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Dietary fish oil normalized glucose-stimulated insulin secretion in isolated pancreatic islets of dyslipemic rats through mechanisms involving glucose phosphorylation, peroxisome proliferator-activated receptor γ and uncoupling protein 2 ☆

M.R. Ferreira, A. Chicco, Y.B. Lombardo *

Department of Biochemistry, School of Biochemistry, University of Litoral. Ciudad Universitaria Paraje El Pozo CC 242 (3000) Santa Fe, Argentina

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ABSTRACT

This study evaluates some possible mechanisms behind the beneficial effects of dietary fish oil (FO) on β cell dysfunction in rats fed a sucrose-rich diet (SRD). Rats were fed a SRD for 6 months. Thereafter, half the rats received a SRD in which corn oil was partially replaced by FO up to 8 months. The other half continued consuming the SRD up to 8 months. A control group was fed a control diet throughout the experimental period. In isolated islets of SRD-fed rats dietary FO normalized the reduced glucose phosphorylation, the altered glucose oxidation, the triglyceride content, the increased protein mass levels of peroxisome proliferator-activated receptor γ (PPARγ) and uncoupling protein 2 without changes in GLUT2 and PPARα. These finding suggest that the changes mentioned above could be involved in the normalization of the altered glucose-stimulated insulin secretion pattern in this nutritional model of dyslipidemia and insulin resistance.

1. Introduction

Impairment of glucose-stimulated insulin secretion (GSIS) is an early failure of type 2 diabetes. It is known that the chronic exposure to high levels of both glucose and fatty acids impairs GSIS "in vivo" and "in vitro" and could negatively influence β cell activity through the so called glucolipotoxicity leading to β cell dysfunction. Several mechanisms have been proposed which could contribute to the β cell failure. Among others, we could mention the following: changes in glucose phosphorylation and/or oxidation, oxidative stress, ceramide biosynthesis, down regulation of several genes including glucokinase (GK), GLUT2, peroxisome proliferator-activated receptor α (PPAR α) or stimulation of sterol regulatory element-binding protein 1-c (SREBP1-c), PPAR γ, uncoupling protein 2 (UCP2) (See Poitout et al. [1] for a review of the literature).

Pancreatic β cells are somewhat more vulnerable to lipid spillover, with limited compensatory fatty acid oxidation as the sole means of disposing of excess fatty acids [2]. The plasma levels of nutrient metabolites vary with dietary composition (e. g type and quantities of fatty acids, carbohydrates, etc). Therefore, the feeding behavior plays an important role in the control of the β cell function [3]. Different studies have demonstrated the beneficial effects of n-3 PUFAs (mainly EPA: 20:5 n-3 and DHA: 22:6 n-3) in preventing ectopic lipid storage in non-adipose tissues (lipotoxicity), which affects organ functions [4,5]. These polyunsaturated fatty acids have the ability to regulate cell metabolism through multiple mechanisms [6]. However, their effects on modulate insulin secretion and islets function still remain unclear. In this regard, Storlien et al. [7] demonstrated that replacement of only 6% of fatty acids with long chain n-3 PUFAs from fish oil prevented the development of insulin resistance in rats fed a high saturated fat diet while Ghafoorunissa et al. [8] reported that dietary n-3 PUFAs prevent the high sucrose diet induced IR. In rats fed a high fat diet, Holness et al. [9] showed that the acute replacement of 7% of dietary fatty acids with long-chain n-3 PUFAs reversed insulin hypersecretion "in vivo". The effect of long term saturated fat feeding to enhance insulin secretion by perifused islets was also completely reversed. Dietary administration of EPA to KK-Ay mice, -a model of obesity and type 2 diabetes-ameliorated impairment of insulin secretion in their islets [10].

Recent studies by Kato et al. [10] showed that incubation of islets from C57BL/6 mice with palmitate caused inhibition of

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* Corresponding author. Tel.: +54 342 457 5211; fax: +54 342 457 5221.
E-mail address: ylombard@fbcb.unl.edu.ar (Y.B. Lombardo).

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glucose and potassium stimulated insulin secretion, but the addition of EPA restored both inhibitions. Palmitate activated and EPA abolished both mRNA and nuclear protein levels of SREBP1-c accompanied by reciprocal changes of SREBP1-c target genes such as IRS-2 and granuphilin. UCP2 was also modulated by palmitate and palmitate-EPA in a similar manner at both mRNA and protein levels [10].

Increased inflammation is also one of the major factors that lead to the development of insulin resistance (IR). Wei et al. [11] recently showed that stable cellular production of n-3 PUFAs and reduction of n-6 to n-3 ratio via mfat-1 transgenic mouse model enhanced insulin secretion and conferred strong resistance to cytokine-induced β cell dysfunction.

