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Journal of Nutritional Biochemistry 13 (2002) 209–218

THE JOURNAL OF Nutritional Biochemistry

Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model☆

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Received 15 June 2001; received in revised form 2 October 2001; accepted 25 October 2001

Abstract

The present work was designed to assess the possible benefits of (7% w/w) dietary fish oil in reversing the morphological and metabolic changes present in the adipose tissue of rats fed an SRD for a long time. With this purpose, in the epididymal fat tissue, we investigated the effect of dietary fish oil upon: i) the number, size and distribution of cells, ii) the basal and stimulated lipolysis, iii) the lipoprotein lipase (LPL) and the glucose 6-phosphate dehydrogenase activities, and iv) the antilipolytic action of insulin. The study was conducted on rats fed an SRD during 120 days with fish oil being isocaloric substituted for corn oil for 90–120 days in half the animals. Permanent hypertriglyceridemia, insulin resistance and abnormal glucose homeostasis were present in the rats before the source of fat in the diet was replaced. The major new findings of this study are the following: i) Dietary fish oil markedly reduced the fat pads mass, the hypertrophy of fat cells and improved the altered cell size distribution. ii) The presence of fish oil in the diet corrected the inhibitory effect of high sucrose diet upon the antilipolytic action of insulin, reduced the "in vitro" enhanced basal lipolysis and normalized isoproterenol-stimulated lipolysis. Fat pads lipoprotein lipase activity decreased reaching values similar to those observed in age-matched controls fed a control diet (CD). These effects were not accompanied by any change in rat body weight. All these data suggest that the dyslipemic rats fed a moderate amount of dietary fish oil constitute a useful animal model to study diet-regulated insulin action.

Keywords: Epididymal adipose tissue; Dyslipidemia; Insulin resistance; Fish oil (n-3) fatty acids; Sucrose-rich diet

1. Introduction

For more than two decades there has been considerable interest in the dietary intake of marine polyunsaturated fatty acids (PUFA) especially 20:5 n-3 and 22:6 n-3 since results of animal and clinical studies have suggested their beneficial health effect. Besides to its role as an energy source and its effects on membrane lipid composition n-3 fatty acids serve as important mediators of gene expression. There is now evidence that fish oil may work via peroxisome proliferator activated receptors (PPARs) controlling the expression of genes involved in lipid and glucose metabolism and adipogenesis. Moreover, it was shown that fish oil regulate the transcription of gene coding for lipogenic and glycolitic enzymes [1]. Fish oil feeding has a significant effect in lowering plasma triacylglycerol and very low density lipoprotein (VLDL) concentrations in experimental animals and in normal as well as hypertriglyceridemic men [2,3]. A potential beneficial effect of dietary fish oil supplementation on peripheral insulin resistance has been suggested by studies in both type II diabetics [4] and animals [5]. In this regard, several studies have shown that long chain n-3 fatty acids prevent the onset of insulin resistance and hypertriglyceridemia when given to rats concomitantly with either high fat or high sucrose diets for a short period of time (3–6 weeks) [6–8].

There is accumulated evidence that the increased availability of free fatty acids in muscle and liver is associated with insulin resistance [9,10]. Adipose tissue is a target tissue to supply fatty acids for whole body utilization. Its metabolic activity is the main contributor to the development of obesity, followed by, or concomitant with, insulin resistance. However, the effect of administration of fish oil upon the metabolic and morphological aspects of adipose

☆ A preliminary report was presented at the 19th International Symposium on Diabetes and Nutrition, June 2001, Dusseldorf, Germany.

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0955-2863/02/$ – see front matter
PII: S0955-2863(01)00214-5

tissue in the presence of insulin resistance has been only partially explored. In the case of rats rendered insulin resistant by feeding a sucrose rich diet, Rizkalla et al. [11] demonstrated an improvement of insulin stimulated glucose incorporation into total lipids and glucose oxidation in adipocytes from epididymal fat pads of rats fed a sucrose rich diet containing 30% of energy as fish oil. A high insulin stimulated glucose transport, oxidation and incorporation into total lipids was shown by Luo et al. [12] using a similar experimental approach. Moreover, insulin action was positively correlated with the fatty acid insaturation index in membrane phospholipids [12]. Peyron et al. [13] have recently observed an increased basal and stimulated lipolysis without changes in cathecholamines excretion in adipocytes of rats fed a sucrose rich diet in the presence of fish oil (30% of total energy). An increased effect of insulin on glucose utilization in fat pad after fish oil (10% w/w) treatment was also observed in rats "in vivo" by Klimes et al. [2].

