Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 935–940
Journal of Nutritional Biochemistry

Effects of dietary fish oil on thyroid hormone signaling in the liver

Luana L. Souza[a], Marcio O. Nunes[a], Gabriela S.M. Paula[a], Aline Cordeiro[a], Vânia Penha-Pinto[b], Jose Firmino N. Neto[b], Karen J. Oliveira[c], Maria das Graças Tavares do Carmo[d], Carmen C. Pazos-Moura[a,*]

[a]Laboratório de Endocrinologia Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS, Bloco G, Rio de Janeiro 21941-902, Brazil
[b]LabLip, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[c]Instituto Biomédico, Universidade Federal Fluminense, Rio de Janeiro, Brazil
[d]Laboratório de Bioquímica Nutricional, Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Received 20 March 2009; received in revised form 6 July 2009; accepted 8 July 2009

Abstract

n−3 polyunsaturated fatty acids (PUFAs) present in fish oil (FO) potently decrease serum lipids, which is also an effect of thyroid hormones. Both PUFAs and thyroid hormones affect hepatic lipid metabolism, and here we hypothesized that a long-term diet rich in *n*−3 PUFAs would enhance thyroid hormone action in the liver. Female rats received isocaloric and normolipid diets containing either soybean oil (SO) or FO during lactation. Male offspring received the same diet as their dams since weaning until sacrifice when they were 11 weeks old. FO group, as compared to SO group, exhibited lower body weight since 5 weeks of age until sacrifice, with no alterations in food ingestion, lower retroperitoneal white fat mass and elevated inguinal fat mass relative to body weight, with unchanged water and lipid but reduced protein percentage in their carcasses. FO diet resulted in lower serum triglycerides and cholesterol. Serum total triiodothyronine, total thyroxine and thyrotropin were similar between groups. However, liver thyroid hormone receptor (TR) β1 protein expression was higher in the FO group and correlated negatively with serum lipids. Liver 5′-deiodinase activity, which converts thyroxine into triiodothyronine, was similar between groups. However, the activity of hepatic mitochondrial glycerophosphate dehydrogenase, the enzyme involved in thermogenesis and a well-characterized target stimulated by T3 via TRβ1, was higher in the FO group, suggesting enhancement of thyroid hormone action. These findings suggest that the increase in thyroid hormone signaling pathways in the liver may be one of the mechanisms by which *n*−3 PUFAs exert part of their effects on lipid metabolism.

Keywords: Thyroid hormone; Fish oil; Liver lipid metabolism; Serum cholesterol; Mitochondrial glycerophosphate dehydrogenase; Thyroid hormone receptor β1

1. Introduction

Epidemiological data have provided clear evidence that fish consumption is associated with a reduced risk of cardiovascular diseases [1], atherosclerosis and hyperlipidemia [2,3]. Moreover, the current trend of consuming diets deficient in *n*−3 polyunsaturated fatty acids (PUFAs) with a high *n*−6:*n*−3 PUFA ratio may contribute to many disease processes [4]. Fish oil (FO), which contains large amounts of long-chain *n*−3 PUFAs, particularly eicosapentaenoic acid [EPA; 20:5(*n*−3)] and docosahexaenoic acid [DHA; 22:6(*n*−3)], has been successfully used in the treatment of hyperlipidemia [2,5,6] and obesity [7]. These highly unsaturated *n*−3 PUFAs from FO have a potent stimulatory effect on fatty acid oxidation and a strong suppressive effect on hepatic lipogenesis and triglyceride synthesis, leading to a decline in plasma triglycerides [8,9]. They also modulate the hepatic metabolism of cholesterol, resulting in an important hypocholesterolemic effect [10,11].

The mechanisms by which long-chain *n*−3 PUFAs exert their effects are only partially understood. It has become clear that these fatty acids have the ability to control the transcriptional activity of nuclear receptors and, thereby, the transcription rate of specific genes related to lipid and carbohydrate metabolism [12]. *n*−3 long-chain PUFAs bind to peroxisome proliferator-activated receptors (PPARs) α, β, γ1 and γ2 [13], and it is accepted that the actions of PUFAs are, in great part, mediated by PPARs [10]. However, PUFAs also bind to other nuclear receptors involved in the control of hepatic lipid metabolism such as liver X receptor α [14], hepatocyte nuclear factor-4 α and γ [15,16], and retinoid X receptor [17]. Another mechanism involved in the actions of PUFAs is control of the nuclear abundance of transcription factors such as sterol receptor element binding protein 1, nuclear factor κB, carbohydrate regulatory element binding protein and Max-like factor X [18–20], which are involved in the regulation of genes of the hepatic metabolism of lipids.

Thyroid hormone receptors (TRs) are ligand-activated transcription factors that regulate gene expression, including that involved in lipid metabolism. An important feature of thyroid hormone deficiency is dyslipidemia, particularly hypercholesterolemia [21,22]. Thyroid hormone acts to increase cholesterol metabolism [23–25] and

* Corresponding author.
E-mail address: cpazosm@biof.ufrj.br (C.C. Pazos-Moura).

0955-2863/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2009.07.008

simultaneously stimulates lipogenesis and fatty acid oxidation, with predominant action on oxidation [26]. In the liver, the β isoform of TRβ is more abundant than the α isoform (TRα) [27]. In addition, studies in TRβ knockout mice and others employing TRβ pharmacological analogs confirm the major importance of TRβ in hepatic lipid metabolism [25,28,29].

Considering the hypolipidemic effect of thyroid hormones and *n*−3 PUFA, together with a previous demonstration of interactions between TR and PPAR pathways in the regulation of lipid metabolism [30–32], we tested, in the present study, the hypothesis that long-term consumption of *n*−3 PUFAs would enhance thyroid hormone action in the liver, and this may be one mechanism contributing to the cholesterol-lowering and triglyceride-lowering effects of *n*−3 PUFAs. In order to test this hypothesis, we evaluated the hepatic expression of TRβ, along with the activity of enzymes that are well-known targets of thyroid hormone action, in a murine model of early and prolonged intake of a diet containing *n*−3 PUFAs from FO.