Our laboratory has recently demonstrated an altered GSIS from isolated islets of Langerhans in rats chronically fed (6–8 months) a sucrose-rich diet (SRD)—an experimental model of dyslipidemia, IR, visceral adiposity and increase of fat storage in non-adipose tissues (e.g. liver, skeletal and heart muscle) [4]. In these animals, a significant decrease of both GK activity and its protein mass levels was accompanied by an altered glucose oxidation (decreased pyruvate dehydrogenase complex: PDHc activity) and a significant increase of triglyceride content within the β cells. Furthermore, both PPAR γ and UCP2 protein mass levels were increased, suggesting that glucolipotoxicity could be at least one possible cellular mechanism contributing to the β cell dysfunction [12]. Interestingly, Pighin et al. [13] demonstrated that in rats fed a SRD for a long term (8–9 months) the partial replacement of corn oil by fish oil (FO) during the last two months on the diet reversed IR, abnormal glucose homeostasis and dyslipidemia and completely normalized both the fat storage and the PDHc activity within the β cell as well as insulin secretion patterns stimulated by glucose.

From the above findings, we considered that it would be worthwhile to study some of the underlying mechanisms involved in the possible beneficial effect of dietary FO upon the β cell dysfunction induced by feeding rats a long-term (6 months) SRD. To achieve this goal, in isolated islets we analyzed the effect of FO administration on: (1) glucose uptake through the protein mass level of GLUT2, phosphorylation and oxidation estimated by the enzymatic activities and protein mass levels of GK and hexokinase (HK) and PDHc activity, respectively. (2) The protein mass levels of: UCP2, PPAR γ—a nuclear receptor that promotes uptake with the subsequent storage of lipid and regulates UCP2- and PPAR α that modulates insulin secretion and in turn regulates the transcription of genes involved in the lipid metabolism. Besides, the triglyceride content within the β cell was also quantified.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats initially weighing 180 to 190 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained under controlled temperature (22 °C ± 1), humidity, and airflow conditions, with a fixed 12-hour light-dark cycle (light, 0700 h–1900 h). They were initially fed a standard non-purified diet (Ralston Purina, St Louis, MO). After 1 week of acclimatization, they were randomly divided into 2 groups: control and experimental. The experimental group received a sucrose-rich diet (SRD) containing by weight (g/100 g): 62.5 sucrose, 8 corn oil CO). The control group received the same semisynthetic diet but with sucrose replaced by corn starch (62.5%, high-starch diet, control diet (CD)). Details of the diet composition are shown in Table 1. The experimental protocol was approved by the Human and Animal Research Investigation Committee of the School of Biochemistry, University of Litoral (Santa Fe, Argentina).

Table 1
Composition of experimental diets[a].

Diet Ingredients	Control diet (CD)		Sucrose-rich diet (SRD)		SRD+fish oil (SRD +FO)	
	% by weight	% of calories	% by weight	% of calories	% by weight	% of calories
Casein free vitamin	17.0	17.5	17.0	17.5	17.0	17.5
Salt mix[b]	3.5		3.5		3.5	
Vitamin mix[c]	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

[a] The composition of experimental diets are based on AIN-93M diet.
[b] Salt mix is based on salt mix AIN-93MX (in g/kg of mix): calcium carbonate, 357.0; potassium phosphate (monobasic) 250.0; sodium chloride, 74.0; potassium sulfate, 46.6; potassium citrate, tri-potassium (monohydrate) 28.0; magnesium oxide, 24.0; 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, 0.275.
[c] Vitamin mix is based on vitamin mix AIN-93VX (in g/kg of mix): 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, 2.5 (0.1% in mannitol); vitamin E (500 IU/g), 15.00; vitamin A (500,000 IU/g) 0.80; vitamin D3 (400,000 IU/g), 0.25; vitamin K, 0.075.

2.2. Experimental design

Rats in the experimental group received the SRD for 6 months, period after which the animals were randomly divided into 2 subgroups. The first subgroup continued on the SRD up to 8 months. The second subgroup (SRD+FO) received a SRD in which the source of fat (CO 8% w/w) had been partially replaced by fish oil (FO) (7% of cod liver oil containing approximately 850 U/g vitamin A and 85 U/g vitamin D and 0.6 mg/g cholesterol) plus CO 1% w/w from months 6 to 8. The control group received the CD throughout the experimental 8-month period. The SRD without the addition of FO used from month 6 to 8 and the CD were balanced for cholesterol and vitamins D and A present in the FO. Diets were isoenergetic (16.3 kJ/g of food) and were available ad libitum. Diets were prepared every day by adding the oils to the base mixture containing the other nutrients. The oils and base mixture were separately stored at 4 °C until preparation of the diet. FO was kept under nitrogen atmosphere during storage. The fatty acid composition of the fat source was analyzed by capillary GC, as previously described [14]. Details of the dietary fatty acid composition of each experimental diet are shown in Table 2. Rats were housed in individual cages and the weight of each animal was recorded twice a week throughout the experimental period. In a separate experiment, the individual caloric intake and weight gain of 8 animals in each group and subgroup were assessed twice a week. At the end of the experimental period, food was removed at the end of the dark period and experiments were performed before 1000 h.

