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ORIGINAL ARTICLE

Fish Oil Normalizes Plasma Glucose Levels and Improves Liver Carbohydrate Metabolism in Rats Fed a Sucrose-Rich Diet

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Abstract A sucrose-rich diet (SRD) induces insulin resistance and dyslipidemia with impaired hepatic glucose production and gluconeogenesis, accompanied by altered post-receptor insulin signaling steps. The aim of this study was to examine the effectiveness of fish oil (FO) to reverse or improve the impaired hepatic glucose metabolism once installed in rats fed 8 months a SRD. In the liver of rats fed SRD in which FO replaced corn-oil during the last 2 months, as dietary fat, several key enzyme activities and metabolites involved in glucose metabolisms (phosphorylation, glycolysis, gluconeogenesis and oxidative and non oxidative glucose pathway) were measured. The protein mass levels of IRS-1 and αp85 PI-3K at basal conditions were also analyzed. FO improved the altered activities of some enzymes involved in the glycolytic and oxidative pathways observed in the liver of SRD fed rats but was unable to restore the impaired capacity of glucose phosphorylation. Moreover, FO reversed the increase in PEPCK and G-6-Pase and reduced the G-6-Pase/GK ratio. Glycogen concentration and GSa activity returned to levels similar to those observed in the liver of the control-fed rats. Besides, FO did not modify the altered protein mass levels of IRS-1 and αp85 PI-3K. Finally, dietary FO was effective in reversing or improving the impaired activities of several key enzymes of hepatic carbohydrate metabolism contributing, at least in part, to the normalization of plasma glucose levels in the SRD-fed rats. However, these positive effects of FO were not observed under basal conditions in the early steps of insulin signaling transduction.

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Abbreviations

AKt	Serine/threonine protein kinase
AMPKα2	AMP activated kinase isoform
C	Cholesterol
CD	High starch diet
ChREBP	Carbohydrate response element binding protein
CO	Corn oil
DAGT	Diacylglycerol acyltransferase
FFA	Unesterified fatty acids
FO	Fish oil
G-6-Pase	Glucose-6-phosphate phosphatase
GK	Glucokinase
Glucose-6-P	Glucose-6-phosphate
Glut2 and Glut4	Glucose transporters 2 and 4
GSa	Glycogen synthase a
HK	Hexokinase
IRS	Insulin receptor substrate
JNK	c-jun N terminal kinase
LXR	Liver X receptor
PDHa	Pyruvate dehydrogenase active form
PDHc	PDH complex
PEPCK	Phosphoenolpyruvate carboxykinase
PI-3K	Phosphoinositide-3 kinase
PK	Pyruvate kinase
PPARα	Peroxisome proliferators activated receptor α
PUFA	Polyunsaturated fatty acid(s)
SRD	Sucrose rich diet
TAG	Triacylglycerol(s)

Introduction

The liver plays a central role in the regulation of glucose metabolism and maintenance of blood glucose homeostasis by regulating glycogen storage and breakdown and gluconeogenesis. It is well known that alterations in the composition of dietary macronutrients (e.g. carbohydrates, lipids, etc.) could deeply and rapidly modify the hepatic glucose metabolism in rats and humans [1–4]. In this regard, several studies have observed reduced insulin suppression of glucose production and hepatic gluconeogenesis, increased capacity for gluconeogenesis, reduced protein mass levels of Glut2 (isolated hepatocytes) and high TAG contents in the liver of rats fed a high sucrose/fructose diet for 1–8 weeks [1, 5–7]. Moreover, sucrose induced the impairment of hepatic insulin action involving multiple post-receptor insulin signaling steps such as tyrosine phosphorylation of insulin receptor substrate proteins-1 and 2 (IRS-1, IRS-2), interaction of phosphoinositide kinase (PI-3K) with IRS protein and phosphorylation of AKt [8]. Recent studies have suggested that the c-jun N terminal kinase (JNK) may also contribute to fructose induced antagonist of insulin signaling in liver [9].

On the other hand, animal studies have demonstrated that the quality of dietary fat is as important as its quantity to induce or prevent insulin resistance [10]. There is accumulated evidence of the beneficial role of n-3 long chain fatty acids (20:5 n-3, 22:6 n-3 PUFA) contained in dietary fish oil on animal and human health, especially concerning their protecting role against the adverse symptoms of the metabolic syndrome [11–13]. It has been shown that dietary fish oil prevents the onset of insulin resistance and dyslipidemia, hepatic steatosis as well as impaired glucose tolerance in rats fed a high-fat or high-sucrose diet [13–15]. However, few studies have analyzed the capacity of dietary fish oil to reverse insulin resistance once installed in rats after a long-term (6 months) feeding of a sucrose-rich diet (SRD). Using this experimental model we have previously demonstrated that partial changes in the dietary fat composition—from corn oil (18% total energy) to fish oil (cod liver oil 16% plus corn oil 2% of total energy)—lead to a normalization of the preexisting state of dyslipidemia, altered glucose homeostasis, whole body peripheral insulin insensitivity and visceral adiposity without detectable changes in plasma insulin levels [11, 16, 17]. However, a study by Podolin et al. [18] found that when the sucrose-rich diet containing a lower amount of menhaden oil (6% total energy) was given to insulin-resistant rats, the insulin action on the glucose metabolism remained impaired. The amount, duration and fatty acid composition of supplemented n-3 PUFA could also contribute to this discrepancy.

