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# Dietary fish oil reverses lipotoxicity, altered glucose metabolism, and nPKCε translocation in the heart of dyslipemic insulin-resistant rats

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**Abstract**

The present study analyzes several markers of energy metabolism in the heart muscle of dyslipemic insulin-resistant rats fed a sucrose-rich diet (SRD, 62.5% wt/wt) for 8 months. It also explores the possible beneficial effects of dietary fish oil supplementation on cardiac lipids and glucose metabolism. With this purpose, male Wistar rats were fed an SRD for 6 months. Whereas half of the animals continued with the same diet for up to 8 months, the other half was fed an SRD in which fish oil (7% + 1% corn oil wt/wt) replaced corn oil (8% wt/wt) from months 6 to 8. The results were compared with rats fed a control diet (starch 62.5% wt/wt). The cardiac muscle of SRD-fed rats showed (1) a significant reduction ($P < .05$) in key enzymes activities and metabolites involved in glucose metabolism, accompanied by a significant ($P < .05$) increase of lipid storage (triglyceride, long-chain acyl coenzyme A, and diacylglycerol), and (2) a significant increase ($P < .05$) of nPKCε protein mass expression in the membrane fraction without changes in the cPKCβII. Dietary fish oil, which reduces the availability of plasma lipid flux and normalizes glucose homeostasis, was able to reverse heart muscle lipotoxicity. Fish oil benefits key enzymes activities in glucose metabolism and normalizes glycogen and glucose-6-phosphate concentration, and the altered nPKCε protein mass expression translocation in the heart of SRD-fed rats. Our findings suggest that manipulation of dietary fats may play a key role in the management of lipid disorders, offering a protection against the development of cardiovascular diseases.


## 1. Introduction

Cardiovascular diseases (CVDs) are one of the most significant lifestyle-related health problems, representing a major cause of premature death in Western countries. The metabolic disorders found in individuals prone to develop CVD are known as *metabolic cardiovascular syndrome* or *insulin resistance (IR) syndrome*, which includes a constellation of abnormalities such as overweight, hypertension, dyslipidemia, glucose intolerance, IR, and type 2 diabetes mellitus [1].

An experimental model that resembles several aspects of the cardiovascular syndrome can be induced in rats by feeding them high-sucrose or high-fructose diets [2,3]. Our laboratory [4-6] has shown that normal rats fed a sucrose-rich diet (SRD) for a long period (6-8 months) develop dyslipidemia, moderate hyperglycemia with normoinsulinemia, IR, visceral adiposity, and slight overweight. We have also demonstrated a substantial increase of triglyceride storage in nonadipose tissues (eg, liver, skeletal muscle, pancreas) [7], including heart muscle [8].

The accumulation of triglyceride in heart, caused by a mismatch between the uptake and oxidation of fatty acids, is associated with several pathophysiologic conditions [9]. Atkinson et al [10] have shown that the increased triglyceride content in the hearts of insulin-resistant JCR: LA-cp male rats is the result of an augmented supply of fatty acids rather than of reduced oxidation. Both plasma free fatty acid (FFA) and triglyceride levels were significantly increased in the insulin-resistant rats. In these animals, the accumulation of triglyceride is associated with a decrease of insulin-stimulated glucose metabolism. In animal models of obesity and diabetes such as db/db and ob/ob mice and ZDF rats, triglyceride accumulation within the cardiomyocytes is also associated with impaired contractile function [11].

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Protein kinase C (PKC) is a member of a large family of serine/threonine kinases that seems to play a role in the pathophysiology of cardiovascular diseases such as myocardial hypertrophy, hypertension, and atherosclerosis. Both hyperglycemia as well as the increased storage of lipid (triglyceride and long-chain acyl coenzyme A [LC ACoA]) within the cardiac muscle and their metabolites (eg, diacylglycerol [DAG]) could modulate PKC activity. There is a significant amount of evidence demonstrating that the increase of protein kinase C ($\beta$ and $\varepsilon$ isoforms) activities and protein contents is associated with subcellular alteration and could contribute to cardiovascular complications in the diabetes heart [12].

In SRD-fed rats, long-lasting hypertriglyceridemia with altered glucose metabolism could have profound effects on myocardial substrate utilization and function. However, these aspects have been only partially investigated in this experimental model [13,14]. On the other hand, the macronutrients composition of the diet is an important environmental determinant in the prevention or improvement of several metabolic disorders included in the plurimetabolic syndrome such as CVD [7,15]. In this regard, a significant body of evidence indicates that the intake of dietary polyunsaturated fatty acids (PUFAs) (20:5 n-3 and 22:6 n-3) has profound heart health benefits [16].