2.3. Analytical methods

Rats from each dietary group were decapitated. The blood samples were collected in tubes containing sodium EDTA as anticoagulant, rapidly centrifuged at 3500 × *g* for 15 min at 4 °C and the plasma was either immediately assayed or stored at −20 °C and examined within 3 days. Plasma triglycerides [15], free fatty acids [16] and glucose levels [17] were determined by

spectrophotometric methods. The immunoreactive insulin assay was calibrated against a rat insulin standard (Novo, Nordisk, Copenhagen, Denmark) and their plasma levels were measured by the method of Herbert et al. [18]. The liver was rapidly removed, frozen, clamped in liquid nitrogen and stored at −80 °C. The homogenates of frozen liver powder were used for the determination of triglyceride content [15]. Pancreases were rapidly removed from all rats and the islets were isolated by collagenase digestion (Sigma, St. Louis, MO) and collected under a stereoscopic microscope [19]. Triglyceride content and the activity of the pyruvate dehydrogenase complex (PDHc) in the isolated islets were determined according to the methodology described by Briaud et al.[20] and Zhou et al. [21], respectively. Glucokinase (GK) and hexokinase (HK) were measured in the citosolic fraction of isolated islets by fluorometric assay [22,23] as the conversion of NAD^+ to NADH by exogenous glucose-6-phosphate dehydrogenase. More details of the methodologies have been described elsewhere [12,13].

2.4. Perifusion of isolated islets

After the islets were washed twice with a Krebs-Ringer bicarbonate (KRB) buffer, groups of 30 to 40 islets isolated from each rat were loaded in a 13-mm chamber containing a 5 μm nylon membrane filter. Islets were perifused with KRB containing 3 mmol/L glucose, 250 mg/L essentially fatty acid—free bovine serum albumin (Sigma), 40 mg/L dextran 70 (Sigma), $pH = 7.4$ at 37 °C (constantly gassed with 95% O_2, 5% CO_2) at a flow rate of 0.9–1.2 mL/min. After a prewash period of 30 min, two basal samples were obtained. Then, the KRB containing 16.5 mmol/L of glucose was used until the end of the perifusion period. Aliquots of the effluent were collected at 1 min intervals until 15 min, and then at 5 min intervals until 40 min and were stored at −20 °C until insulin analysis. More details of the methodology used have been described elsewhere [23,13].

2.5. Western blot analysis of pancreatic islets protein mass levels of glucokinase (GK), hexokinase (HK) and glucose transporter 2 (GLUT2)

Protein extracts from isolated islets were performed as previously described by Shao et al. [24] to quantify GK, HK and GLUT2 protein mass levels. Total protein samples were resolved on SDS-PAGE according to Laemmli using 9% polyacrylamyde gel for GK and HK and 10% polyacrylamyde gel for GLUT2. For immunoblotting, proteins in the SDS-PAGE gel were transferred to polyvinyldifluoride (PVDF) membranes (Amersham Biosciences, Piscataway, NJ). The membranes were probed with specific antibodies (rabbit polyclonal antibody anti-GK, goat polyclonal anti-HK or rabbit polyclonal anti-GLUT 2,, 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). β actin (Sigma) was used as a loading control. The intensity of the bands was quantified by the National Institute of Health (Bethedsa, MD) imaging software. The linearity of Western blot analysis was from 20 to 100 μg of protein and the relationship between the amount of sample subjected to immunoblotting and the signal intensity observed was linear under the conditions described above. The correlation between the amount of protein and the enhanced chemiluminiscence image intensity was 0.94 for GK, 0.97 for HK and 0.96 for GLUT2.

Table 2
Fatty acid composition of the experimental diets (% w/w).

Fatty acids	CD and SRD[a]	SRD+Fish oil[b]
14:0	traces	0.328
16:0	0.832	1.000
16:1 n-7		0.760
18:0	0.208	0.236
18:1 n-9	2.568	2.071
18:2 n-6	4.120	0.697
18:3 n-3	0.032	
20:0	0.032	0.067
20:1 n-9	0.128	0.975
20:4 n-3		0.245
20:5 n-3		0.720
22:5 n-3		0.042
22:6 n-3		0.651
Total		
Saturated	1.072	1.633
Monounsaturated	2.696	3.806
Polyunsaturated		
n-6	4.120	0.697
n-3	0.032	1.658
P/S	3.870	1.444
n-3/n-6	0.008	2.378

Other minor fatty acids have been excluded.

[a] CD and SRD: includes Corn Oil (Mazola, Best Foods Canada Starch, Montreal, Quebec, Canada).

[b] SRD+Fish oil: includes 1% Corn oil plus 7% Fish oil. (Fish oil: cod liver oil, ICN Biomedical, Costa Mesa, CA).