2. Materials and methods

2.1. Animal care and experimental design

Wistar rats were kept in a room with controlled temperature and artificial dark–light cycle (lights on from 0700 to 1900 h). Our protocol was approved by the institutional animal care committee.

During the entire lactation period, female rats were fed an isoenergetic diet (4.1 kcal/g dry diet) and a normolipid diet containing either 9% soybean oil (SO; rich in *n*−6 PUFA) or 8% FO (rich in *n*−3 PUFAs) plus 1% corn oil as the source of lipids. Within 24 h of delivery, pups were counted and weighed. Postnatal litter size was maintained at six pups per litter, all males if possible. Weaning occurred on Day 21 of lactation; after weaning, pups were fed the same type of diet that was fed to their dams, adjusting the SO to 7% and the FO to 6%. FO (ROPUFA −75; Roche/DSM Nutritional, Indianapolis, IN) contained minima of 42% EPA and 22% DHA. The composition of the diets is shown in Table 1, and the fatty acid composition is listed in Table 2. The diet formulations complied with the general recommendations of the American Institute of Nutrition [33].

After the dams had been weaned, they were excluded from the study, and male pups continued on the diet until the end of the experiment when the animals were 11 weeks old. Four animals were kept in each cage. Body weight and chow ingestion were measured once or twice a week, respectively. Rats were sacrificed by decapitation, and liver, heart, interscapular brown adipose tissue, retroperitoneal white adipose tissue and subcutaneous inguinal white adipose tissue (adhering to the skin in the inguinal region) were excised and weighed. Adipose tissues were returned to the carcasses, which were eviscerated for body composition analysis. Samples of liver tissue were frozen in liquid nitrogen and stored at −70°C prior to extraction of total protein. Serum was obtained from trunk blood and kept frozen at −20°C for measurements of hormones and biochemical parameters.

Table 1
Composition of diets based on SO or FO for dams during lactation and for pups after weaning

Ingredients (%)	Dams		Pups	
	SO	FO	SO	FO
Cornstarch	59.95	59.95	68.07	68.07
Casein	21.0	21.0	15.0	15.0
Cellulose	5.0	5.0	5.0	5.0
Choline bitartrate	0.25	0.25	0.25	0.25
L-Cystine	0.3	0.3	0.18	0.18
Mineral mix[a]	3.5	3.5	3.5	3.5
Vitamin mix[b]	1.0	1.0	0.1	0.1
Butylhydroquinone	0.0014	0.0014	0.0014	0.0014
SO[c]	9.0	–	7.0	–
FO[d]	–	8.0	–	6.0
Corn oil[c]	–	1.0	–	1.0

[a] Mineral mix (g/kg): calcium, 357.0; phosphorus, 250.0; potassium, 74.6; sodium, 74.0; sulfur, 300; magnesium, 24.0; iron, 5.21; copper, 0.3; manganese, 0.63; zinc, 1.65; chromium, 0.27; iodine, 0.01; selenium, 0.01; boron, 0.08; molybdenum, 0.01; silicon, 1.45; nickel, 0.03; lithium, 0.02; vanadium, 0.007 (AIN-93 mineral mix; DYETS 210025; Dyets, Inc., , Bethlehem, PA, USA).

[b] Vitamin mix (g/kg diet): thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; niacin, 3.0; calcium pantothenate, 1.60; folic acid, 0.20; biotin, 0.02; vitamin B12, 2.5; vitamin A palmitate, 0.80 (500.00 IU/g); vitamin E acetate, 15.0 (500 IU/g); vitamin D3, 0.25 (400.00 IU/g); vitamin K1, 0.75 (AIN-93 vitamin mix; DYETS 310025; Dyets, Inc.).

[c] Rich in *n*−6 PUFA (Liza, Brazil).

[d] Rich in *n*−3 PUFA (contained minima of 42% EPA and 22% DHA; Roche/DSM Nutritional).

Table 2
Fatty acid composition of diets based on SO or FO

Fatty acids (%)	SO	FO
C14:0	1.34	1.44
C16:0	14.45	6.90
C18:0	3.71	3.07
C18:1 *n*−9 *trans*	ND	ND
C18:1 *n*−9 *cis*	19.03	9.51
C18:1 other *cis*-isomers[a]	ND	0.38
C20:1	0.52	1.70
C24:1	ND	0.60
C18:2 *n*−6 *trans*	ND	ND
C18:2 *n*−6 (linoleic)	55.87	10.85
C18:3 *n*−3 (α-linolenic)	3.59	1.13
C20:5 *n*−3 (eicosapentaenoic)	ND	35.04
C22:6 *n*−3 (docosahexaenoic)	ND	17.24
Total saturated fatty acid	19.50	11.41
Total monounsaturated fatty acid *cis*	19.55	12.19
Total PUFA *cis*	59.46	64.26
Total *trans*-fatty acid	ND	ND
PUFA:saturated fatty acid	3.04	5.63

ND, not determined.

[a] Includes all positional *cis*-isomers of 18:1, except for 18:1 ω-9.

2.2. Body composition analysis

The procedures followed previously established protocols [34,35], and all reagents were of analytical grade. Eviscerated carcasses were weighed, autoclaved and homogenized in distilled water (1:1). Aliquots of homogenate were used for the measurement of water, protein and fat contents. Water content was determined in 1 g of homogenate that was weighed before and after drying for 72 h at 90°C. Three grams of homogenate was used to determine fat mass gravimetrically [34]. Samples were hydrolyzed in a shaking water bath at 70°C for 2 h with 30% KOH and ethanol. After the addition of 9 M sulfuric acid, total lipids were extracted by three successive washes with petroleum ether (Vetec, Rio de Janeiro, RJ, Brazil). The samples were dried at room temperature until constant weight had been obtained. Protein was extracted from 1 g of homogenate using 0.6 N KOH at 37°C for 1 h. After that, the samples were centrifuged at 800×*g* for 10 min, and the supernatant was collected to measure protein concentration by employing Bradford reagent [35].