The studies mentioned above were conducted with the simultaneous administration of fish oil and sucrose during a short period of time (3–6 weeks). Animal studies focused on the effect of fish oil to reverse a preexisting sucrose induced insulin resistance are scarce and not related to adipose tissue metabolism [8,14,15]. A recent work by Soria et al. [16] in rats fed an SRD for a long term (up to 15 weeks) demonstrated that the length of time on the diet plays an important role in the evolution of the adiposity and metabolic changes observed in the fat pad tissue. In this vein, increased fat pad weight and hypertrophy of adipocytes with clear alteration in the cell size distribution were observed only after long-term SRD feeding, while increased HSL activity under basal and stimulated conditions was present after short (3 weeks) or long periods on the diet. Furthermore, our laboratory has reported that the metabolic environment of hypertriglyceridemic rats chronically fed an SRD evolve from normoglycemia with hyperinsulinemia (3–5 weeks on SRD) to moderate hyperglycemia with normoinsulinemia (13–15 up to 40 weeks on SRD) [17,18]. Besides, a more pronounced increase of plasma free fatty acid level was also observed after long- term feeding [18].

Thus, the present work was designed to assess the possible benefits of (7% w/w) dietary fish oil in reversing the morphological and metabolic changes present in the adipose tissue of rats fed an SRD for a long time. With this purpose, in the epididymal fat tissue, we investigated the effect of dietary fish oil upon: i) the number, size and distribution of cells, ii) the basal and stimulated lipolysis, iii) the lipoprotein lipase (LPL) and the glucose 6-phosphate dehydrogenase activities, and iv) the antilipolytic action of insulin. The study was conducted on rats fed an SRD during 120 days in which a permanent hypertriglyceridemia, insulin resistance and abnormal glucose homeostasis had been present, before the source of fat (corn oil) in the diet was replaced by an isocaloric amount of cod liver oil for 90–120 days in half of the animals.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats, weighing 180 to 200 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were maintained in an animal room under controlled temperature (22 ± 1°C), humidity, and air flow conditions, with a fixed 12.00 hr light/dark cycle (light 7:00 AM–7:00 PM). They were initially fed a rat standard laboratory chow (Ralston Purina, St Louis, MO, USA). After a week of acclimatization, rats were randomly divided into two groups: control and experimental. The experimental group was fed a semisynthetic sucrose-rich diet (SRD) containing by weight (% w/w): 62.5 sucrose (64.1% of calories), 17 of casein free of vitamins (17.4% of calories), 8 corn oil (18.5% of calories), 7.5 cellulose, 3.5 salt mixture (AIN-93AM-MX), 1 vitamin mixture (AIN-93M-VX), 0.2 choline chloride, and 0.3 methionine. The composition of both the vitamin and salt mixtures added to the SRD complied with the recommendations made by the Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76 A Rodent Diet. Details of the methodology used have been described elsewhere [15]. The control group received the same semisynthetic diet but with sucrose replaced by starch (high starch diet: CD). The experimental group received the SRD for 90 days after which the animals were randomly divided into two subgroups. The first subgroup (SRD) continued on the SRD up to 120 days. The second subgroup (SRD + cod liver oil) received an SRD in which the source of fat (corn oil 8% w/w) had been replaced by cod liver oil 7% w/w (16% of calories) plus corn oil 1% w/w from days 90 to 120. The control group received the CD throughout the experimental 120-day period. The SRD without the addition of cod liver oil used from days 90 to 120 and the CD were balanced for cholesterol and vitamins D and A present in the cod liver oil. Details on the composition of the diets were previously given [15]. Diets were isoenergetic, providing approximately 16.3 kJ/g of food and were available ad libitum. Diets were prepared every day by adding the oils to a base mixture containing the other nutrients. The oils and base mixture were separately stored at 4°C until preparation of the diet. Cod liver oil was kept under an atmosphere of nitrogen during storage. Details of dietary fatty acid composition of each diet were previously given [14]. 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 eight animals in each group and subgroup were assessed twice a week.