2.6. Western blot analysis of pancreatic islets protein mass levels of peroxisome proliferator-activated receptor gamma and alpha (PPAR γ, PPAR α) and uncoupling protein 2 (UCP2)

Protein extracts from lysed pancreatic islets were used for quantification of the protein mass levels of PPAR γ or PPAR α [25]. Islet homogenates were used for quantification of the protein mass levels of UCP2 as previously described [26]. Total protein samples were resolved on SDS-PAGE according to Laemmli (10%, 12% or 14% polyacrylamyde gel for PPAR γ, PPAR α and UCP2 respectively). Proteins in the SDS-PAGE gel were transferred to PVDF membranes (Amersham Biosciences). For immunoblotting, the membranes were probed with specific antibodies (rabbit polyclonal antibody anti-PPAR γ and PPAR α and goat polyclonal anti-UCP2, from Santa Cruz Biotechnology, Inc.). 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). β actin (Sigma) was used as a loading control. The intensity of the bands was quantified by the National Institute of Health (Bethedsa, MD) imaging software. The relationship between the amount of sample subjected to immunoblotting and the signal intensity observed was linear under the conditions described above. The linearity of Western blot assay was from 20 to 100 μg of protein, and the correlation coefficients were 0.93, 0.95 and 0.92 for PPAR γ, PPAR α and UCP2, respectively.

2.7. 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 [6,12,13,27] considering an 80% power. Results were expressed as mean ± SEM. Statistical comparisons were done transversely between different dietary groups at the end of the experimental study. 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

Table 3
Body weight, energy intake, plasma metabolites, insulin levels, insulin/glucose ratio and liver triglyceride content of rats fed a control (CD), a sucrose-rich (SRD) or a SRD +fish oil (SRD+FO) diet[1].

		CD	SRD	SRD+FO
Initial body weight (g)	(8)	190.0 ± 3.4	187.1 ± 4.3	191.4 ± 3.2
Body weight at 6 months (g)	(8)	417.4 ± 9.9^{b}	484.1 ± 10.8^{a}	476.2 ± 9.0^{a}
Body weight at 8 months (g)	(8)	448.9 ± 8.6^{b}	517.4 ± 15.8^{a}	492.0 ± 7.5^{a}
Weight gain (g) (months 6–8)	(8)	33.3 ± 4.9^{a}	32.2 ± 4.8^{a}	15.9 ± 4.4^{b}
Energy intake (Initial to 6 months) (kJ/d)	(8)	282.3 ± 16.0^{b}	352.0 ± 15.2^{a}	326.6 ± 8.3^{a}
Energy intake (months 6–8) (kJ/d)	(8)	278.5 ± 7.6^{b}	347.6 ± 13.3^{a}	317.6 ± 7.3^{a}
PLASMA				
Triglyceride (mM)	(6)	0.89 ± 0.07^{b}	2.60 ± 0.16^{a}	1.00 ± 0.07^{b}
FFA (μM)	(6)	290.0 ± 17.8^{b}	822.6 ± 37.0^{a}	317.0 ± 44.3^{b}
Glucose (mM)	(6)	6.47 ± 0.31^{b}	8.25 ± 0.20^{a}	6.60 ± 0.35^{b}
Insulin (pM)	(6)	389 ± 57	442 ± 76	378 ± 33
Insulin/ Glucose ratio (μU/μmol)	(6)	8.72 ± 0.64^{a}	7.33 ± 0.40^{b}	8.24 ± 0.30^{a}
LIVER				
Triglyceride (μmol/g wet weight)	(6)	12.60 ± 0.64^{b}	22.70 ± 2.00^{a}	11.10 ± 0.43^{b}

[1] Values are expressed as mean ± SEM, ( ) number of rats. Values in a line 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.

means by the Newman-Keuls' test [28]. Differences having P values lower than 0.05 were considered to be statistically significant (SPSS 15.0 for Windows, SPSS INC. Chicago, Illinois). All reported P values are two-sided.

3. Results

3.1. Body weight gain, energy intake, plasma metabolites and insulin levels, liver triglyceride content and glucose-stimulated insulin secretion from isolated islets.

Body weight gain and energy intake were carefully monitored in all groups of rats throughout the experimental period. In agreement with previous publications [13,29], the increase in body weight (16%) and energy intake recorded in rats chronically fed a SRD for 6 month was still present when the SRD was fed for 8 months ($P < 0.05$) (Table 3). When FO replaced CO in the SRD from month 6 to 8, a moderate reduction of weight gain without significant changes in energy intake was observed when compared with SRD and CD groups. In agreement with previous publications [13,29], at the end of the experimental period, basal plasma triglyceride, FFA and glucose levels were significantly higher in the SRD-fed rats compared with age-matched controls fed a CD (Table 3). Similar values were obtained from rats fed a SRD during 6 months (data not shown). The presence of FO as the principal source of dietary fat in the SRD normalized all the above parameters. No statistically significant differences in plasma insulin levels were observed at the end of the experimental period between the three dietary groups (Table 3). Under the experimental conditions, plasma insulin/glucose ratio showed a significant decrease ($P < 0.05$) in the SRD group compared with the CD and SRD+FO groups. Furthermore, in the SRD-fed rats the enhanced triglyceride content within the liver was completely normalized after FO administration (Table 3). As previously reported, rats fed the CD showed the classic biphasic pattern of glucose (16.5 mM) stimulated insulin secretion. Perifused islets from SRD-fed rats showed an alteration of the biphasic pattern with an absence of the first peak and an increase in the second phase of hormone secretion compared with CD-fed rats. On the other hand, in the presence of FO in the SRD group, the glucose-induced insulin secretion showed a pattern comparable with that of CD-fed rats (Fig. 1).