In rats fed a long-term SRD, we have previously demonstrated that partial changes in the fat composition (from corn oil to cod liver oil; fish oil (FO)] of the diet lead to a normalization of the preexisting state of dyslipemia, altered glucose homeostasis, and whole-body insulin insensitivity [17,18]. However, the effect of dietary fish oil upon glucose and lipid utilization in the heart muscle of this experimental model has been only partially evaluated [19,20]. Thus, the goals of this study are (1) to analyze in the cardiac muscle of rats fed an SRD for 8 months the following parameters: (*a*) triglyceride, LC ACoA, DAG, glucose-6-phosphate, and glycogen contents; (*b*) the activities of the enzymes involved in the nonoxidative (hexokinase and glycogen synthase [GSa]) and oxidative (pyruvate dehydrogenase complex [PDHc]) pathways of glucose metabolism; and (*c*) the protein mass and subcellular localization of the PKC isoforms (cPKC$\beta$II and nPKC$\varepsilon$) and (2) to investigate the effect of the replacement of corn oil by FO in the diet upon the parameters mentioned above.

## 2. Materials and methods

### 2.1. Animals and diets

Male Wistar rats initially weighing 170 to 185 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained under controlled temperature (22°C ± 1°C), humidity, and air flow conditions, with a fixed 12-hour light-dark cycle (light 7:00 AM to 7:00 PM). They were initially fed a standard nonpurified diet containing by weight (grams per 100 g) the following: 63 starch (corn, sorghum, wheat, oats, and barley), 22.5 protein, 3.5 fat, 6 fiber, 1 vitamin mixture, and 4 salt mixture (Ralston Purina, St Louis, MO). After 1 week of acclimation, the rats were randomly divided into 2 groups. The experimental group received a purified SRD containing by weight (grams per 100 g) the following: 62.5 sucrose, 17 casein free of vitamins, 8 corn oil, 7.5 cellulose, 3.5 salt mixture (AIN-93M-MX), 1 vitamin mixture (AIN-93-VX), 0.2 choline, and 0.3 DL-methionine. The control group received the same purified diet, but with sucrose replaced by cornstarch (62.5 g/100 g) (high-starch diet [CD]). The experimental group received the SRD for 6 months, after which the rats were randomly subdivided into 2 groups (Fig. 1). The rats of the first subgroup continued the SRD up to 8 months of feeding. The second subgroup (SRD + FO) received the SRD in which the source of fat (corn oil, 8 g/100 g) had been replaced by FO (7 g of cod liver oil per 100 g plus 1 g/100 g of corn oil) from months 6 to 8. The 1% corn oil in the last subgroup was maintained to provide the adequate amount of essential fatty acids. The control group received the CD throughout the experimental period. Cod liver oil was purchased from ICN, Biomedical Pharmaceutical (Costa Mesa, CA). The SRD without the addition of FO used from months 6 to 8 and the CD was balanced for the cholesterol and vitamins D and A present in the FO. Diets were isoenergetic, providing approximately 16.3 kJ/g of food, and were consumed by the rats ad libitum. Diets were prepared every day by adding the oils and the base mixture containing the other nutrients. The oils and base mixture were separately stored at 4°C until preparation of the diet. Dietary fats were analyzed by capillary gas chromatography, as previously described [17]. The weight of each rat was recorded twice each week during the experimental period. In a separate experiment, the individual energy intakes and weight gains of at least 8 animals in each group and subgroup were assessed twice each week. At the end of the 8-month feeding period, food was removed at 7:00 AM (the end of the dark period); and experiments were performed between 7:00 and 9:00 AM.

All experimental procedures on animals were approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina.

### 2.2. Analytical methods

Rats were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight). Blood samples were

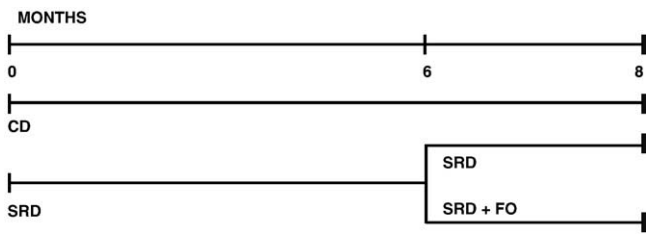


Fig. 1. Experimental design: CD, SRD, or SRD + FO.

obtained from the jugular vein and rapidly centrifuged at 3500 rpm for 15 minutes at 4°C; and the plasma was either immediately assayed or stored at −20°C and examined within 3 days.

Plasma triglycerides [21], FFA [22], and glucose [23] levels were determined by spectrophotometric methods. Immunoreactive insulin was measured by the method of Herbert et al [24]. The immunoreactive insulin assays were calibrated against a rat insulin standard (Novo Nordisk, Copenhagen, Denmark). The heart muscle was rapidly removed from all the anesthetized rats, weighted, frozen, clamped in liquid nitrogen, and stored at −80°C.