2.3. Biochemical parameters

Serum cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were measured using commercial kits (Applied BioSystems, Foster City, CA) following the recommendations of the manufacturer.

2.4. Hormone assays

Serum T_4 and T_3 concentrations were determined by using a radioimmunoassay kit (ICN Pharmaceuticals, Costa Mesa, CA) in accordance with the manufacturer's instructions. Serum thyroid-stimulating hormone (TSH) was measured in 100-μl serum samples in duplicate determination by a specific rat TSH radioimmunoassay using reagents acquired from the National Hormone and Pituitary Program (Torrance, CA), as detailed previously [36]. The within-assay variation was less than 5.3% in all hormone assays. All samples that were compared were measured within the same assay.

2.5. Analysis of TRβ1 liver expression

Liver samples were homogenized in lyses buffer (pH 6.4; 50 mM Hepes, 1 mM $MgCl_2$, 10 nM EDTA and 1% Triton X) with protease inhibitor cocktail complete (Roche/DSM Nutritional). Forty micrograms of total protein per sample was resolved by SDS-PAGE on a 10% gel and transferred onto a polyvinylidene difluoride membrane (Westran; Whatman, Kent, UK). The membrane was blocked with 5% nonfat dry milk (Molico; Nestle, São Paulo, SP, Brazil) and incubated overnight at room temperature with anti-TRβ1 (1:1000 dilution; Upstate Biotechnology, Inc., Lakeplacid, NY) or with internal control anti-cyclophilin B antibody (1:20,000 dilution; Affinity Bioreagents,

Golden, CO). Membranes were then washed and incubated with peroxidase-labeled anti-rabbit IgG antibody (1:15,000 dilution; Amersham Biosciences, Inc., Piscataway, NJ) for 3 h at room temperature. All blots were then washed and incubated with a luminogen detection reagent (ECL; Amersham Biosciences, Inc.) for further exposure on an autoradiograph film (Kodak). Protein bands were evaluated by densitometry using the software Kodak 1D3.5. The membranes were stained with Rouge Ponceau to evaluate the relative amounts of transferred proteins.

2.6. Liver mitochondrial α-glycerophosphate dehydrogenase (mGPD) activity

The enzymatic activity of mGPD in the liver was measured in the mitochondrial fraction by using phenazine methosulfate (PMS; Sigma, St. Louis, MO) as an electron transporter between the reduced enzyme and iodonitrotetrazolium choride violet (INT; Sigma). The assay was performed in the presence of 0.1 M DL-α-glycerophosphate (Sigma) diluted in KCN/potassium phosphate buffer and a solution of 0.12 mM PMS and 7.9 mM INT [37,38]. Samples were analyzed with a spectrophotometer at 500 nm, and the values were expressed as absorbance (OD) per minute per milligram of mitochondrial protein.

2.7. Liver 5′-deiodinase type 1 (D1) activity measurements

D1 activity was measured in liver homogenates by the release of ^{125}I from ^{125}I reverse T_3 (rT_3), as previously reported [39,40]. Liver homogenates were prepared in Tris–HCl buffer (pH 6.8) containing 10 mM DTT and 1 mM EDTA. The assay was performed in duplicate in phosphate buffer containing 1 mM EDTA (pH 6.9) in the presence of 1.5 μM rT_3 and 12.5 mM DTT. Equal volumes of ^{125}I rT_3 (1.07mCi/g specific activity; Perkin Elmer Life Sciences, Boston, MA) purified by paper electrophoresis before each set of assays were added to each assay tube. After 1 h of incubation at 37°C, the reaction was stopped, and free ^{125}I from enzymatic deiodination was eluted from Dowex 50 W-X2 columns with 10% acetic acid. Deiodination percentual in the presence of the enzyme was approximately 20–30%, and the blank was less than 1–2% of total radioactivity in the reaction mixture. Specific enzyme activity was expressed as picomoles of rT_3 deiodinated per hour per milligram of protein.

2.8. Statistical analysis

Data are reported as mean±S.E.M. Student's *t* test was used for comparisons between groups (GraphPad Prism; GraphPad Software, Inc., San Diego, CA). For body weight and chow ingestion, data were analyzed by two-way analysis of variance, followed by Bonferroni posttest. Serum TSH was analyzed by Mann–Whitney test.

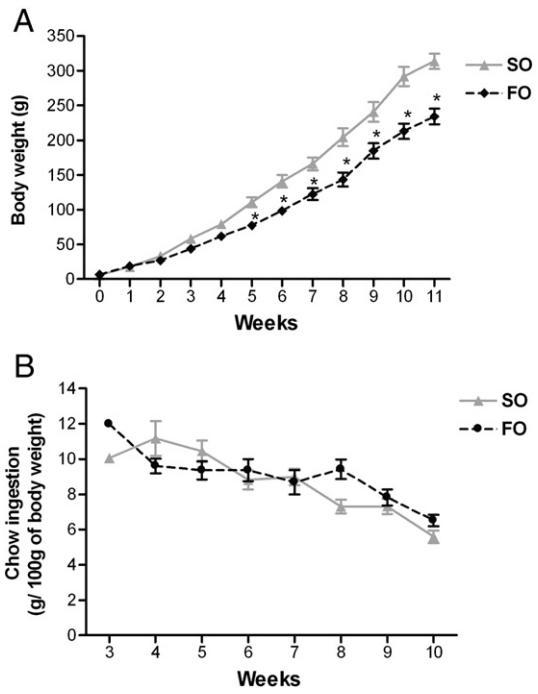

Fig. 1. Body weight (A) and chow ingestion (B) of rats fed diets rich in SO or FO since weaning until 11 weeks of age and whose dams were fed the same diet during lactation. n=8 animals per group. Data are presented as mean±S.E.M. *P<.05.