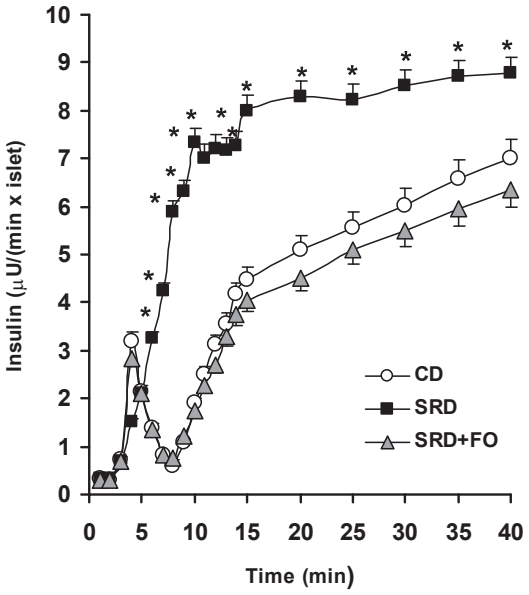

Fig. 1. Insulin secretion under the stimulus of 16.5 mM glucose in perifused pancreatic islets of rats fed a control (CD), a sucrose-rich (SRD) or a SRD+fish oil (SRD+FO) diet. Values are mean ± SEM, $n = 6$. *$P < 0.05$ SRD vs CD and SRD+FO at each time point.

3.2. Enzyme activities, protein mass levels of glucokinase (GK), hexokinase (HK) and GLUT2 and triglyceride content in isolated islets.

The effect of FO on the activity of the key enzymes of glucose phosphorylation (GK and HK) as well as their protein mass levels is depicted in Table 4. FO was able to normalize the reduced activity of GK recorded in the SRD-fed rats as well as the enhanced HK/GK ratio (the levels of HK activity did not change within the experimental groups, data no shown). This was accompanied by an increase of the GK protein mass levels which reached values similar to those shown in the CD-fed group. Besides, no changes in the protein mass level of HK and the glucose transporter GLUT2 were observed in the three dietary groups.

In agreement with previous results [12], the triglyceride content within the islets increased 2.5 fold in the SRD-fed rats and returned to control values after FO administration. Furthermore, the active form of PDHc (PDHa), which was significantly decreased

Table 4
Glucokinase, hexokinase, and pyruvate dehydrogenase activities, GLUT 2, glucokinase and hexokinase protein mass levels and triglyceride content, in isolated islets of rats fed a control (CD), a sucrose-rich (SRD) or a SRD+fish oil (SRD+FO) diet[1].

	CD	SRD	SRD+FO
Enzyme activities			
Glucokinase (pkat/μg protein)	0.064 ± 0.004^{a}	0.043 ± 0.005^{b}	0.063 ± 0.007^{a}
Hexokinase/Glucokinase ratio	3.48 ± 0.14^{b}	5.47 ± 0.64^{a}	2.96 ± 0.30^{b}
Pyruvate dehydrogenase complex PDHc (pkat/islet)	0.142 ± 0.007	0.165 ± 0.009	0.135 ± 0.012
PDH active form (% of total PDH complex)	70.0 ± 5.3^{a}	38.0 ± 3.6^{b}	72.0 ± 4.0^{a}
Protein mass levels (% of control)			
GLUT 2	100.0 ± 3.2	96.0 ± 4.5	96.0 ± 6.0
Glucokinase	100.0 ± 3.8^{a}	76.5 ± 4.3^{b}	94.9 ± 4.5^{a}
Hexokinase	100.0 ± 2.3	93.0 ± 3.3	90.0 ± 9.0
Metabolite			
Triglyceride (ng/islet)	67.5 ± 6.23^{b}	173.5 ± 14.0^{a}	88.8 ± 18.0^{b}

[1] Values are expressed as mean ± SEM, $n=6$. Values in a line 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.

in the SRD-fed rats, reached values similar to those observed in the CD-fed group when FO replaced CO during the last 2 months of the experimental period without changes in total PDHc activity (Table 4).