The homogenate of frozen muscle powder was used for the determination of triglycerides [21], LC ACoA [25], DAG [26], glycogen [27], and glucose-6-phosphate [23], as well as the cPKC$\beta$II and nPKC$\varepsilon$ protein mass expression [28]. The activities of the GSa, PDHc, and PDH kinase were also determined as previously described [4,8]. The activity of hexokinase was determined according to Thompson and Cooney [29].

### 2.3. Extraction and assay of cPKCβII and nPKCε

The preparation of tissue extracts for PKC was carried out by the method described by Liu et al [28]. All procedures were carried out at 4°C. Briefly, aliquots of frozen heart were homogenized in Tris-HCl buffer (pH 7.5), sonicated, and centrifuged at 105 000*g* for 60 minutes to separate the soluble and particulate-bound enzyme. The resulting supernatant was labeled as the *cytosolic fraction*, whereas the pellet was resuspended in an ice-cold homogenization buffer with 1% Triton X-100 and incubated on ice for 60 minutes. The resuspended pellet was centrifuged at 105 000*g* for 60 minutes, and this supernatant was labeled as the *membrane fraction*. Protein concentrations of the different fractions were measured according to the Bradford method [30].

### 2.4. Electrophoresis and immunoblotting of membrane and cytosol fractions

Protein samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Bio-Rad, CA, USA) using an 8 and 4 (wt/vol) acrylamide gel [5]. Proteins were transferred to nitrocellulose membranes using a Bio-Rad Transblot cell. Blocked membranes were incubated with primary polyclonal antibodies against cPKC$\beta$II or nPKC$\varepsilon$ (1:500 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA), followed by anti–rabbit immunoglobulin G secondary antibody linked to horseradish peroxidase (1:3000 dilution). The bound antibody was detected by autoradiography using an enhanced chemiluminescence kit (Amersham, Little Chalfont, Buckinghamshire, UK). The intensity of the bands was quantified by the National Institutes of Health (Bethesda, MD) imaging software. Density was corrected for variation in extraction between samples using a ratio of dry weight postextraction to wet weight preextraction. Preliminary studies showed linearity of Western blot assay from 50 to 150 $\mu$g of protein for cytosol and membrane fractions. The correlation coefficients between the amount of protein and the enhanced chemiluminescence image intensity were 0.98 and 0.95 for cytosol and membrane fractions of cPKC$\beta$II and 0.97 and 0.94 for cytosol and membrane fractions of nPKC$\varepsilon$, respectively ($P < .01$). The relationship between the amount of sample subjected to immunoblotting and the signal intensity observed was linear under the conditions described above.

### 2.5. Glycogen synthase activity

In vitro GSa was determined as previously described in detail [4]. The fractional velocity of GSa was calculated as the rate of incorporation of labeled [U-$^{14}$C] UDP (uridine 5′-diphosphate) (New England Nuclear, Boston, MA) into glycogen at 0.1 mmol/L glucose-6-phosphate divided by the rate at 10 mmol/L and expressed as a percentage.

### 2.6. Extraction and assay of PDHc and PDH kinase activities

The extraction and assays of PDHc from heart muscles have been previously described in detail [8]. The isolation and assay of PDH kinase were also done as previously described [8,18]. The PDH kinase activity was assayed by determining the adenosine triphosphate–dependent inactivation of the PDH activity as a function of time. The apparent first-order rate constant (K per minute) was calculated from a least-squares linear regression analysis of ln (inactivation by the adenosine triphosphate) against time of incubation.

### 2.7. Hexokinase activity

Muscle cardiac samples still frozen were homogenized with trietanolamine buffer (pH 7.5), using a hand-held glass homogenizer, according to Thompson and Cooney [29], with slight modifications. Briefly, the homogenate was centrifuged for 2 minutes at 13 000 rpm; and the supernatant was used without further purification for the assay of enzyme activity. The hexokinase activity was assayed spectrophotometrically at 37°C, and the reaction was started by the addition of muscle extract. The rate of appearance of β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH) was monitored at 340 nm. The hexokinase activity was expressed as milliunits per milligram of protein.

### 2.8. Statistical analysis

Sample sizes were calculated on the basis of measurements previously made in our laboratory with rats fed a CD or an SRD [4-6,8,17,18] considering 80% power. Results were expressed as mean ± SEM. Statistical comparisons were done transversely between different dietary groups at the end of the experimental diet. Statistical significance between groups was determined by 1-way analysis of variance, with one factor (diet) followed by the inspection