N-3 PUFA regulate the expression of a number of genes involved in lipid and carbohydrate metabolism by modulating the activity or expression of several transcription factors [19]. In the liver of rats fed a SRD during 8 months, Hein et al. [20] have recently demonstrated a decrease in the protein mass expression of liver X receptor α (LXRα) while enhancing peroxisome proliferators activated receptor α (PPARα) after shifting the source of dietary fat from corn oil to fish oil during the last 2 months of the experimental period. This was accompanied by a decrease in the activities of hepatic enzymes involved in "de novo" lipogenesis, stimulating fatty acid oxidation and therefore decreasing liver steatosis, hyperlipidemia and normalizing the whole body peripheral insulin resistance produced by the SRD. Among the effects of n-3 PUFA on liver carbohydrate metabolism, Liimatta et al. [21] demonstrated that n-3 PUFA decrease liver pyruvate kinase activity while Jump et al. [22] showed that they suppress mRNA encoding glucokinase. Recently, it was shown that a carbohydrate response element binding protein (ChREBP) is a pivotal transcription factor implicated in the reciprocal regulation of lipogenesis and glycolysis by glucose and n-3 PUFA [23].

To our knowledge, no studies have been reported focusing on the possible beneficial effect of dietary n-3 PUFA to improve/reverse the impaired hepatic glucose metabolism and insulin insensitivity in rats fed a SRD for a long time in which a well-established dyslipidemia and insulin resistance was present at 6 months of diet before the source of dietary fat was partially replaced by fish oil during the following 2 months (6–8 months). Therefore, the aim of this study was to analyze the effect of fish oil on: (i) the enzyme activities and metabolites involved in glucose metabolisms (phosphorylation, glycolysis, gluconeogenesis and oxidative and non oxidative glucose disposal pathways); and (ii) the protein mass levels on the early proximal insulin signaling steps: insulin receptor substrate-1 (IRS-1) and the subunit α p85 of phosphoinositol-3 kinase (PI-3K) in the liver of rats chronically fed a SRD under the experimental conditions described above.

Materials and Methods

Animals and Diets

Male Wistar rats initially weighing 180–190 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained under controlled environmental conditions (temperature 22 ± 1 °C; humidity and air flow condition; 12-h light/dark cycle, light-on 0700 to 1900). After 1-week period of acclimation, the rats were randomly divided into two groups (control

and experimental). The sucrose-rich diet (SRD) group ($n = 72$) received a purified high-sucrose diet containing by weight, 62.5% sucrose, and 8% corn oil (CO). The control group ($n = 36$) received the same semisynthetic diet, but sucrose was replaced by cornstarch (62.5%) [high starch diet (CD)]. The SRD group was fed the diet for 6 months, after which the rats were randomly subdivided into two subgroups. The rats of the first subgroup continued on the SRD up to month 8 of feeding. The second subgroup (SRD + FO) received the SRD in which the source of fat (CO 8 g/100 g) had been replaced by FO (7 g/100 g of cod liver oil, ICN Biomedical, Costa Mesa, CA, plus 1 g/100 g of CO) from month 6 to 8 (Table 1). The control group received the CD during 8 months. The SRD without the addition of FO used for the 2 last months and the CD were balanced for cholesterol and vitamins D and A, present in the FO. Dietary fats were analyzed by capillary GC as previously described [24]. The fatty acid composition of the different fat sources is shown in Table 2. Diets were isoenergetic, providing approximately 16.3 kJ/g of food and were available ad libitum as previously described [20]. The weight of each rat was recorded twice each week over 8 months. In a separate experiment, the individual energy intakes and weight gains of eight rats in each group and subgroup were assessed twice each week. At the end of the 8-month dietary period, experiments were performed before 10:00 h. Rats were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg of body weight). Blood and liver samples were obtained as previously described [20]. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina.

Analytical Methods

Plasma triacylglycerols (TAG), cholesterol (C) free fatty acids (FFA) and glucose levels were measured by spectrophotometric methods as previously described [25, 26]. The immunoreactive insulin assays were calibrated against a rat insulin standard (Novo Nordisk, Copenhagen, Denmark) as previously described [25, 26]. Homogenates of frozen liver powder were used for the determination of TAG, glycogen, glucose-6-P and fructose-2,6-bisphosphate by standard spectrophotometric methods as previously described [25]. Fructose-1-P was assayed by the method of Eggleston L [27].