3.3. Protein mass levels of PPAR γ,PPAR α and UCP2 in isolated islets

To examine whether or not FO feeding could alter the protein mass of PPAR γ, PPAR α and UCP2, the protein mass levels of these transcription factors and UCP2 were measured in isolated islets of rats fed a SRD and SRD+FO. The immunoblots of isolated islets revealed a single 67 kDa band consistent with PPAR γ, 56 kDa band for PPAR α and 29 kDa band for UCP2. Each gel contained equal number of samples from CD, SRD and SRD+FO (Fig. 2A–C, upper panel). After densitometry of immunoblots the PPAR γ, PPAR α and UCP2 of the CD group were normalized to 100% and the level of PPAR γ, PPAR α and UCP2 of both SRD and SRD+FO groups were expressed relative to this. The qualitative and quantitative analysis of the Western blot showed that the relative abundance of PPAR γ and UCP2 was significantly higher ($P < 0.05$) in the isolated islets of the SRD group when compared with rats fed the CD (Fig. 2A and C lower panel). The addition of FO to the SRD during the last 2 months of the experimental period significantly decreased ($P < 0.05$) the protein mass levels of both PPAR γ and UCP2, which reached values similar to those recorded in the CD-fed rats. No changes in the PPAR α protein mass levels were observed in isolated islets among the three dietary groups.

4. Discussion

The long-term exposure to fatty acids in the presence of hyperglycemia impairs insulin release and leads to β cell dysfunction. The present study provides new information on some of the potential mechanisms behind the beneficial effects of dietary FO on the reversion/improvement of β cell dysfunction, which develops in rats rendered dyslipemic and insulin resistant by chronically (8 months) feeding them with a sucrose-rich diet. Expanding our previous research, the major new findings arising from these investigations are the following: in the isolated islets of SRD fed rats (1) the addition of FO normalized both the observed reduced GK activity and the protein mass level. The activity of HK and the relative abundance of protein mass level of both HK and GLUT2 that remained within the control value in rats fed a SRD were not modified when FO replaced CO as a dietary fat. (2) FO was able to normalize the increased protein mass level of PPAR γ and UCP2 recorded in the rats fed a SRD without significant changes in the relative abundance of protein mass level of PPAR α. In addition, and confirming previous results, the presence of FO was able to both normalize glucose oxidation (estimated by the activity of PDH complex) and the triglyceride content within the islets and correct the impaired GSIS from isolated islets induced by a high-sucrose diet. All the changes mentioned above were achieved by shifting the source of fat in the SRD from CO to FO during the last two months of the experimental period.

Pancreatic islets from rats fed a SRD were chronically exposed to a metabolic milieu characterized by moderate hyperglycemia and dyslipidemia (oversupply of triglyceride and FFA). This feeding behavior plays an important role in the control of the β cell function. In this regard, confirming and extending previous findings [12], the present work shows that the substitution of starch by sucrose in the diet unfavorably affected β cell functions such as glucose phosphorylation and GSIS, among others, from isolated pancreatic islets.

Recent studies from animal models suggest that oral dosing of n-3 PUFAs (EPA and DHA) could contribute to protect again β cell lipotoxicity [10,13,30]. However, the underlying mechanisms involved in the effect of FO were only partially analyzed [10,11,13,31]. The present data show that the replacement of dietary CO by FO in the SRD-fed rats normalized both the decreased GK activity—a rate limiting step for glucose metabolism—and their protein mass level. Furthermore, the HK/GK ratio suggests that the impaired glucose phosphorylation observed in the isolated islets of SRD-fed rats could be reversed by increasing the dietary n-3/n-6 fatty acid ratio (from 0.008 to 2.300).

Gremlich et al. [32] demonstrated that exposure of isolated rat pancreatic islets to palmitic acid induced a decrease of both GK mRNA and protein mass level as well as GLUT2 mRNA. Nascimento et al. [33] recently showed that fish oil treatment improved or prevented the alteration of GLUT2 expression in C57BL/6 mice fed a high-fat-high-sucrose diet. Moreover, in these animals dietary fish oil reduced plasma triglyceride levels and fat pad hypertrophy. Our results demonstrate that the protein mass level of glucose transporter GLUT2 in isolated islets of rats chronically fed a SRD was similar to that recorded in rats fed a CD. This was an unexpected finding since a high increase of plasma FFA accompanied with IR was observed in this dietary group. Dietary FO did not modify the protein mass level of GLUT 2. However, after FO administration the increasing plasma glucose, triglyceride and FFA levels were normalized reaching values similar to those observed in the control group. Thus, reduction of glucolipotoxicity throughout the diminished availability of plasma lipids and the restored glucose homeostasis after dietary FO could contribute to normalize β cell glucose phosphorylation.