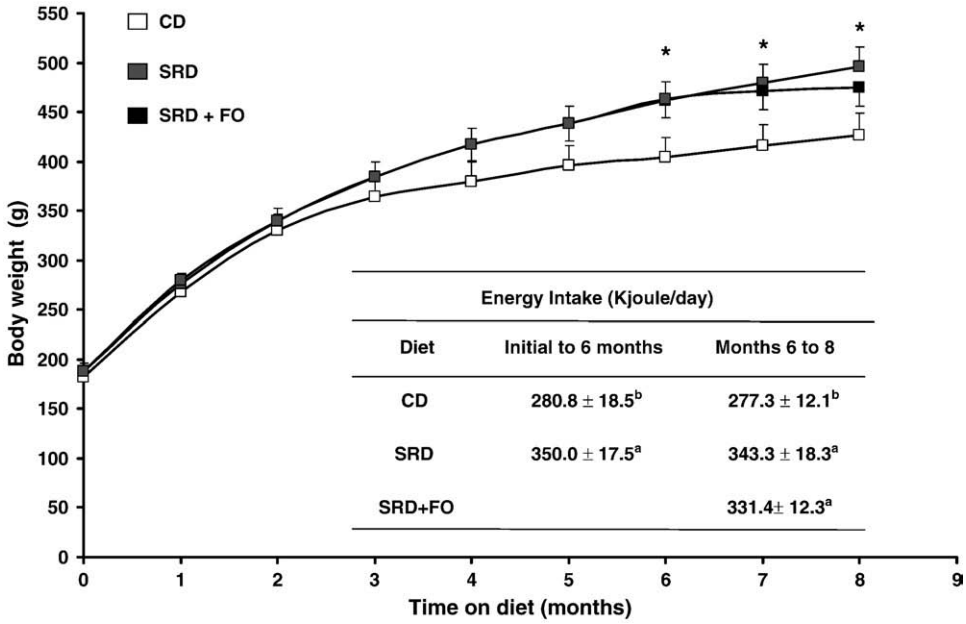


Fig. 2. Body weight and energy intake in rats fed CD, SRD, or SRD + FO. Values are expressed as mean ± SEM (n = 8). *$P < .05$, SRD and SRD + FO vs CD at each time point. In the table inserted in Fig. 1, values in each column that do not share the same superscript letter differ ($P < .05$) when one variable at a time was compared by Newman-Keuls test. For more details, see animals and diets in Materials and methods.

of all differences between pairs of means by the Newman-Keuls test [31]. Differences with $P$ values < .05 were considered statistically significant. In all cases, the interclass correlation coefficients were ≥.73.

## 3. Results

### 3.1. Body weight gain and energy intake

Energy intakes and body weights were carefully monitored in all groups of rats throughout the experimental period. Similarly, as we have previously shown [4,32], an increase ($P < .05$) in body weights (18%) and energy intakes was recorded in the present work in rats chronically fed an SRD for 6 months compared with CD (Fig. 2). When the diet is prolonged for 2 more months (months 6 through 8), the significant difference of body weight between the 2 dietary groups is maintained. However, the weight gain increases at a lower rate in both groups of animals compared with values obtained from the initial time until 6 months on the diet (Fig. 2). The age of animals at this time of the experiment could contribute to these differences. Rats fed an SRD + FO from 6 to 8 months developed a moderate reduction of weight gain compared with both SRD and CD groups. Values were as follows (grams, mean ± SEM; n = 8; months 6 to 8): 35.2 ± 6.1 in CD, 33.5 ± 5.0 in SRD, and 15.0 ± 5.1 in SRD + FO ($P < .05$, CD and SRD vs SRD + FO). Total heart weights recorded in each experimental group showed a significant increase in SRD- and SRD + FO–fed groups vs CD. Conversely, relative heart weights (grams per 100 g body weight) were similar in all dietary groups (Table 1).

### 3.2. Plasma metabolites and insulin levels

At the end of the dark period (7:00 AM), plasma triglyceride, FFA, and glucose concentrations were higher in rats fed the SRD for 8 months compared with age-matched controls fed a CD. All these variables returned to control values in the group of SRD rats in which fish oil replaced corn oil during the last 2 months of the experimental period. No statistical differences in plasma insulin levels were observed at the end of the experimental period among the 3 dietary groups (Table 2).

### 3.3. Heart muscle metabolites concentrations and enzyme activities

Glycogen and glucose-6-phosphate concentrations were significantly decreased in the cardiac muscle of SRD-fed rats. A significant decrease of GSa expressed as percentage of fractional activity as well as hexokinase activity was also observed in this dietary group as compared with control rats fed a CD. A complete normalization of all these variables

Table 1
Total and relative heart weights in rats fed CD, SRD, or SRD + FO

| Diet | Total weight (g) | Relative weight (g/100 g body weight) |
|---|---|---|
| CD | 1.09 ± 0.03b | 0.2505 ± 0.02 |
| SRD | 1.27 ± 0.05a | 0.2579 ± 0.01 |
| SRD + FO | 1.21 ± 0.03a | 0.2544 ± 0.01 |

Values are expressed as mean ± SEM; n = 8. Values in each column that do not share the same superscript letter are significantly different ($P < .05$) when one variable at a time was compared by Newman-Keuls test.