On the day of the experiment, otherwise indicated, food was removed at 7:00 AM (at the end of the dark period) and the experiments were performed between 7:00 AM and 10:00 AM. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina.

2.2. Preparation of isolated adipocytes

The epididymal fat pads were removed via midline abdominal incision and rinsed in isotonic saline a 37°C. Adipocytes were isolated according to the Rodbell method [19], with minor modifications. Briefly, the fat pads were minced with scissors, placed in a plastic flask and incubated at 37°C in a Dubnoff shaking water bath, (Precision Scientific Group, GCA Corporation, Chicago Illinois, USA) at 60 cycles/min for one hour in Krebs-Henseleit phosphate buffer (KHB) (pH = 7.4), containing 1.25 mM Ca^{+2}, 4% bovine serum albumin essentially free of fatty acids, 5.5mM glucose and crude collagenase (1–2 mg/g of tissue) from Clostridium histolyticum. Subsequently, adipocytes were gently filtered through an approximately 200-mm diameter nylon mesh to remove stroma and blood vessels, washed three times in fresh collagenase-free KHB and allowed to separate from the infranadant by low speed centrifugation (600 rpm). Isolated cells were then resuspended in collagenase-free buffer at 37°C.

2.3. Determination of fat cell size and fat cell number

The microscopic method of Di Girolamo et al. [20] was used to measure cell diameters. The sizing of 100 adipocytes from epididymal fat tissue was conducted by the same operator throughout the present study to improve precision as suggested by Khan et al. [21]. For each experimental group of animals the number of fat cells in similar class intervals of 2.5 μm was treated as a single variable and the average value obtained for each class interval was used to construct a histogram representing the group. The mean diameter and volume for the entire fat cell population were calculated from the histogram according to Di Girolamo [20]. For the estimation of the fat pad cell number, the lipid content of 100–200 mg of fat tissues was extracted by the method of Folch et al. [22]. Total cell number in fat pad was calculated dividing the fat pad lipid content by the mean cell lipid weight. The lipid weight of the average fat cell was calculated from the mean cell volume assuming a lipid density of 0.915 (density of triolein).

2.4. Basal and stimulated lipolysis of the fat cells

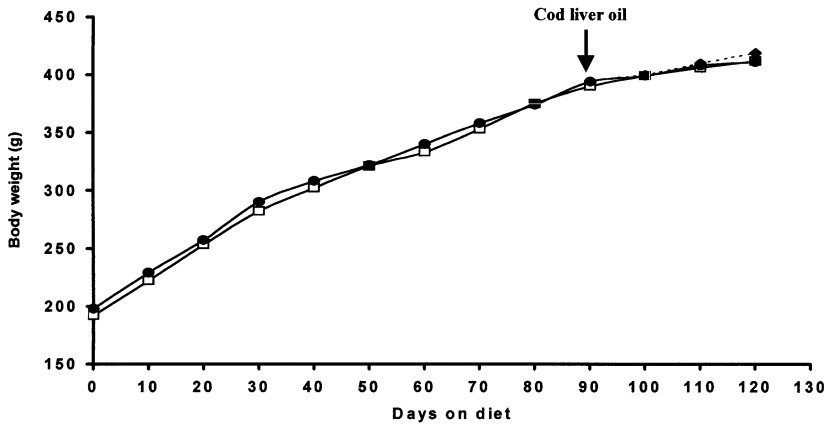
For the study of the lipolysis, aliquots of diluted epididymal fat cells in the same buffer as described in the preparation of adipocytes were placed into plastic vials (1×10^5 cells/ml), and the incubation was performed in a shaking Dubnoff water bath (60 cycles/min) at 37°C for one hour under an atmosphere of O_2:CO_2 95:5. Under these conditions, there was a time-dependent increase in lipolysis for 60 min. Therefore, this time period was chosen for the incubation. A pure β-agonist -isoproterenol- was used for the stimulation of lipolysis. In a preliminary study, the isoproterenol (0 to 10^{-5} M) dose-dependent increase in lipolysis was evaluated. No statistically significant differences in the maximal lipolytic response were found at an isoproterenol concentration of 10^{-5} or 10^{-6} M (data not shown). Thus, to measure the lipolysis, the glycerol release was measured over one hour at 15-min intervals, as described by Rodbell et al. [19], in both the basal state and in the presence of isoproterenol (10^{-6} M), so that the maximal lipolytic responsiveness could be examined. Because the adipose tissue has a very low level of glycerol kinase, only a very small fraction of the glycerol produced by intracellular lipolysis can be utilized and converted to α-glycerophosphate for use in triglyceride synthesis [23]. Glycerol release is therefore a valid index of lipolysis. At the end of the incubation, three aliquots of infranadant (200 μl) were removed from each incubation mixture for the measurement of glycerol by the enzymatic method of Wieland [24].