Table 3
Body composition and weight of tissues of animals fed diets based on SO or FO

Parameter	SO	FO
Weight of tissues		
Heart (mg/mg body weight)	3.04±0.13	3.7±0.21 *
Liver (mg/mg body weight)	35.99±0.57	38.94±1.20 *
Retroperitoneal adipose tissue (mg/mg body weight)	11.22±1.59	6.43±0.67 **
Inguinal adipose tissue (mg/mg body weight)	4.67±0.54	6.71±0.39 **
Brown adipose tissue (mg/mg body weight)	0.98±0.18	1.44±0.17
Body composition		
Water (g/100 g carcass)	41.46±0.18	41.11±0.25
Protein (g/100 g carcass)	27.63±0.66	23.66±0.94 *
Fat (g/100 g carcass)	10.42±0.87	9.79±0.82

Data are presented as mean±S.E.M. n=8 animals per group.
* P<.05.
** P<.01.

Spearman test was used for correlation analysis. Differences were considered significant at P<.05.

3. Results

We compared animals fed normolipid diets enriched in either SO (n−6 PUFA) or FO (n−3 PUFA) from 3 to 11 weeks of age and whose mothers had received the same type of diet during lactation. The FO group presented lower body weight than the SO group starting at 5 weeks of age until sacrifice when the animals were 11 weeks old (25% lower in 11-week-old rats, P<.001; Fig. 1A). The lower body weight of the FO group was not due to reduced food intake, since both groups showed similar chow ingestion from weaning until adulthood (Fig. 1B). As shown in Table 3, in the FO group, heart and liver mass corrected to body weight was 1.2-fold and 1.1-fold higher (P<.05), respectively, although lower in absolute values (heart: SO: 0.96±0.04 g; FO: 0.85±0.03 g; liver: SO: 11.2±0.4 g; FO: 9.1±0.5 g), and the relative mass of brown adipose tissue showed the trend to be higher and showed no changes in absolute values. The mass of the retroperitoneal white adipose depot of FO-fed animals was reduced in absolute (SO: 3.5±0.5 g; FO: 1.5± 0.2 g) and relative (42.7% reduction; P<.01) values. However, the relative mass of inguinal subcutaneous white fat was higher in the FO group (44%; P<.01), although there was no change in absolute values (SO: 1.7±0.3 g; FO: 1.6±0.4 g). Carcass analysis revealed that the FO group had no significant alteration in the relative percentage of fat or water, but exhibited a lower percentage of the protein compartment compared with the SO group (Table 3).

As expected, the FO group exhibited 28.6% and 60.3% lower serum cholesterol and triglycerides, respectively, compared with the SO group (Table 4). HDL was reduced; however, the cholesterol/HDL ratio remained similar between groups.

Serum T_4, T_3 and TSH concentrations were similar in animals fed the FO diet and animals fed the SO diet (Table 4).

We analyzed next the protein expression of TRβ1 in the liver. As depicted in Fig. 2, animals from the FO diet group presented a 24% higher expression of TRβ1 protein in hepatic tissue as compared to animals of the SO diet group (P<.0001). In addition, there was a negative significant correlation between serum triglycerides and the level of TRβ1 expression (r=−.71, P<.05), as well as between serum cholesterol concentrations and the level of TRβ1 expression (r=−.57, P<.05).

In order to investigate whether the higher TRβ1 expression is accompanied by increased TR activation, we evaluated two well-known targets of TRβ1 in the liver: the enzymatic activity of mGPD and the enzymatic activity of 5′-deiodinase (D1), and the results are depicted in Fig. 3A and B, respectively. Although liver D1 activities were similar between the groups, liver mGPD activity was

Table 4
Serum parameters of animals fed diets based on SO or FO

Parameters	SO	FO
Serum lipids		
Triglycerides (mmol/L)	1.24±0.14	0.49±0.04***
Cholesterol (mmol/L)	1.73±0.09	1.24±0.04***
HDL (mmol/L)	0.65±0.05	0.48±0.03*
Total cholesterol:HDL	2.73±0.18	2.62±0.11
Serum hormones		
T_3 (nmol/L)	1.28±0.06	1.24±0.07
T_4 (nmol/L)	57.5±5.1	52.2±5.2
TSH (nmol/L)	0.051±0.009	0.073±0.008

Data are presented as mean±S.E.M. n=8 animals per group.
* P<.05.
*** P<.0001.

significantly higher in the FO group than in the SO group (1.93-fold increase; P<.0001).

4. Discussion

Results of the present study show that the type of dietary fatty acid chronically consumed, since early in life, in normolipid diets affects body weight, serum lipids and thyroid hormone action pathways in the liver of adult rats.

The animals fed the diet containing $n-3$ PUFA (FO) as the main source of lipids, as compared to those receiving diets based on $n-6$ PUFA (SO), presented low blood cholesterol and triglycerides, as expected [41]. In agreement with other reports [42–47], they presented lower body mass and seemed to accumulate less visceral adipose tissue mass, as evaluated by retroperitoneal depot (which reflected alterations in the abdominal fat of these animals). However, the relative mass of subcutaneous depot in the inguinal region was increased. Other studies have shown regional differences in the response of white adipose tissue to diets enriched in $n-3$ PUFA [48], probably reflecting different characteristics of adipose tissue depending on the anatomical site. These results raise the hypothesis that the FO diet may induce a redistribution of fat from visceral depots to subcutaneous depots, which may be metabolically beneficial [49,50]. This also may explain why the percentage of lipid in the eviscerated carcass, which included both types of fat, showed a nonsignificant