Table 2
Plasma metabolites and insulin concentrations in rats fed CD, SRD, or SRD + FO

| Diet | Triglyceride (mmol/L) | FFA (μmol/L) | Glucose (mmol/L) | Insulin (pmol/L) |
|---|---|---|---|---|
| CD | 0.56 ± 0.04[b] | 335 ± 48[b] | 6.44 ± 0.08[b] | 373 ± 21 |
| SRD | 2.07 ± 0.18[a] | 872 ± 42[a] | 8.28 ± 0.15[a] | 365 ± 27 |
| SRD + FO | 0.57 ± 0.04[b] | 305 ± 40[b] | 6.57 ± 0.20[b] | 372 ± 28 |

Values are expressed as mean ± SEM; n = 6. Values in each column that do not share the same superscript letter are significantly different ($P < .05$) when one variable at a time was compared by Newman-Keuls test.

occurred in rats fed an SRD + FO during the last 2 months of the study (Table 3).

On the other hand, triglyceride and LC ACoA levels within the cardiac muscle were significantly higher ($P < .05$) in the SRD-fed animals. This was accompanied by both a reduced active form of the PDHc and increased PDH kinase activities. However, in the SRD + FO–fed group, neither parameter differed from those of the CD group (Table 4). Moreover, the total PDHc activity expressed per gram of wet tissue did not differ among the groups (units per gram wet tissue, mean ± SEM; n = 6): 5.57 ± 0.27 in the CD group, 5.51 ± 0.12 in the SRD group, and 5.62 ± 0.20 in the SRD + FO group.

### *3.4. Diacylglycerol contents and protein mass expression of PKCs isoenzymes*

Fig. 3 depicts an increase of DAG concentration in the heart muscle of SRD-fed rats compared with the values recorded in the CD group. However, in the SRD + FO group, DAG levels reach values similar to those observed in rats fed a CD.

Immunoblotting of cardiac muscle membrane and cytosol fractions revealed a single 83-kd band consistent with nPKCε isoenzyme. Each muscle fraction was run on separate gels, with each gel containing equal number of samples from CD, SRD, and SRD + FO (Fig. 4, upper panel).

After densitometry of immunoblots, both the membrane and the cytosolic nPKCε content of the CD-fed rat group were normalized to 100%; and the membrane and cytosolic levels of both the SRD and SRD + FO groups were expressed relative to this. Qualitative and quantitative analyses of Western blots showed that the relative abundance of nPKCε isoenzyme was significantly increased in the membrane fraction of cardiac muscle of the SRD group when compared with rats fed a CD, whereas the relative abundance of the cytosol fraction was slightly but not significantly decreased (Fig. 4, lower panel). The addition of FO to the SRD-fed rats normalized the relative abundance of nPKCε in the membrane fraction without changes in the cytosol fraction as compared with the CD group. No changes of

Table 3
Glycogen and glucose-6-phosphate concentrations and hexokinase and GSa activities in heart muscle in rats fed CD, SRD, or SRD + FO

| | CD | SRD | SRD + FO |
|---|---|---|---|
| Glycogen (μmol/g wt tissue) | 16.20 ± 0.70[a] | 12.80 ± 0.60[b] | 15.40 ± 0.50[a] |
| Glucose-6-phosphate (μmol/g wt tissue) | 0.88 ± 0.12[a] | 0.62 ± 0.08[b] | 0.97 ± 0.23[a] |
| GSa * (% of fractional activity) | 26.80 ± 2.55[a] | 16.89 ± 2.14[b] | 24.50 ± 1.50[a] |
| Hexokinase † (mU/mg protein) | 105.85 ± 3.19[a] | 79.06 ± 5.27[b] | 121.32 ± 9.88[a] |

Values are expressed as mean ± SEM; n = 6. Values in each line that do not share a superscript letter are significantly different ($P < .05$) when one variable at a time was compared by Newman-Keuls test.

* GSa was expressed as percentage of fractional activity (see Materials and methods).

† Hexokinase: total activity.

Table 4
Triglyceride and LC ACoA concentrations and PDHc and PDH kinase activities in heart muscle in rats fed CD, SRD, or SRD + FO

| | CD | SRD | SRD + FO |
|---|---|---|---|
| Triglyceride (μmol/g wt tissue) | 3.39 ± 0.17[b] | 5.72 ± 0.54[a] | 3.71 ± 0.13[b] |
| LC ACoA * (μmol/L) | 36.56 ± 2.21[b] | 53.37 ± 5.47[a] | 32.18 ± 6.92[b] |
| PDHa † (% of total PDH) | 66.00 ± 8.83[a] | 40.00 ± 4.30[b] | 64.96 ± 4.10[a] |
| PDH kinase ‡ (K/min) | 1.30 ± 0.10[b] | 2.50 ± 0.11[a] | 1.50 ± 0.09[b] |

Values are expressed as mean ± SEM; n = 6. Values in a line that do not share a superscript letter differ ($P < .05$) when one variable at a time was compared by Newman-Keuls test.