Enzymes Activity Assays

Hepatic glucokinase (GK) (EC 2.7.1.2) and hexokinase (HK) (EC 2.7.1.1) activities were assayed in homogenates of frozen liver by the spectrophotometric method described by Barzilai and Rossetti [28]. Glucose-6-Phosphate phosphatase (G-6-Pase) (EC 3.1.3.9) activity was assayed by the method described by Harper [29]. The rate of reaction was measured by the increase in inorganic phosphate with time according to the method of Fiske and Subbarow [30]. The extraction of phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) was performed according to Blakely et al. [31], and the determination of PEPCK

Table 1 Composition of control (CD), sucrose-rich (SRD) or SRD + fish oil (FO) experimental diets fed to male Wistar rats

Ingredients	CD (%)		SRD (%)		SRD + FO (%)	
	Weight	Calories	Weight	Calories	Weight	Calories
Casein free vitamin	17.0	17.5	17.0	17.5	17.0	17.5
Salt mix[a]	3.5		3.5		3.5	
Vitamin mix[b]	1.0		1.0		1.0	
Choline chloride	0.2		0.2		0.2	
Methionine	0.3		0.3		0.3	
Cellulose	7.5		7.5		7.5	
Corn-starch	62.5	64.0				
Sucrose			62.5	64.0	62.5	64.0
Corn oil	8.0	18.5	8.0	18.5	1.0	2.3
Fish oil					7.0	16.2

Diets were based on the AIN-93M diet

[a] Salt mix is based on salt mix AIN-93MX (in g/Kg of diet): calcium carbonate, 37.0; potassium phosphate (monobasic) 250.0; sodium chloride, 74.0; potassium sulfate, 46.6; potassium citrate, tri-potassium (monohydrate) 28.0; magnesium oxide, 34.0; ferric citrate, 6.06; zinc carbonate, 1.65; manganese carbonate, 0.63; cupric carbonate, 0.30; potassium iodate, 0.01; sodium selenate, 0.01025; ammonium paramolybdate, 0.00795; chromium potassium sulfate, 0.2174

[b] Vitamin mix is based on vitamin mix AIN-93VX (in g/Kg of diet): niacin 3.00; calcium pantothenate, 1.60; pyridoxine HCl, 0.70; thiamin HCl, 0.60; riboflavin, 0.60; folic acid, 0.20; d-biotin, 0.02; vitamin B-12 (0.1% triturated in mannitol) 2.5; vitamin E (500 IU/g), 15.00; vitamin A (500,000 IU/g) 0.80; vitamin D3 (400,000 IU), 0.25; vitamin K, 0.075

Table 2 Fatty acid composition of the fat source included in each experimental diet

Fatty acids	CO[a]	CO + FO[b]	FO[c]
	g/100 g total fatty acids		
14:0	Tr	4.1	4.7
16:0	10.4	12.8	12.8
16:1 n-7		9.5	10.8
18:0	2.6	2.8	3.0
18:1 n-9	32.1	26.0	25.0
18:2 n-6	51.5	9.0	2.6
18:3 n-3	0.4		Tr
20:0	0.4	0.9	0.9
20:1 n-9	1.6	12.0	13.7
20:4 n-3		3.1	3.5
20:5 n-3		9.0	10.3
22:5 n-3		0.5	0.6
22:6 n-3		8.1	9.3
Total			
Saturated	13.4	20.6	21.4
Monounsaturated	33.7	47.5	49.5
Polyunsaturated			
n-6	51.50	9.00	2.60
n-3	0.40	20.70	23.70
P/S	3.87	1.44	1.23
n-3/n-6	0.008	2.30	9.11

Other minor fatty acids have been excluded

[a] Corn Oil (CO), Mazola (Best Foods Canada Starch, Montreal, Quebec, Canada)

[b] Includes 1% CO plus 7% FO

[c] FO: cod liver oil (ICN Biomedical, Costa Mesa, CA)

activity according to Petrescu et al. [32]. Pyruvate kinase (PK) (EC 2.7.1.40) activity was assayed in homogenates of fresh liver according to Osterman et al. [33]. Glycogen synthase (GSa) (EC 2.4.1.11) activity was determined and the fractional velocity of GSa was calculated as previously described in detail [9]. The extraction and determination of pyruvate dehydrogenase (PDHc) (EC 1.2.4.1) activity from liver tissue has been previously described in detail [34].

Western Blot Analysis of Liver Protein Mass Levels of Insulin Receptor Substrate-1 (IRS-1), αp85 Subunit of Phosphoinositide-3 kinase (PI-3K), Glucokinase (GK) and Hexokinase (HK)

Animals were killed before 10:00 h. To minimize differences in time being euthanized between treatments, three rats were chosen randomly in the three different dietary groups and were killed simultaneously. Frozen liver powder (100 mg) was homogenized and centrifuged at 16,000*g* for 20 min to remove insoluble materials [2] for IRS-1 and αp85 PI-3K. Liver homogenates were prepared as described by Zhang et al. [35] for GK and HK protein mass levels analysis. Total protein samples were resolved on SDS-PAGE and transferred to PVDF membranes. For immunoblotting, the membranes were probed with specified antibodies (rabbit polyclonal antibody anti-IRS-1, anti-αp85, anti-GK or polyclonal goat antibody anti-HK, from Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The blots were then incubated with horseradish peroxidase-linked secondary antibody followed by chemiluminescence detection according to the manufacturer's instructions (Super Signal West Pico chemiluminescence detection, Pierce Biotechnology, Rockford, IL). The protein levels were normalized to β actin. The intensity of the bands was quantified by NIH imaging software. The relationship between the amount of sample subjected to immunoblotting and the signal intensity observed was linear under the conditions described above.