The normalization of plasma lipid observed in the SRD+FO group also impacted on β cell triglyceride storage and glucose

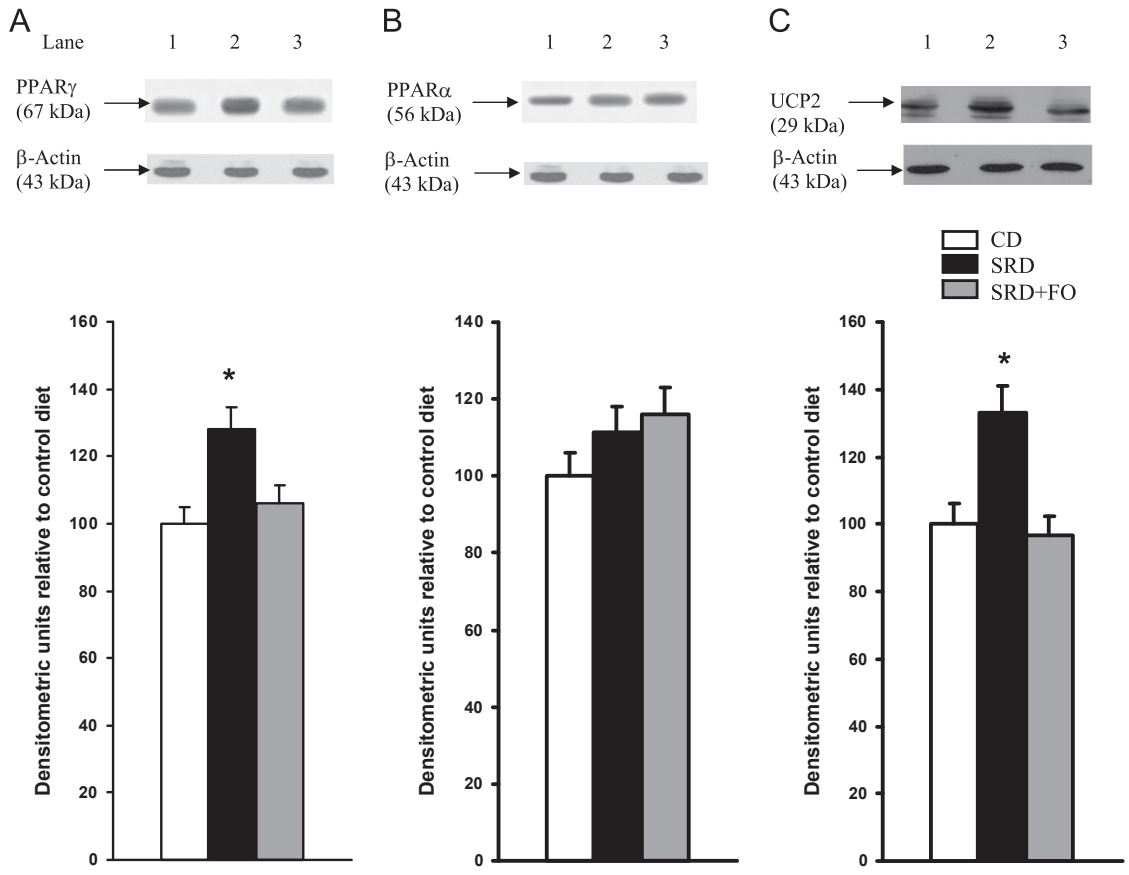

Fig. 2. Isolated islets protein mass levels of PPARγ, PPARα, and UCP2 of rats fed a control (CD), a sucrose-rich (SRD) or a SRD+fish oil (SRD+FO) diet. (A) Upper part: Immunoblots of PPARγ of isolated islets from CD, SRD and SRD+FO rats. Molecular marker is shown on the right. Lane 1 CD; lane 2 SRD; lane 3 SRD+FO. Lower part: densitometric immunoblots analysis of PPARγ protein mass in isolated islets of rats fed a CD, SRD, or SRD+FO. (B) Upper part: Immunoblots of PPARα of isolated islets from CD, SRD and SRD+FO rats. Molecular marker is shown on the right. Lane 1 CD; lane 2 SRD; lane 3 SRD+FO. Lower part: densitometric immunoblots analysis of PPARα protein mass in isolated islets of rats fed a CD, SRD, or SRD+FO. (C) Upper part: Immunoblots of UCP2 of isolated islets from CD, SRD and SRD+FO rats. Molecular marker is shown on the right. Lane 1 CD; lane 2 SRD; lane 3 SRD+FO. Lower part: densitometric immunoblots analysis of UCP2 protein mass in isolated islets of rats fed a CD, SRD, or SRD+FO. β-actin was used as a loading control. Values are mean with their errors depicted by vertical bars (6 animals per group) and expressed as percentage relative to the control diet. *$P < 0.05$ vs CD and SRD+FO.

oxidation since both the increased triglyceride content within the β cell and the decreased PDHc activity recorded in the SRD-fed rats reached values similar to those observed in the CD group after FO administration, thus confirming previous results. This could be at least partially responsible for restoring GSIS in the SRD-fed rats. Abnormal lipid partitioning resulting in either depletion of islet lipid accumulation or excessive islet lipid is potentially important in the development of pancreatic β cell failure. It is known that SREBP-1c up regulates lipogenic enzymes within the β cell as it does in the liver. Activation of SREBP-1c has been shown to promote triglyceride accumulation and to be involved in impaired insulin secretion and glucose intolerance [34]. In islets of obese diabetic rodents SREBP-1c may contribute to lipotoxicity by promoting triglyceride storage and removing fatty acids derived signaling factors from the cellular pool [35]. The present study does not provide data concerning the effect of dietary FO on the expression and protein mass level of SREBP-1c in rats chronically fed a SRD that could contribute to the reduction of triglyceride islets content.