* LC ACoA is expressed as micromoles per liter, assuming that 80% of the tissue was water.

† PDHa: active form of PDH complex, expressed as percentage of total PDHc activity (PDHa: basal activity ×100/total activity).

‡ PDH kinase activity was assayed by determining the adenosine triphosphate–dependent inactivation of PDHc activity as a function of time (K per minute) and was calculated from the first-order kinetic constant.

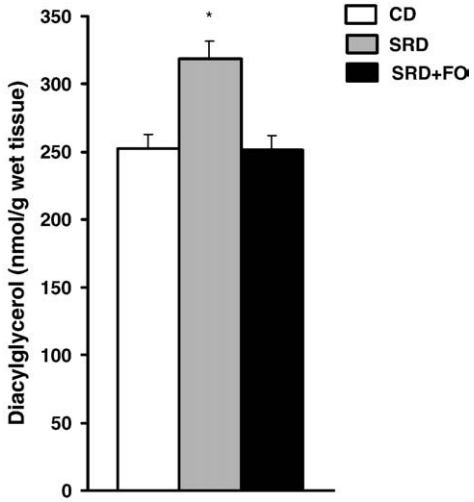


Fig. 3. Heart muscle DAG concentrations in rats fed CD, SRD, or SRD + FO. Values are expressed as mean ± SEM (n = 6). *$P < .05$, SRD vs CD and SRD + FO.

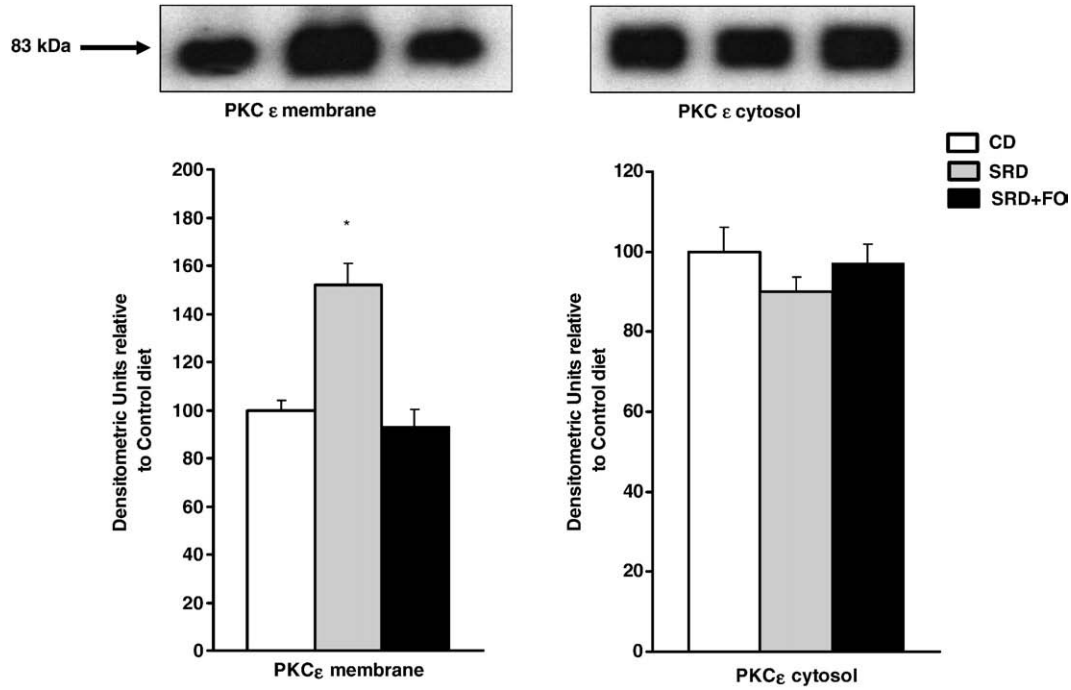


Fig. 4. Heart muscle protein mass expression of nPKCε from rats fed CD, SRD, and SRD + FO. Upper panel, Immunoblots of nPKCε in membrane and cytosol fractions of heart muscle from CD, SRD, and SRD + FO. Molecular mass marker is shown on the right. Lower panel, Densitometric immunoblots analysis of nPKCε protein mass in membrane and cytosol fractions of rats fed CD, SRD, and SRD + FO. Values are expressed as mean ± SEM (n = 6) and expressed as percentage relative of control diet in both membrane and cytosol fractions. *$P < .05$, SRD vs CD and SRD + FO.

immunoblotting of both cytosol and membrane fractions of cPKC βII isoenzyme were observed in the 3 dietary groups. Values expressed as percentage of control were as follows (mean ± SEM ; n = 6): membrane fraction—100.0 ± 1.3 for CD, 104.4 ± 5.8 for SRD, and 97.7 ± 4.8 for SRD + FO; cytosol fraction—100.0 ± 2.1 for CD, 106.4 ± 7.0 for SRD, and 107.4 ± 8.5 for SRD + FO.