Statistical Analysis

Sample sizes were calculated on the basis of measurements previously made in our laboratory with rats fed either a CD or a SRD [16, 20, 34], considering an 80% power. Results were expressed as means ± SEM. Statistical comparisons were done transversely between different dietary groups. The statistical significance between groups was determined by one-way ANOVA, with one factor (diet) followed by the inspection of all differences between pairs of means by the Newman–Keuls test [36]. Differences having p values lower than 0.05 were considered to be statistically significant. In all cases the interclass correlation coefficients were at least 0.73.

Results

Body-Weight Gain, Energy Intake, Plasma Metabolites and Insulin Levels

Energy intake and body weight were carefully monitored in all groups of rats throughout the experimental period. As we have previously shown [17, 20] a significant increase ($p < 0.05$) in body weight and energy intake occurred in rats fed a SRD for 6 months compared with rats fed a CD (Table 3). These differences in body weight and energy intake were still present in the SRD group when the diet was extended till month 8 of feeding. The presence of FO did not modify the body weight of the SRD-fed rats. However, in spite of a similar energy intake between the SRD and SRD + FO fed rats at month 8, the weight gain was moderately decreased in the latter group (Table 3). The daily individual intake of fatty acids in the three dietary groups was as follows (mean ± SEM, $n = 8$): monounsaturated: CD:

0.455 ± 0.016 g; SRD: 0.565 ± 0.028 g and SRD + FO: 0.743 ± 0.024 g; polyunsaturated: CD: 0.700 ± 0.024 g (0.77% of n-3); SRD: 0.869 ± 0.043 g (0.77% of n-3) and SRD + FO: 0.0466 ± 0.150 g (69.6% of n-3).

In agreement with previous publications [17, 20] plasma levels of TAG, FFA, C and glucose were higher in rats fed the SRD for 8 months compared with the age-matched controls fed a CD (Table 3). Similar values were obtained in rats fed a SRD for 6 months (data not shown). All the variables returned to control values in the SRD-fed rats in which FO replaced corn oil for the last 2 months of feeding. No statistically significant differences in plasma insulin levels were observed at the end of the experimental period among the three dietary groups. Under these experimental conditions, plasma insulin/glucose ratio showed a significant decrease $p < 0.05$ in the SRD fed group compared with both CD and SRD + FO (Table 3). Furthermore, the enhanced liver TAG content in the SRD-fed rats decreased when the diet was switched to SRD + FO (Table 3).

Liver Enzymes Activities and Metabolites Concentration

Table 4 depicts the activities of GK and HK (enzymes involved in glucose phosphorylation); PK (glycolytic pathway); the active form of PDHc (PDHa)—as an estimation of glucose oxidation—, the protein mass levels of GK and HK and the concentration of fructose-1-P and fructose-2,6-bisphosphate in the liver of the three dietary groups at the end of the experimental period.

SRD-fed rats showed a significant decrease ($p < 0.05$) of GK without changes in HK activities and their protein mass levels compared to the CD-fed rats. However, PK and the PDHa activities increased approximately 4 and

Table 3 Body weight, energy intake, plasma metabolites, insulin levels, insulin/glucose ratio and liver TAG content of rats fed a control (CD), sucrose-rich (SRD) or SRD + fish oil (FO)

	CD	SRD	SRD + FO
Body weight at 6 months (g)	403.4 ± 19.8[b]	467.4 ± 11.3[a]	461.7 ± 10.9[a]
Energy intake (initial to month 6) (kJ/d)	272.0 ± 12.5[b]	340.0 ± 12.8[a]	338.7 ± 11.9[a]
Body weight at 8 months (g)	438.5 ± 11.1[b]	506.3 ± 18.8[a]	479.6 ± 6.9[a]
Energy intake (months 6–8) (kJ/d)	275.0 ± 9.4[b]	341.2 ± 17.0[a]	318.8 ± 10.5[a]
Weight gain (g) (months 6–8)	34.6 ± 4.9[a]	33.6 ± 5.1[a]	17.2 ± 6.1[b]
Plasma			
TAG (mM)	0.61 ± 0.06[b]	2.04 ± 0.10[a]	0.78 ± 0.06[b]
FFA (μM)	304.4 ± 17.5[b]	805.5 ± 42.0[a]	328.0 ± 39.6[b]
C (mM)	2.19 ± 0.06[b]	3.30 ± 0.15[a]	2.06 ± 0.11[b]
Glucose (mM)	6.37 ± 0.13[b]	8.24 ± 0.13[a]	6.55 ± 0.19[b]
Insulin (μU/ml)	58.30 ± 4.03	60.10 ± 5.37	52.60 ± 3.26
Insulin/Glucose ratio (μU/μmol)	9.30 ± 0.56[a]	7.03 ± 0.54[b]	8.43 ± 0.30[a]
Liver			
TAG (μmol/g wet weight)	12.60 ± 0.64[b]	22.70 ± 2.00[a]	11.10 ± 0.43[b]