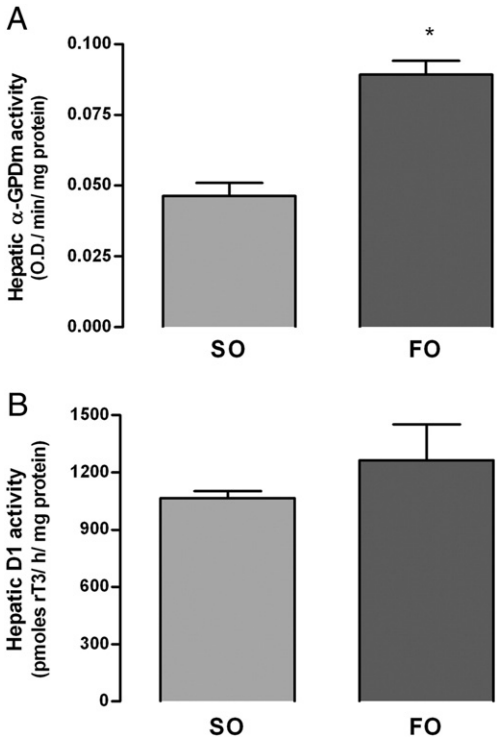

Fig. 3. Liver mGPD activity (A) and liver 5′-deiodinase (D1) activity (B) of animals fed diets rich in SO or FO since weaning until 11 weeks of age and whose dams were fed the same diet during lactation. n=8 rats per group. Data are presented as mean±S.E.M. *P<.0001.

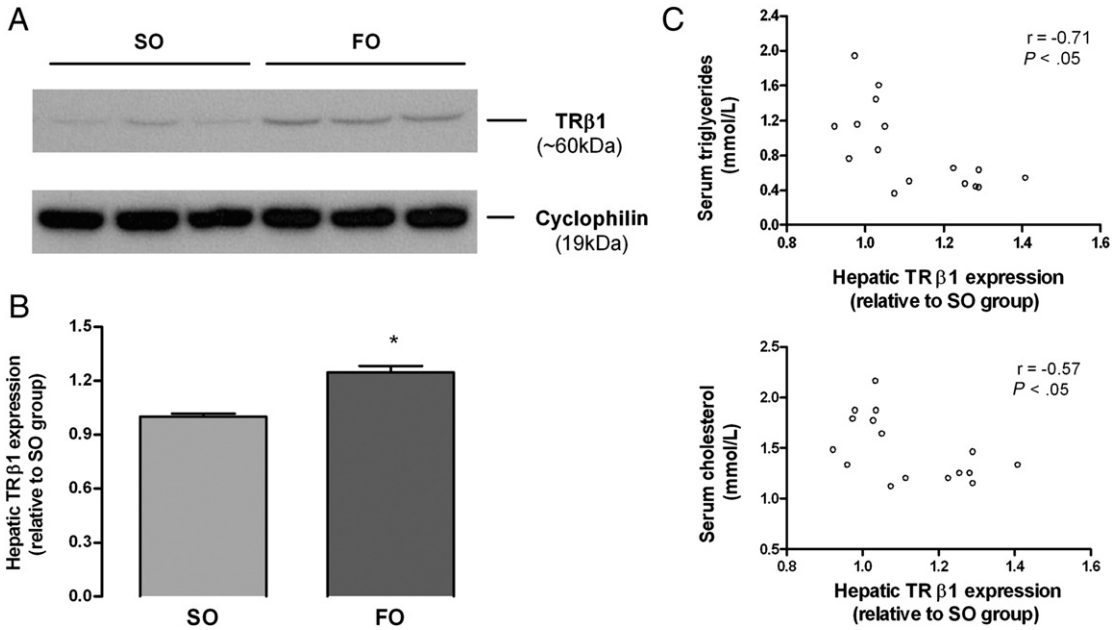

Fig. 2. Liver TRβ1 expression of animals fed diets rich in SO or FO since weaning until 11 weeks of age and whose dams were fed the same diet during lactation. (A) Representative autoradiograph of liver TRβ1 (~60 kDa) and cyclophilin (19 kDa; internal control) analyzed by Western blot analysis. (B) Relative expression of TRβ1 (normalized by cyclophilin). Densitometric values of the FO group relative to those of the SO group. (C) Negative correlations between hepatic TRβ1 expression and serum lipids. Data are presented as the mean±S.E.M. of three independent measurements. n=8 animals per group. *P<.0001.

trend to reduction. On the other hand, the lower percentage of protein in the carcass of the FO group suggests that their reduced body weight was also related to lower skeletal muscle mass. Other studies on the influence of FO diets on body composition employing the carcass method are scarce and controversial [51–53], probably due to many differences in experimental conditions. Decreases [51], increases [53] or no alterations [52,54] in the percentage of lipid and protein content of carcass from animals fed FO diets have been reported.

Thyroid hormones and TSH serum levels remained the same regardless of whether the diet chronically consumed is based on $n-6$ or $n-3$ PUFAs. However, there was a consistently higher abundance of TRβ1 in the liver of the animals fed the FO diet, which potentially may lead to enhancement of thyroid hormone action. The extreme importance of TRβ1 in the ability of T_3 to reduce serum cholesterol was revealed by the demonstration that mice lacking TRβ are insensitive to the cholesterol-lowering effect of T_3, even when they overexpress TRα in the liver [25]. In contrast to our finding, others [55] did not find a significant increase in TRβ expression in the liver of animals that received a high-carbohydrate diet with FO for 3 h/day for 5 days. The differences in experimental conditions may explain the different findings.

In favor of the view that a higher liver TRβ1 expression was accompanied by an enhancement of TR-mediated actions is the finding of a higher activity of liver mGPD, which is a well-characterized T_3 target gene. T_3 acts, via TRβ1, directly at the gene promoter level in order to up-regulate the transcription rate of mGPD [56]. Hypothyroidism is associated with reduced mGPD mRNA expression and enzymatic activity, and the opposite is observed in hyperthyroidism [57]. Therefore, we suggest that, although thyroid hormone levels were not increased in rats fed the FO diet, the higher expression of TRβ1 was responsible, at least in part, for the higher enzyme expression. As far as we know, there has been no previous report on the effect of FO ingestion on the activity of mGPD, although recently, others [58] demonstrated an increase in cytosolic GPD activity in fasted animals fed the FO diet in relation to those fed diets containing saturated fats.