Cumulative evidence shows that β cell UCP2 expression is up-regulated by glucolipotoxic conditions [36]. Contributions of UCP2 to ATP depletion and impaired insulin secretion have been well established [36,37]. It has been suggested that FFA are the physiological endogenous regulators of β cell UCP2 and this explains the partial uncoupling of β cell observed after FFA exposure [26]. The present data demonstrate that dietary FO was able to reduce the increased protein mass level of UCP2 recorded in the isolated islets of SRD-fed rats. In this regard, Kato et al. [10] showed that EPA suppressed the increased protein mass level and the over expression of UCP2 induced by palmitate in pancreatic isolated islets from C57BL/6 mice. Moreover, when the effect of palmitate-EPA on GSIS was estimated in knockdown experiments using adenoviral siRNA of UCP2, a significant inhibition of mRNA and protein levels of UCP2 was obtained. In addition, the reduction in ATP to ADP ratio was restored by UCP2 suppression [10].

On the other hand, UCP2 is also up-regulated by PPAR γ in islets [38,39]. PPAR γ has lipogenic potential promoting fatty acids disposal in pancreatic β cell and compromises glucose-stimulated insulin secretion [38,40]. Patanè et al. [41] demonstrated that chronic exposure to FFA caused an increase of PPAR γ expression in islets leading to a reduction of ATP production mediated through the increase of UCP2 expression in the mitochondrial inner membrane. In this regard, confirming and enlarging previous findings, an increase of PPAR γ protein mass levels was observed in the islets of SRD-fed rats. However, when FO replaced CO as a dietary source of fat the protein mass levels of PPAR γ in the isolated islets returned to the values observed in the CD-fed group. Therefore, the decrease of triglyceride content in the islets reducing the release of fatty acids within the β cell together with the

normalization of the lipid and glucose environment might contribute to the down regulation of PPAR γ and UCP2 and this may contribute to the normalization of GSIS in the isolated islets of the SRD-fed rats after FO administration.

Another possible modulator of insulin secretion is PPAR α. This nuclear transcription factor controls genes involved in lipid metabolism in islets and islets cell lines. The main physiological function of PPAR α appears to reside in the regulation of lipid uptake and oxidation [40]. In pancreatic islets PPAR α mRNA expression is induced by high concentration of fatty acids; conversely, high glucose "in vitro" or hyperglycemia "in vivo" suppresses the expression [42]. In Zucker diabetic fatty rat islets, Unger et al. [43] reported a decrease in the expression of PPAR α, acyl CoA oxidase and CPT-1 mRNA despite the presence of chronic hyperlipidemia. In type 2 diabetic mice given dietary W14, 643 a PPAR α agonist showed a normalization of serum lipids and GSIS with a reduction of β cell proliferation and mass when compared to untreated controls [44]. However, although an increase of plasma FFA levels and altered glucose homeostasis was present in the SRD-fed rats, no changes in the protein mass level of PPAR α was recorded in isolated islets from this dietary group compared to animals fed a CD. At present, we are unaware of any data concerning the expression of acyl CoA oxidase and CPT-1 both target enzymes of PPAR α in the islets of this experimental model. Therefore, we cannot discard the possibility that a balance between the moderate chronic hyperglycemia in face of increased plasma FFA may play a role in the behavior of this nuclear receptor in these animals. Furthermore, the normalization of plasma glucose and lipid levels was not accompanied by a significant change in the protein levels of PPAR α after FO administration. Interestingly, in the same experimental model, we recently demonstrated that dietary FO significantly increased the protein mass level of liver PPAR α and decreased liver steatosis [6].

The effect of dietary n-3 PUFA could also be related to the activity reduction of the proinflammatory process [45]. Therefore, we cannot discard the possibility that the administration of fish oil in the dyslipemic, insulin-resistant rats fed a SRD could inhibit the generation of proinflammatory precursors and may contribute to restore β cell dysfunction.

In brief, expanding our previous research, the present work suggests that some of the possible mechanisms concerning the beneficial effects of FO on the improvement/reversal of β cell dysfunction in the SRD-fed rats involved: a normalization of the protein mass level and enzymatic activity of GK, without changes in the protein mass levels of GLUT2—that in turn contribute to improve the impaired glucose phosphorylation—and a down regulation of UCP2 and PPAR γ protein mass levels. In addition, FO reverses both the higher plasma FFA and triglyceride levels and the increased lipid storage within the β cell and normalizes the impaired glucose oxidation. The effects of dietary FO mentioned above are at least in part involved in the normalization of glucose-stimulated insulin secretion in this nutritional rat model of dyslipidemia and insulin resistance. Finally, although caution is warranted before extrapolating these findings from rodents to human subjects, given the adverse consequence of a prolonged elevation of FFA on insulin sensitivity and secretion in humans, it would appear that nutritional interventions able to reduce the elevated fatty acids may be beneficial.

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