Values are expressed as means ± SEM; $n = 8$. Values in each lane that do not share the same superscript letter are significantly different ($p < 0.05$) when one variable at a time was compared by the Newman–Keuls test

Table 4 Glucokinase, hexokinase, pyruvate kinase and pyruvate dehydrogenase activities, glucokinase and hexokinase protein mass levels and metabolites concentration in the liver of rats fed a control (CD), sucrose-rich (SRD) or SRD + fish oil (FO)

	CD	SRD	SRD + FO
Enzyme activities			
Glucokinase (mU/mg protein)	7.24 ± 0.42[a]	5.14 ± 0.38[b]	5.15 ± 0.41[b]
Hexokinase (mU/mg protein)	2.09 ± 0.08	1.82 ± 0.12	1.97 ± 0.07
Pyruvate Kinase (mU/mg protein)	69.63 ± 3.41[c]	305.25 ± 4.15[a]	153.42 ± 8.81[b]
Pyruvate Dehydrogenase active form (% of total PDH complex)	35.02 ± 1.61[b]	50.55 ± 3.04[a]	36.33 ± 1.54[b]
Protein mass levels			
Glucokinase (% of control)	100.0 ± 2.8	103.0 ± 7.1	98.6 ± 6.1
Hexokinase (% of control)	100.0 ± 3.1	91.3 ± 5.7	90.3 ± 11.6
Metabolites			
Fructose-1-P (μmol/g wet tissue)	0.27 ± 0.02[b]	0.53 ± 0.07[a]	0.33 ± 0.03[b]
Fructose-2,6-bisphosphate (μmol/g wet tissue)	10.66 ± 0.26	9.40 ± 0.57	10.57 ± 0.62

Values are expressed as means ± SEM; $n = 6$. Values in each lane that do not share the same superscript letter are significantly different ($p < 0.05$) when one variable at a time was compared by the Newman–Keuls test

1.4-fold, respectively compared to those fed a CD. Besides, a twofold increase in fructose-1-P without changes in fructose-2,6-bisphosphate concentration was recorded in the SRD fed rats. Dietary fish oil did not modify the behavior of both HK and GK activities. The latter stayed similarly lower than those observed in the SRD fed rats. Interestingly, no statistically significant differences in the protein mass levels of GK and HK were observed in the liver of SRD + FO rats (Table 4).

On the other hand, under FO administration, the PK activity significantly decreased ($p < 0.05$) while both the activity of PDHa and fructose-1-P contents reached values similar to those recorded in the CD group. FO did not induce changes in fructose-2,6-bisphosphate levels.

Table 5 shows the activities of key enzymes related to glycogen synthesis, gluconeogenesis and glucose homeostasis and the concentration of glycogen and glucose-6-P in the liver of the three dietary groups. Glycogen synthase (GSa)—expressed as a percentage of the fractional activity—as well as glycogen levels were significantly increased in the SRD-fed rats. A reduction in both GSa activity and glycogen content was observed when the source of fat in the SRD (corn oil) was partially replaced by FO. However, while the glycogen content returned to values similar to those recorded in the CD group, the GSa activity was still significantly higher than that in the CD-fed rats. No changes in liver glucose-6-P concentration were observed among all dietary groups.

In addition, Table 5 depicts that the liver of the SRD-fed rats showed a significant increase in the activities of two enzymes involved in the gluconeogenesis pathway: PEPCK and G-6-Pase. Both enzyme activities reached values similar to those observed in the CD-fed rats in the group of rats fed a SRD + FO.

On the other hand, the terminal step of glucose release from the liver is a function of the activities of G-6-Pase and GK. The addition of FO that normalizes G-6-Pase activity was able to significantly decrease G-6-Pase/GK ratio. Values were as follows (means ± SEM, $n = 6$) 13.45 ± 2.1 for CD, 27.24 ± 3.2 for SRD and 19.81 ± 2.3 for SRD + FO; $p < 0.05$ CD vs SRD and SRD + FO; $p < 0.05$ SRD vs SRD + FO.

Protein Mass Levels of IRS-1 and αp85 PI-3K

The immunoblotting of liver tissue revealed a single 175 kDa band consistent with IRS-1 and 85 kDa bands for αp85 PI-3K. Each gel contained an equal number of samples from the CD, SRD and SRD + FO groups (Fig. 1a). After densitometry of immunoblots, both the IRS-1 and αp85 PI-3K of the CD group were normalized to 100% and the levels of IRS-1 and αp85 PI-3K from SRD and SRD + FO were expressed relative to this. The qualitative and quantitative analysis of the Western blot showed that the relative abundance of IRS-1 and αp85 PI-3K were significantly decreased ($p < 0.05$) in the liver of the SRD group when compared with rats fed a CD (Fig. 1b). The addition of FO to the diet of the SRD-fed rats did not induce any changes in either IRS-1 or αp85 PI-3K; values were similar to those recorded in the SRD-fed rats.