2.5. Assay of the antilipolytic action of insulin

To study the antilipolytic action of insulin, isoproterenol (10^{-7} M) and adenosine deaminase (1 U/ml) were added to the epididymal fat cells suspensions (1×10^5 cells/ml), and incubations were conducted during 1 hr at 37°C under an atmosphere of O_2:CO_2 95:5 both in the absence or presence of insulin (purified porcine insulin, Novo Laboratory) at a final concentration of 2.0 nM. A preliminary dose dependent (0.5 to 6.0 nM) study showed that the maximal antilipolytic action of the hormone reached a plateau between 1.7 and 6.0 nM. At the end of the incubation, three aliquots of infranadant (200 μl) were removed from each incubation mixture and the glycerol release measured as described above [24]. The antilipolytic action of insulin was expressed as the ratio of the value of the insulin-inhibited lipolysis to that of the isoproterenol-stimulated lipolysis in the absence of the hormone as percentage (%).

2.6. Euglycemic clamp studies

The whole body peripheral insulin sensitivity was measured using the euglycemic hyperinsulinemic clamp technique as previously described elsewhere [15]. Briefly, the rats were anesthetized after a 5 hr fasting period. Then, a blood sample was obtained, on which glucose and insulin levels were assessed. Afterward, an infusion of highly purified porcine neutral insulin (Actrapid, Novo Industry, Denmark) at 0.8 $U.kg^{-1} \cdot h^{-1}$ for 2 hr was started. Glycemia was maintained at an euglycemic level by injecting at a variable rate 0.2 g/ml glucose solution. The glucose infusion rate began 5 min after the insulin infusion started. The blood glucose concentration was measured using the glucose oxidase method (Glucose Accutrend Sensor, Boehringer Mannheim Biochemicals, Buenos Aires, Argentina) within 2 min after the samples were obtained. The glucose infusion rate (GIR) during the second hour of the clamp was taken as the net steady state of whole-body glucose. In all studies, blood samples (0.3 ml) for insulin determination [25] were obtained at 60, 90 and 120 min.



Diet	Time on Diet (days)	Caloric Intake (Kjoules/day)
Control	1 to 120	276.0 ± 25.4
Sucrose-rich	1 to 90	279.0 ± 23.0
Sucrose-rich	90 to 120	269.0 ± 19.9
Sucrose-rich+ cod liver oil	90 to 120	270.0 ± 15.7

Fig. 1. Body weight and caloric intake of rats fed a control diet (CD: days 1–120), sucrose-rich diet (SRD: days 1–120) or SRD (days 1–120) + cod liver oil (days 90–120). □——□ CD. ●——● SRD. ●·····● SRD + cod liver oil.