## 4. Discussion

In this study, we analyzed several markers of energy metabolism in the heart muscle of rats fed a long-term SRD and explored the possible beneficial effects of dietary FO supplementation on cardiac lipids and glucose metabolism. Two major findings arose from this investigation: (1) A significant decrease of metabolites and the activities of key enzymes involved in glucose metabolism is accompanied by an increase of lipid storage (triglyceride, LC ACoA, and DAG) within the heart muscle. Moreover, the nPKCε protein mass was significantly increased in the membrane fraction of cardiac cells, without changes in the protein mass expression of PKCβ2. (2) The dietary administration of FO, which lowers plasma triglyceride and FFA levels and increases whole-body insulin sensitivity [32], was able to normalize all the metabolic changes mentioned above.

Insulin and fatty acids are important modulators of cardiac substrate utilization. In the present study, and in agreement with previous results [8,32], plasma triglyceride and FFA levels were significantly elevated in the insulin-resistant rats fed an SRD, indicating that there is an increased availability of fatty acids to nonadipose tissues including heart muscle. Moreover, the substantial increase of myocardial lipid deposition present in this dietary group suggests that the heart muscle of the SRD-fed rats is capable of increased fatty acid uptake and esterification. In this regard, Atkinson et al [10] showed that, in the heart of insulin-resistant JCR:LA-cp rats, the cardiac fatty acid–binding protein expression was significantly higher and that triglyceride storage within the cardiomyocyte was increased.

In the SRD-fed rats, the increase of triglyceride and LC ACoA stores was accompanied by a significant increase of PDH kinase and a reduction of PDHc activities. A decreased flux through PDHc limits the conversion of pyruvate derived from glycolysis to acetyl CoA and impairs the oxidative glucose metabolism [33]. Similarly, Chatham and Seymour [34] confirmed these findings, showing that, in the heart muscle of Zucker diabetic fatty rats, the reduction in glucose oxidation was associated with reduced flux through PDHc.

Impaired glycolytic flux in the heart muscle could also include independent effects on other rate-limiting steps involved in glycolysis such as the enzymatic activity of hexokinase. Our results show a significant decrease of hexokinase activity accompanied by a decrease of glucose-6-phosphate concentration in the cardiac muscle of SRD-fed rats. The lower enzyme activity was present in the face of a significant increase of LC ACoA concentrations. In this regard, we have demonstrated a decrease of hexokinase

activity with an increase of LC ACoA and triglyceride levels in the skeletal muscle of rats fed an SRD for a similar period [5]. Furthermore, in "in vitro" studies, Thompson and Cooney [29] showed that LC ACoA reduced the hexokinase activity in the skeletal muscle of normal rats by allosteric inhibition. An increase of the LC ACoA concentration could also affect translocation of the glucose transport by acylating proteins involved in membrane fusion processes [35]. So far, we have not measured the activity of the glucose transporter; however, our group [8] has previously shown a reduced basal and insulin-stimulated glucose uptake in isolated perfused hearts of rats fed a 15-week SRD.

The molecular mechanisms responsible for the impaired insulin action in the presence of elevated plasma FFA levels are not yet completely understood. Soltys et al [36] demonstrated that, in perfused rat hearts, palmitate induced inhibition of insulin-stimulated glucose uptake and utilization, and reduced the rate of glucose oxidation. Similarly, Terruzzi et al [37] showed that, in heart muscle, Intralipid (Fresnius Kabi, Stockholm, Sweden) blocked the insulin effect on glucose uptake and reduced the activity of the IRSs/PI3-kinase/AKT/GSK3 axis involved in the activation of glucose transport (uptake) and glycogen synthesis. Long-chain acyl CoA could also inhibit the GSa activity. The present study shows a significant reduction of both GSa activity and glycogen contents in the heart of SRD-fed rats, suggesting that the nonoxidative pathway of glucose metabolism was also altered.

The heart of SRD-fed rats also showed an increase of DAG concentration. An increase in the activation of the DAG-sensitive PKC pathways has been reported in different tissues including heart muscle from diabetic animals and humans (see reviews in Idris et al [12]). Our data show a significant increase in the expression of nPKCε protein mass in the membrane fraction without changes in the cytosol. Although we did not directly measure the nPKCε activity, there are numerous studies [38,39] making the assumption that translocation is tantamount to activation. Similarly, Malhotra et al [40] reported an increase of the expression of nPKCε protein mass in the membrane fraction of cardiocytes of diabetic rats. In parallel, troponin I phosphorylation was also increased. Interestingly, both changes were prevented by insulin or by blocking the angiotensin II receptor. Studies using cardiocytes in vitro indicated that activation of nPKCε and nPKCδ is associated with myocardial ventricular hypertrophy [41,42]. Thus, the increased protein mass of nPKCε in the membrane fraction of the heart of the SRD-fed rats in the absence of cardiac hypertrophy suggests the presence of an underlying alteration of cardiac function. In this vein, Dutta et al [13] showed a specific myocyte mechanical defect including slower rates of shortening and relengthening, and slower cytosolic $Ca^{++}$ clearing in rats fed a sucrose diet for 7 to 10 weeks.