Discussion

The main purpose of this work was to explore whether dietary FO can improve or reverse the altered hepatic glucose metabolism and insulin resistance induced in rats by the chronic administration of a sucrose-rich diet, to investigate the key enzymatic steps and early proximal insulin signaling responsible for these changes. The new major findings in the present study are as follows: (i) The significant increase in PK and PDHa activities, key enzymes of the glycolytic and oxidative glucose pathways, as well as fructose-1-P level observed in the liver of the SRD-fed rats were significantly improved or normalized, reaching values similar to those of the CD-fed group in which FO replaced corn oil as a dietary fat during the last 2 months of the experimental period (8 months). However, FO was unable to restore the altered capacity of glucose phosphorylation (decreased GK activity) present in the

Table 5 Glycogen and glucose-6-phosphate concentration and glycogen synthase, glucose-6-phosphate phosphatase and phosphoenolpyruvate carboxykinase activities in the liver of rats fed a control (CD), sucrose-rich (SRD) or SRD + fish oil (FO)

	CD	SRD	SRD + FO
Metabolites and enzyme activities			
Glycogen (μmol/g wet tissue)	239.3 ± 22.3[b]	390.4 ± 25.0[a]	292.0 ± 30.8[b]
Glucose-6-phosphate μmol/g wet tissue)	0.23 ± 0.02	0.20 ± 0.01	0.22 ± 0.02
Glycogen Synthase (% of fractional activity)	13.16 ± 1.16[b]	18.50 ± 0.50[a]	15.70 ± 1.55[ab]
Glucose-6-P-phosphatase (mU/mg protein)	97.4 ± 15.4[b]	140.0 ± 6.1[a]	102.0 ± 11.5[b]
Phosphoenolpyruvate Carboxykinase (mU/mg protein)	2.29 ± 0.17[b]	3.21 ± 0.20[a]	2.49 ± 0.17[b]

Values are expressed as means ± SEM; $n = 6$. Values in each lane that do not share the same superscript letter are significantly different ($p < 0.05$) when one variable at a time was compared by the Newman–Keuls test

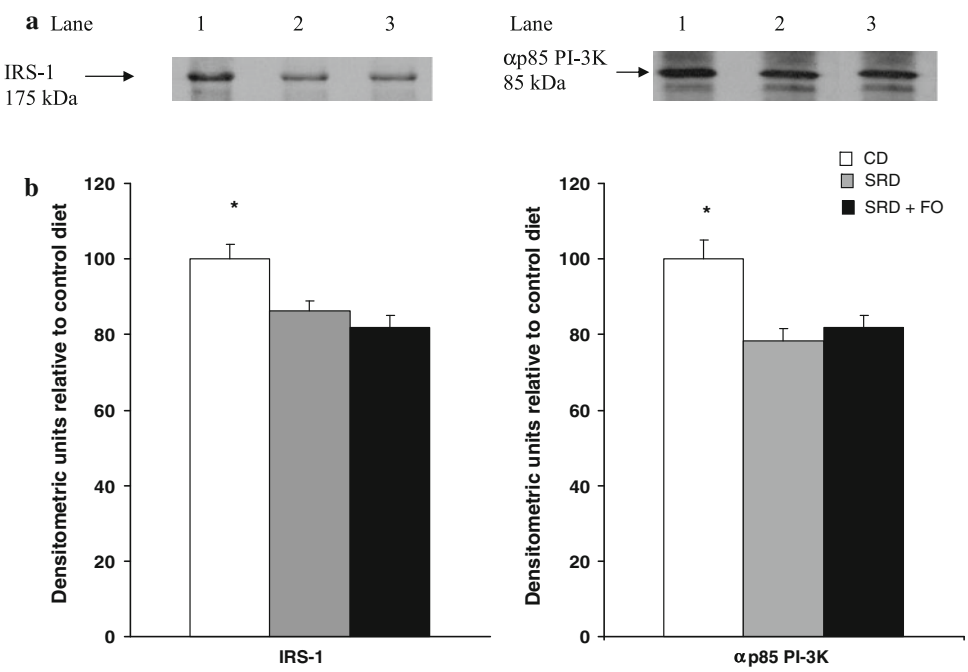

Fig. 1 Liver protein mass levels of IRS-1 and αp85 PI-3K of rats fed a control (CD), sucrose-rich (SRD) or a SRD + fish oil (FO). **a** Immunoblots of liver IRS-1 and αp85 PI-3K from CD, SRD and SRD + FO. Molecular marker is shown on the right. *Lane 1* CD, *lane 2* SRD and *lane 3* SRD + FO. **b** Densitometric immunoblot analysis of IRS-1 and αp85 PI-3K protein mass in liver tissue of rats fed a CD, SRD or SRD + FO. Values are expressed as means ± SEM ($n = 6$) and expressed as percentage relative to control diet in both IRS-1 and αp85 PI-3K. *$p < 0.05$ CD versus SRD and SRD + FO

liver of the SRD-fed rats. (ii) FO was able to reverse the increased activities of PEPCK and G-6-Pase, enzymes involved in the gluconeogenesis pathway. FO significantly reduced the G-6-Pase/GK ratio, which led to decreased hepatic glucose output. In addition, increased glycogen concentration and GSa activity returned to levels similar to those recorded in the CD-fed rats. (iii) Dietary FO did not modify the altered protein mass levels of IRS-1 and the subunit of α p85 PI-3K.

