been shown to bind specific receptors to decrease obesity-related inflammation and insulin resistance [49].

In summary, the present study demonstrated that rats fed with high-fat diet enriched with soy oil developed increased fat mass and serum leptin. The acute hypophagia in response to ICV insulin was attenuated by the soy diet, and insulin signal transmission was blocked at the level of PKB/Akt. Contrastingly, consumption of high-fat diet enriched with fish oil prevented body adiposity and hyperleptinemia and improved serum lipid profile and corticosterone levels. Insulin hypophagia as well as hypothalamic insulin signaling to PKB/Akt was well preserved. Since only the fish diet is rich in n-3 PUFAs, it is suggested that, rather than the energy density alone, the fat type was an operant aspect of high-fat feeding.

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