2.7. Analytical methods

The rats were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight). Blood samples were obtained from the jugular vein and immediately centrifuged at 4°C. The plasma samples obtained were either immediately assayed or stored at −20°C and examined within the following 3 days. Plasma glucose [26], triglyceride [27] and free fatty acids (FFA) [28] were determined by spectrophotometric methods. Immunoreactive insulin (IRI) was measured by Herbert et al's method [25]. The IRI assay was calibrated against the rat insulin standard (Novo Nordisk, Copenhagen, Denmark). The epididymal and retroperitoneal adipose tissues were removed and weighed. The activities of Glucose-6-Phosphate Dehydrogenase (G-6-PDH) and Lipoprotein lipase (LPL) were measured in epididymal tissue as previously described [16,29].

2.8. Statistical analysis

Results were expressed as mean ± SEM. The statistical significance between groups was determined by one way Analysis of variance followed by the inspection of all differences between pairs of mean by the Newman-Keuls′test [30]. Differences having p values lower than 0.05 were considered to be statistically significant.

2.9. Reagents

Enzyme for assays, substrate and coenzyme were purchased from Sigma Chemical Co (St. Louis, MO, USA) or from Boehringer Mannheim Biochemical (Indianapolis, IN, USA). Cod liver oil was purchased from ICN (Costa Mesa, CA, USA). All other chemicals were reagent grade.

3. Results

3.1. Body weight gain and caloric intake

Food intake and weight gain were carefully monitored in all groups of animals throughout the experimental protocol. Animals seemed to find all diets palatable. The food intake (expressed as caloric intake in kilojoules per day) was comparable in all groups over the entire experimental period (see Table included in Fig. 1). The weight gain in all dietary groups was also comparable (Fig. 1).

3.2. Plasma metabolite levels and euglycemic-hyperinsulinemic clamp studies

In agreement with previous reports from our laboratory [14,15] and confirmed by the present findings (Table 1), the high plasma levels of triglyceride, FFA and glucose reported in rats fed the SRD for 120 days returned to control values when cod liver oil replaced corn oil in the diet from days 90 to 120. On the other hand, similar plasma immunoreactive insulin (IRI) levels were recorded in all groups of animals. The effect of dietary substitution on whole body peripheral insulin sensitivity (euglycemic-hyperinsulinemic clamp studies) was assessed at the end of the experimental period. Blood glucose was clamped at 5.5–6.0 mM. Values

Table 1
Plasma metabolites and insulin concentration and Glucose Infusion rate (GIR) in rats fed a control diet (CD), sucrose-rich (SRD) or SRD + cod liver oil

Diet	Triglyceride (mM)	Free fatty acids (μM)	Glucose (mM)	Insulin (μU/ml)	GIR* (mg/Kg/min)
CD (days 1 to 120)	0.51 ± 0.07^{a}	245.3 ± 19.4^{a}	6.10 ± 0.12^{a}	44.1 ± 5.2^{a}	10.50 ± 0.90^{a}
SRD (days 1 to 120)	2.25 ± 0.18^{b}	788.3 ± 51.2^{b}	8.13 ± 0.18^{b}	54.3 ± 5.0^{a}	4.98 ± 0.35^{b}
SRD (1 to 120) + cod liver oil (days 90 to 120)	0.49 ± 0.10^{a}	288.3 ± 30.0^{a}	5.94 ± 0.13^{a}	48.3 ± 4.9^{a}	12.30 ± 0.98^{a}

Values are expressed as mean ± SEM. Six animals were included in each experimental group. Values in each column 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.

* Steady state of blood glucose and insulin concentration during the last 60 min of the clamp were as follows (mean ± SEM, n = 6) Glucose (mM): CD: 5.80 ± 0.20, SRD: 5.85 ± 0.22, SRD + cod liver oil: 5.90 ± 0.23. Insulin (μU/ml): CD: 715 ± 43, SRD: 748 ± 37, SRD + cod liver oil: 733 ± 40.

at 5 hr post prandial blood glucose (mM) before the clamp were (mean ± SEM, n = 6) CD: 5.35 ± 0.15, SRD: 7.85 ± 0.13 and SRD + cod liver oil 5.50 ± 0.17. These results were not associated with changes in plasma insulin concentration (mU/ml, mean ± SEM, n = 6): CD: 40.8 ± 6.5; SRD: 43.3 ± 5.9 and SRD + cod liver oil: 45.0 ± 6.3. The glucose infusion rate (GIR), which measures insulin action "in vivo", was significantly decreased ($p < 0.01$) in the SRD group compared to animals fed a CD for the same period of time. GIR returned to values similar to those recorded in rats fed a CD when cod liver oil replaced corn oil in the SRD from days 90 to 120 (Table 1).