However, the participation of other mechanisms in the FO-induced rise in mGPD activity cannot be excluded. The administration of the PPARα agonist clofibrate has been shown to stimulate mGPD activity in rats [59,60]. However, the magnitude of this response was reduced in hypothyroid animals [60], suggesting a functional interdependence between PPAR and TR signaling pathways in the control of mGPD activity. PPAR and TR interactions in gene regulation, including some genes involved in lipid oxidation and thermogenesis, have been reported [30,31].

Even though the liver 5′-deiodinase is another well-established target for TRβ1 [61], enzyme activities were similar in both groups, suggesting that induction of TRβ1 expression by $n-3$ PUFAs from FO did not result in an overall enhancement of thyroid hormone signaling in the liver; rather, it may be target specific. This seems functionally conceivable since T_3 and PUFAs may act synergically in regard to some effects such those related to oxidative metabolism, but have opposite effects with respect to lipid synthesis [62]. TRβ1 is the major TR responsible for the up-regulation of liver D1 mRNA and activity, even though TRα has also some contributions [63]. To date, we have not verified the expression of TRα.

The higher hepatic mGPD activity in the FO diet group can contribute to the stimulatory effect of $n-3$ PUFAs from FO on oxidative metabolism. mGPD represents a rate-limiting component of the glycerophosphate shuttle, an important metabolic pathway connecting glycolysis with the mitochondrial respiratory chain [56,64]. Mice lacking the mGPD gene exhibited a reduction in oxygen consumption [64], showing the contribution of the enzyme to increasing energy expenditure, which is also an action attributed to long-chain $n-3$ PUFAs from FO. In the present study, in agreement with others [45,53], the reduction in body weight seems to be due to higher energy expenditure, since food ingestion was similar in both diets groups.

In conclusion, the present study shows that normolipidic diets rich in $n-3$ PUFAs from FO started early in life and maintained until adulthood resulted in lower body weight and hypolipidemia associated with a higher expression of TRβ1 in the liver, accompanied by a higher activity of mGPD. These findings suggest that the increase in thyroid hormone signaling in the liver may be one of the mechanisms by which $n-3$ PUFAs exert part of their effects on lipid metabolism.

Acknowledgment

Funding for this work was provided by FAPERJ, CNPq and CAPES.

References

[1] Schmidt EB, Skou HA, Christensen JH, Dyerberg J. *N*-3 fatty acids from fish and coronary artery disease: implications for public health. Public Health Nutr 2000;3:91–8.
[2] Saito Y, Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Ishikawa Y, et al. Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: sub-analysis of primary prevention cases from the Japan EPA Lipid Intervention Study (JELIS). Atherosclerosis 2008;200(1):135–40.
[3] Russo GL. Dietary *n*-6 and *n*-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 2009;77(6):937–46.
[4] Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002;56(8):365–79.
[5] Stalenhoef AF, De Graaf J, Wittekoek ME, Bredie SJ, Demacker PN, Kastelein JJ. The effect of concentrated *n*-3 fatty acids versus gemfibrozil on plasma lipoproteins; low density lipoprotein heterogeneity and oxidizability in patients with hypertriglyceridemia. Atherosclerosis 2000;153(1):129–38.
[6] Chan DC, Watts GF, Mori TA, Barrett PH, Redgrave TG, Beilin LJ. Randomized controlled trial of the effect of *n*-3 fatty acid supplementation on the metabolism of apolipoprotein B-100 and chylomicron remnants in men with visceral obesity. Am J Clin Nutr 2003;77(2):300–7.
[7] Gunnarsdottir I, Tomasson H, Kiely M, Martinéz JA, Bandarra NM, Morais MG, et al. Inclusion of fish or fish oil in weight-loss diets for young adults: effects on blood lipids. Int J Obes (London) 2008;32(7):1105–12.
[8] Willumsen N, Skorve J, Hexeberg S, Rustan AC, Berge RK. The hypotriglyceridemic effect of eicosapentaenoic acid in rats is reflected in increased mitochondrial fatty acid oxidation followed by diminished lipogenesis. Lipids 1993;28(8):683–90.
[9] Ikeda I, Cha JY, Yanagita T, Nakatani N, Oogami K, Imaizumi K, et al. Effects of dietary alpha-linolenic, eicosapentaenoic and docosahexaenoic acids on hepatic lipogenesis and beta-oxidation in rats. Biosci Biotechnol Biochem 1998;62(4):675–80.
[10] Sugiyama E, Ishikawa Y, Li Y, Kagai T, Nobayashi M, Tanaka N, et al. Eicosapentaenoic acid lowers plasma and liver cholesterol levels in the presence of peroxisome proliferators-activated receptor alpha. Life Sci 2008;83(1–2):19–28.
[11] Al-Shurbaji A, Larsson-Backström C, Berglund L, Eggertsen G, Björkhem I. Effect of *n*-3 fatty acids on the key enzymes involved in cholesterol and triglyceride turnover in rat liver. Lipids 1991;26(5):385–9.
[12] Jump DB. *N*-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Curr Opin Lipidol 2008;19:242–7.
[13] Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, et al. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. Mol Cell 1999;3(3):397–403.
[14] Ou J, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, et al. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci USA 2001;98(11):6027–32.
[15] Dhe-Paganon S, Duda K, Iwamoto M, Chi YI, Shoelson SE. Crystal structure of the HNF4 alpha ligand binding domain in complex with endogenous fatty acid ligand. J Biol Chem 2002;277(41):37973–6.
[16] Wisely GB, Miller AB, Davis RG, Thornquest Jr AD, Johnson R, Spitzer T, et al. Hepatocyte nuclear factor 4 is a transcription factor that constitutively binds fatty acids. Structure 2002;10(9):1225–34.
[17] Lengqvist J, Mata De Urquiza A, Bergman AC, Willson TM, Sjövall J, Perlmann T, et al. Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain. Mol Cell Proteomics 2004;3(7):692–703.
[18] Dentin R, Benhamed F, Pégorier JP, Foufelle F, Viollet B, Vaulont S, et al. Polyunsaturated fatty acids suppress glycolytic and lipogenic genes through the inhibition of ChREBP nuclear protein translocation. J Clin Invest 2005;115(10):2843–54.