Glucose release from hepatocytes is a function of the enzymatic activities of GK and G-6-Pase. The present work shows a decrease in GK and conversely, an increase in G-6-Pase activities in the SRD-fed rats. This could in part contribute to losing control of the hepatic glucose output. Interestingly, at that time, the reduction in the hepatic GK activity was not accompanied by changes in their protein mass level. Although we did not evaluate the GK translocation in the present study, Shin et al. [37] in ZDF-rats recently demonstrated a lack of GK translocation from the nucleus to the cytoplasm altered expression of the enzyme leading to an inadequate endogenous glucose production and glucose uptake. When FO replaced corn oil as a source of fat in the SRD, no further changes in the already decreased GK activity and protein mass level were observed. However, in mice fed 2–5% of n-3 PUFA in vivo and in vitro Dentin et al. [23] showed that hepatic GK gene expression is inhibited decreasing the amount of total GK protein content and activity. They observed that the suppressive effect of n-3 PUFA on the gene expression of GK and liver PK is caused by an alteration of ChREBP nuclear translocation. On the other hand, FO decreases the activity and mRNA of G-6-Pase in rats fed a high-fat diet [38]. The present data shows that the increase in G-6-Pase activity in the liver of rats fed a SRD was significantly decreased when FO replaced corn oil in this diet. Therefore, although glucose phosphorylation is still impaired in the SRD + FO group, an inhibiting effect of FO on the G-6-Pase activity leading to a decreased G-6-Pase/GK ratio could improve the altered hepatic glucose homeostasis present in the SRD-fed rats.

Several studies showed that the hepatic disposal of a large amount of fructose increased the liver PK and PEPCK activities and expression as well as the PDHc activity [31, 39–41]. The current study in which rats were fed a SRD for an extended period (8 months) shows a substantial increase in PK, PEPCK and G-6-Pase activities that leads to an elevated hepatic glucose production. Moreover, the glucose oxidation estimated by PDHc activity as well as fructose-1-P concentration was also increased without changes in the fructose-2,6-bisphosphate level. No change in the latter metabolite was observed in sucrose-fed rats by Sommercorn et al. [42]. The present data shows that FO oil was able to normalize the hepatic fructose-1-P level, and decrease the PK activity thus decreasing the flux through pyruvate and in turn diminishing the PDHc activity that reached values similar to those of the CD-fed group. A decrease in both hepatic PK activity and gene expression was observed by Higuchi et al. [43] in mice fed dietary fish oil compared to those fed a lard diet. Dietary fish oil significantly decreased the PEPCK activity reaching values similar to those recorded in the CD fed group. Neschen et al. [44] showed that FO decreased PEPCK mRNA when

compared with safflower oil. On the other hand, FO significantly reduced the high plasma FFA levels measured in the SRD-fed rats, since fatty acids provide fuel for gluconeogenesis, thus a reduction in the hepatic availability of these metabolites could contribute, at least in part, to the normalization of this metabolic pathway.

On the other hand, we recently demonstrated [20] that under the same experimental conditions, FO was able to inhibit hepatic lipogenesis through the up regulation of PPARα and down regulation of LXRα and normalized liver TAG content.

Gergely et al. [45] showed that fructose-1-P stimulates glycogen synthesis in normal rats intravenously injected with fructose. Moreover, fructose-1-P concentration inhibited glycogen phosphorylase and regulated glycogen accumulation when fructose delivery was increased [5]. Similarly, our results show an increase in glycogen synthase activity, and both glycogen and fructose-1-P contents in the liver of the SRD-fed rats. Dietary FO decreased all these parameters which reached values similar to those of the CD group.

Hepatic changes in the early steps of insulin signaling transduction may play a role in the insulin resistance induced by high sucrose or fructose diet. Under basal conditions Bezerra et al. [2] reported no differences in IR and IRS-1 protein levels in both liver and muscle of rats fed a high fructose diet for a short time (4 weeks). However, after insulin stimulation, they demonstrated a significant reduction in tyrosine phosphorylation of IR and IRS-1 phosphorylation levels in both tissues. A reduction in IRS-1/PI-3kinase association was also observed in liver and muscle. Besides, in rats fed a high sucrose diet for 5 weeks, Wei et al. [9] showed that hepatic insulin resistance was mediated in part via activation of the JNK activity through changes in serine phosphorylation of IRS-1. Our present results extend those described by Bezerra et al. [2] since when the SRD was administered for 8 months instead of 4 weeks, a reduction in liver protein mass levels of IRS-1