The activation of the cPKC$\beta$2 isoenzyme can also cause vascular dysfunction. Substantial hyperglycemia may increase DAG contents and could contribute to activated PKC isoenzymes in heart [12]. Unexpectedly, the expression of cPKC$\beta$2 protein mass in both membrane and cytosol fractions of the cardiac muscle of SRD-fed rats was similar to those observed in rats fed a CD. Inoguchi et al [43] reported a preferential increase of cPKC$\beta$2 in the membrane of the aorta and heart muscle in streptozotocin diabetic rats. Therefore, it is possible that the differences between the results of Inoguchi et al and our data may be due to the significant differences in both plasma glucose (24 mmol/L in Inoguchi et al vs 8.2 mmol/L in the SRD-fed rats) and insulin levels (nihil or very low in the study of Inoguchi et al and similar to CD in the present work).

Dietary fish oil returned plasma triglyceride, FFA, and glucose to control levels without detectable changes in plasma insulin concentration in rats fed an SRD. The hypolipemic effect of fish oil may work via the peroxisome proliferator–activated receptor α (PPAR-α) that controls the expression of genes involved in hepatic fatty acid oxidation and the transcription factor SREBP1c that is required for suppression of de novo lipogenesis and monounsaturated fatty acids synthesis [7].

The normalization of dyslipidemia and glucose homeostasis after FO administration [18,44] completely reversed the altered metabolites and enzymes activities involved in the oxidative and nonoxidative glucose pathways; and the increase of triglyceride, LC ACoA, and DAG in the cardiac muscle of SRD-fed rats was completely reversed. Furthermore, to the best of our knowledge, the present study is the first to show a reversal of the increased nPKCε protein mass expression in the heart membrane fraction of SRD-fed rats by dietary interventions with FO. Davidoff et al [45] showed that, in rats fed an SRD for a shorter period, lipid-lowering interventions (eg, exercise, bezafibrate) prevented or reversed the development of IR and cardiomyocyte dysfunction. The PPAR-α is also expressed in the heart muscle, and studies in neonatal cardiomyocyte in vitro showed that activation of PPAR-α increased the transcription of genes involved in fatty acids oxidation [46,47]. Thus, we cannot discard the possibility that the administration of FO could also contribute to normalize the glucose and lipid metabolisms in the heart of SRD-fed rats via activation of PPAR-α. Besides, de Jonge et al [48] showed in cultured rat ventricular myocyte that n-3 PUFAs directly induce changes in the fatty acid composition of the membrane phospholipids. This could alter the endogenous substrates for the membrane-associated phospholipase C-$\beta$ and $A_2$, resulting in altered molecular species of 1,2-diacylglycerols and non-esterified PUFAs that in turn evoke changes in events downstream of the signaling cascades [49]. Although the present study did not analyze the fatty acid composition of the heart muscle phospholipids, we have previously demonstrated an increase in n-3 eicosapentaenoic acid and docosahexaenoic acid as well as in the n-3/n-6 ratio in the skeletal muscle of SRD-fed rats after FO administration [5,44]. These effects could be involved in the mechanisms related to the beneficial effects of dietary PUFAs on cardiac

dysfunction. Interestingly, an increase of the 22:6 n-3/18:2 n-6 ratio in the membrane phospholipids of heart muscle was also observed by Cefalu et al [50] in rats subjected to chronic caloric restriction. In these animals, insulin sensitivity was improved. On the other hand, Rousseau et al [51] suggested that, in rats fed a high-sucrose diet, the cardioprotective effect of fish oil (especially docosahexaenoic acid) occurs through mechanisms that may involve the cardiac adrenergic system.

In brief, this study demonstrates that, in the heart muscle of SRD-fed rats, lipotoxicity is accompanied by changes of several markers involved in glucose and lipid metabolism and altered nPKCε protein mass expression translocation. This metabolic profile suggests an underlying alteration of cardiac function. Dietary fish oil that reduces the availability of plasma lipid flux and normalizes glucose homeostasis is able to reverse lipotoxicity. Fish oil restores the activities of key enzymes involved in glucose metabolism and the expression of nPKCε in the membrane fraction of the cardiac muscle. Furthermore, our findings suggest that the manipulation of dietary fats may play a key role in the management of lipid disorders, thus protecting against the development of cardiovascular diseases.

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