[19] Jump DB. Fatty acid regulation of gene transcription. Crit Rev Clin Lab Sci 2004;41 (1):41–78.
[20] Xu J, Christian B, Jump DB. Regulation of rat hepatic L-pyruvate kinase promoter composition and activity by glucose, *n*-3 polyunsaturated fatty acids, and peroxisome proliferator-activated receptor-alpha agonist. J Biol Chem 2006;281 (27):18351–62.
[21] Erem C, Değer O, Bostan M, Orem A, Sönmez M, Ulusoy S, et al. Plasma lipoprotein (a) concentrations in hypothyroid, euthyroid and hyperthyroid subjects. Acta Cardiol 1999;54(2):77–81.
[22] Al-Tonsi AA, Abdel-Gayoum AA, Saad M. The secondary dyslipidemia and deranged serum phosphate concentration in thyroid disorders. Exp Mol Pathol 2004;76(2):182–7.
[23] Lopez D, Abisambra Socarrás JF, Bedi M, Ness GC. Activation of the hepatic LDL receptor promoter by thyroid hormone. Biochim Biophys Acta 2007;1771(9): 1216–25.
[24] Ness GC, Pendleton LC, Li YC, Chiang JY. Effect of thyroid hormone on hepatic cholesterol 7 alpha hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysectomized rats. Biochem Biophys Res Commun 1990;172(3):1150–6.
[25] Gullberg H, Rudling M, Saltó C, Forrest D, Angelin B, Vennström B. Requirement for thyroid hormone receptor beta in T_3 regulation of cholesterol metabolism in mice. Mol Endocrinol 2002;16(8):1767–77.
[26] Keyes WG, Heimberg M. Influence of thyroid status on lipid metabolism in the perfused rat liver. J Clin Invest 1979;64(1):182–90.
[27] Oetting A, Yen PM. New insights into thyroid hormone action. Best Pract Res Clin Endocrinol Metab 2007;21(2):193–208.
[28] Gullberg H, Rudling M, Forrest D, Angelin B, Vennström B. Thyroid hormone receptor beta-deficient mice show complete loss of the normal cholesterol 7alpha-hydroxylase (CYP7A) response to thyroid hormone but display enhanced resistance to dietary cholesterol. Mol Endocrinol 2000;14(11):1739–49.
[29] Johansson L, Rudling M, Scanlan TS, Lundåsen T, Webb P, Baxter J, et al. Selective thyroid receptor modulation by GC-1 reduces serum lipids and stimulates steps of reverse cholesterol transport in euthyroid mice. Proc Natl Acad Sci USA 2005;102 (29):10297–302.
[30] Liu YY, Heymann RS, Moatamed F, Schultz JJ, Sobel D, Brent GA. A mutant thyroid hormone receptor alpha antagonizes peroxisome proliferator-activated receptor alpha signaling *in vivo* and impairs fatty acid oxidation. Endocrinology 2007;148 (3):1206–17.
[31] De Lange P, Feola A, Ragni M, Senese R, Moreno M, Lombardi A, et al. Differential 3;5;3′-triiodothyronine-mediated regulation of uncoupling protein 3 transcription: role of fatty acids. Endocrinology 2007;148(8):4064–72.
[32] Bogazzi F, Hudson LD, Nikodem VMA. Novel heterodimerization partner for thyroid hormone receptor. Peroxisome proliferator-activated receptor. J Biol Chem 1994;269(16):11683–6.
[33] Reeves FG, Nielsen FH, Fahey JR. AIN-93 purified diets for laboratory rodents final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. American Institute of Nutrition (AIN), purified rodent diets. J Nutr 1993;123:1939–51.
[34] Stansbie D, Brownsey RW, Crettaz M, Denton RM. Acute effects *in vivo* of anti-insulin serum on rates of fatty acid synthesis and activities of acetyl-coenzyme A carboxylase and pyruvate dehydrogenase in liver and epididymal adipose tissue of fed rats. Biochem J 1976;160(2):413–6.
[35] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
[36] Ortiga-Carvalho TM, Polak J, Mccann S, Pazos-Moura CC. Effect of thyroid hormones son pituitary neuromedin B and possible interaction between thyroid hormones and neuromedin B on thyrotropin secretion. Regul Pept 1996;67(1): 47–53.
[37] Toste FP, Alves SB, Dutra SC, Bonomo IT, Lisboa PC, Moura EG, et al. Temporal evaluation of the thyroid function of rats programmed by leptin treatment on the neonatal period. Horm Metab Res 2006;38(12):827–31.
[38] Lee YP, Lardy HA. Influence of thyroid hormones on L-GPD and other dehydrogenases in various organs of the rat. J Biol Chem 1965;240:1427–36.
[39] Pazos-Moura CC, Moura EG, Dorris ML, Rehnmark S, Melendez L, Silva JE, et al. Effect of iodine deficiency and cold exposure on thyroxine 5′-deiodinase activity in various rat tissues. Am J Physiol 1991;260:E175–82.
[40] Lisboa PC, Oliveira KJ, Cabanelas A, Ortiga-Carvalho TM, Pazos-Moura CC. Acute cold exposure, leptin, and somatostatin analog (octreotide) modulate thyroid 5′-deiodinase activity. Am J Physiol Endocrinol Metab 2003;284(6): E1172–6.
[41] Dulloo AG, Mensi N, Seydoux J, Girardier L. Differential effects of high-fat diets varying in fatty acid composition on the efficiency of lean and fat tissue deposition during weight recovery after low food intake. Metabolism 1995;44(2):273–9.