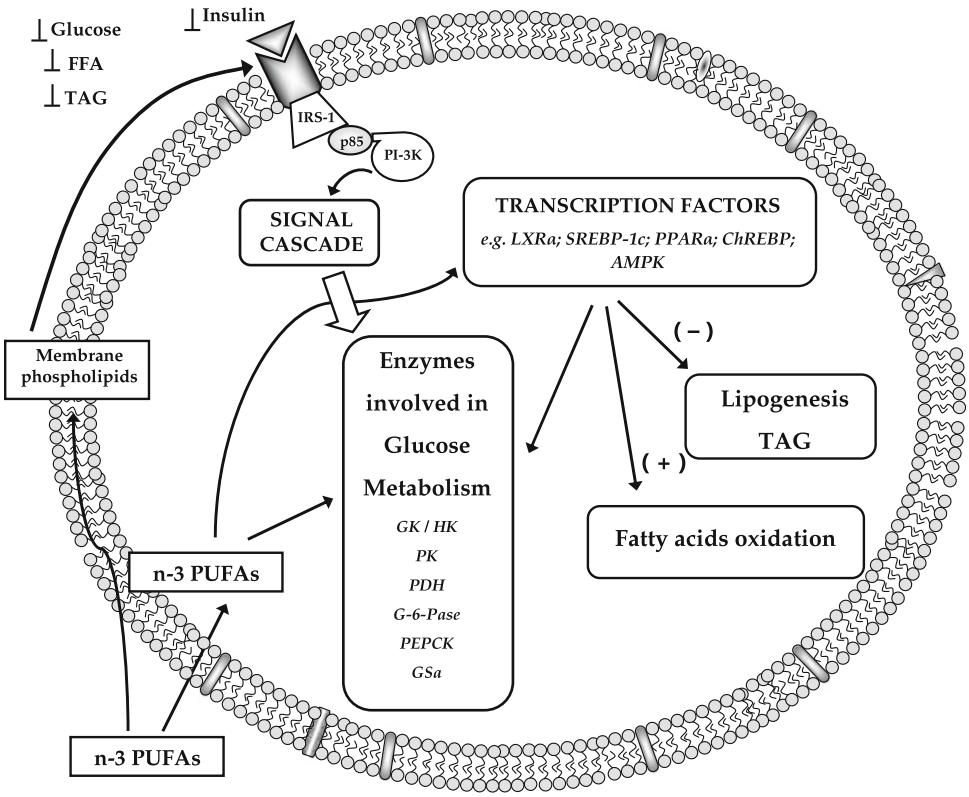

Fig. 2 Integrated effect of fish oil (FO) on liver glucose and lipid metabolism in the SRD-fed rats. FO regulates glucose and lipid metabolism through changes in the activity or abundance of different transcription factors families (e.g. PPARs, SREBPs, LXRs, ChREBP). In the SRD-fed rats FO enhanced hepatic fatty acid oxidation and decreased lipogenesis, leading to normalization of liver and plasma TAG levels, thus contributing to its hypolipemic effects. Several liver key enzymes activities and metabolites involved in hepatic glucose metabolism were improved. FO reversed the increased activities of PEPCK and G-6-Pase and reduced the G-6-Pase/GK ratio which leads to reduce hepatic glucose output but was unable to restore the impaired capacity of glucose phosphorylation. FO restored glucose oxidation and GSa activity. Besides, FO did not modify protein mass levels of IRS-1 and αp85 PI-3K involved on the early steps of insulin signaling transduction. FO normalized glucose homeostasis without changes in circulating insulin levels

and α p85 PI-3K was observed just under basal conditions, without the stimulus of insulin. The replacement of corn oil by FO did not improve the decreased protein mass levels of IRS-1 and α p85 PI-3K. This was a particularly unexpected result, since FO normalizes both the altered plasma basal insulin/glucose ratio and hyperglycemia without changes in insulinemia. Moreover, as previously demonstrated [11], it reverts the low plasma adiponectin levels and insulin resistance (euglycemic–hyperinsulinemic clamp), thus normalizing whole body peripheral insulin sensitivity. Klimes et al. [46] showed no changes in insulin binding and autophosphorylation of the insulin receptor beta subunit under basal conditions and/or after insulin stimulation from the liver of rats fed a SRD or SRD plus FO diet for 3 weeks. However, FO improved insulin-stimulated tyrosine kinase activity and insulin action. On the other hand, a decrease in PI-3K activity in the liver and muscle with an increase in adipose tissue was observed by Corporeau et al. [47] in rats fed a low amount of FO into a normolipidic diet. Taouis et al**.** [14] showed that a high fat diet enriched in n-3 PUFA in rats maintained IR, IRS-1 tyrosine phosphorylation, PI-3K activity and total Glut4 in muscle but not in liver. Figure 2 depicts the effect of n-3 PUFA on liver glucose metabolism in the SRD fed rats.

Finally, dietary FO was effective in reversing or improving the impaired activities of several key enzymes of hepatic carbohydrate metabolism contributing at least in part to the normalization of plasma glucose levels in the experimental model of dyslipidemia and insulin resistance. FO reversed liver steatosis and dyslipidemia. However, these positive effects of FO were not observed under basal conditions in the early steps of insulin signaling transduction. Further studies are needed in order to clarify this matter.

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