[42] Hill J, Peters J, Lin D, Yakubu F, Greene H, Swift L. Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. Int J Obes Relat Metab Disord 1993;17(4):223–36.
[43] Hun C, Hasegawa K, Kawabata T, Kato M, Shimokawa T, Kagawa Y. Increased uncoupling protein2 mRNA in white adipose tissue, and decrease in leptin, visceral fat, blood glucose, and cholesterol in KK-Ay mice fed with eicosapentaenoic and docosahexaenoic acids in addition to linoleic acid. Biochem Biophys Res Commun 1999;259:85–90.
[44] Wang H, Storlien LH, Huang XF. Effects of dietary fat types on body fatness; leptin; and ARC leptin receptor; NPY; and AgRP mRNA expression. Am J Physiol Endocrinol Metab 2002;282(6):1352–9.
[45] Duque-Guimarães DE, de Castro J, Martinez-Botas J, Sardinha FL, Ramos MP, Herrera E, do Carmo MD. Early and prolonged intake of partially hydrogenated fat alters the expression of genes in rat adipose tissue. Nutrition 2009 [Epub ahead of print].
[46] Cha MC, Jones PJ. Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats. J Lipid Res 1998;39(8):1655–60.
[47] Pellizzon M, Buison A, Ordiz Jr F, Santa Ana L, Jen KL. Effects of dietary fatty acids and exercise on body-weight regulation and metabolism in rats. Obes Res 2002;10 (9):947–55.
[48] Belzung F, Raclot T, Groscolas R. Fish oil *n*-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am J Physiol 1993;264(6):R1111–8.
[49] Rodríguez A, Catalán V, Gómez-Ambrosi J, Frühbeck G. Visceral and subcutaneous adiposity: are both potential therapeutic targets for tackling the metabolic syndrome? Curr Pharm Des 2007;13(21):2169–75.
[50] Arner P. Regional differences in protein production by human adipose tissue. Biochem Soc Trans 2001;29:72–5.
[51] Gaíva MH, Couto RC, Oyama LM, Couto GE, Silveira VL, Riberio EB, et al. Polyunsaturated fatty acid-rich diets: effect on adipose tissue metabolism in rats. Br J Nutr 2001;86(3):371–7.
[52] Awad AB, Bernardis LL, Fink CS. Failure to demonstrate an effect of dietary fatty acid composition on body weight; body composition and parameters of lipid metabolism in mature rats. J Nutr 1990;120(11):1277–82.
[53] Soriguer F, Moreno F, Rojo-Martínez G, Cardona F, Tinahones F, Gómez-Zumaquero JM, et al. Redistribution of abdominal fat after a period of food restriction in rats is related to the type of dietary fat. Br J Nutr 2003;89(1): 115–22.
[54] Lombardo YB, Chicco AG. Effects of dietary polyunsaturated *n*-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. J Nutr Biochem 2006;17(1):1–13.
[55] Jump DB, Clarke SD, Thelen A, Liimatta M. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J Lipid Res 1994;35(6): 1076–84.
[56] Weitzel JM, Kutz S, Radtke C, Grott S, Seitz HJ. Hormonal regulation of multiple promoters of the rat mitochondrial glycerol-3-phosphate dehydrogenase gene: identification of a complex hormone-response element in the ubiquitous promoter B. Eur J Biochem 2001;268(14):4095–103.
[57] Rauchová H, Zacharová G, Soukup T. Influence of chronically altered thyroid status on the activity of liver mitochondrial glycerol-3-phosphate dehydrogenase in female inbred Lewis rats. Horm Metab Res 2004;36(5): 286–90.
[58] Higuchi T, Shirai N, Saito M, Suzuki H, Kagawa Y. Levels of plasma insulin; leptin and adiponectin; and activities of key enzymes in carbohydrate metabolism in skeletal muscle and liver in fasted ICR mice fed dietary *n*-3 polyunsaturated fatty acids. J Nutr Biochem 2008;19(9):577–86.
[59] Shoemaker RL, Yamazaki RK. Thyroid hormone-independent regulation of mitochondrial glycerol-3-phosphate dehydrogenase by the peroxisome proliferator clofibric acid. Biochem Pharmacol 1991;41(4):652–5.
[60] Pacot C, Charmoillaux M, Goudonnet H, Truchot RC, Latruffe N. Role of thyroid state on induction by ciprofibrate of laurate hydroxylase and peroxisomal enzymes in rat liver microsomes. Biochem Pharmacol 1993;45(7):1437–46.
[61] Zavacki AM, Ying H, Christoffolete MA, Aerts G, So E, Harney JW, et al. Type 1 iodothyronine deiodinase is a sensitive marker of peripheral thyroid status in the mouse. Endocrinology 2005;146(3):1568–75.
[62] Deshpande N, Hulbert AJ. Dietary omega 6 fatty acids and the effects of hyperthyroidism in mice. J Endocrinol 1995;144(3):431–9.
[63] Amma LL, Campos-Barros A, Wang Z, Vennström B, Forrest D. Distinct tissue-specific roles for thyroid hormone receptors beta and alpha1 in regulation of type 1 deiodinase expression. Mol Endocrinol 2001;15(3):467–75.
[64] Alfadda A, Dossantos RA, Stepanyan Z, Marrif H, Silva JE. Mice with deletion of the mitochondrial glycerol-3-phosphate dehydrogenase gene exhibit a thrifty phenotype: effect of gender. Am J Physiol Regul Integr Comp Physiol 2004;287 (1):R147–56.