3.3. Fad pad morphology, triglyceride content and lipoprotein lipase (LPL) and Glucose-6-Phosphate Dehydrogenase (G-6-PDH) activities

We have previously reported [16] an increase of epididymal tissue weight associated with a hypertrophy of the adipose cells in rats fed an SRD for 90 days. The present study confirmed and enlarged these results when the diet was extended up to 120 days. Table 2 shows that at the end of the experimental period, epididymal tissue weight of SRD fed rats was significantly increased as compared to age matched controls fed a CD. The adipocyte of the SRD fed animals was almost twofold more voluminous and a significant reduction of the adipose cell number, expressed per gram of tissue, was observed. This showed a hypertrophy of fat pad cells in this dietary group. Triglyceride content (μmol/cell) in fat pads was significantly higher in the SRD fed group. Moreover, this dietary group showed a significant increase in LPL and G-6PDH activities, both expressed on a total cell basis or per total tissue weight (Table 2). When the source of fat -corn oil- in the SRD was replaced by cod liver oil, fat pad total weight, relative weight (g/100 g body weight), cell volume and triglyceride content were significantly decreased compared to the SRD groups. However, all these parameters were still slightly above the values recorded in the age matched control rats fed the CD. Besides, the observed hypertrophy of the fat pad was reduced by the substitution of corn oil by cod liver oil in the diet. No differences in total cell number was observed when expressed as total epididymal fat weight in all dietary groups. On the other hand, while adipose LPL activity reached similar values to the CD fed rats, the G-6-PDH activity dramatically decreased and reached values lower than those of the CD fed animals.

Table 2
Epididymal adipose tissue weight, cellularity, triglyceride content and Lipoprotein Lipase (LPL) and Glucose-6-Phosphate Dehydrogenase (G-6-PDH) activities of rats fed a control diet (CD), sucrose-rich (SRD) or SRD + cod liver oil

	CD (days 1–120)	SRD (days 1–120)	SRD (days 1–120) + cod liver oil (days 90–120)
Total weight (g)	$5.80 \pm 0.30^{1,a}$	9.29 ± 0.54^{b}	7.16 ± 0.40^{c}
Relative weight (g/100 g body weight)	1.41 ± 0.10^{a}	2.22 ± 0.11^{b}	1.76 ± 0.04^{c}
Cell volume (pl)	254.0 ± 13.0^{a}	460.0 ± 18.0^{b}	349.0 ± 29.0^{c}
Cell number $\times 10^6$/g tissue	4.34 ± 0.24^{a}	2.71 ± 0.11^{b}	3.29 ± 0.13^{c}
Cell number $\times 10^6$/total weight	25.25 ± 2.52	25.31 ± 2.39	23.52 ± 3.41
Triglyceride (μmol/cell)	0.28 ± 0.02^{a}	0.55 ± 0.05^{b}	0.38 ± 0.03^{c}
LPL (pktal/total weight)	16643 ± 2495^{a}	34335 ± 4335^{b}	19816 ± 2712^{a}
LPL (pktal/cell number $\times 10^6$)	659 ± 98^{a}	1356 ± 171^{b}	842 ± 115^{a}
G-6-PDH (U/total weight)	1.03 ± 0.10^{a}	2.08 ± 0.08^{b}	0.74 ± 0.06^{c}
G-6-PDH (mU/cell number $\times 10^6$)	40.03 ± 1.83^{a}	83.43 ± 2.84^{b}	30.73 ± 2.7^{c}

[1]: Values are expressed as mean ± SEM. Six animals were included in each experimental group. 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.

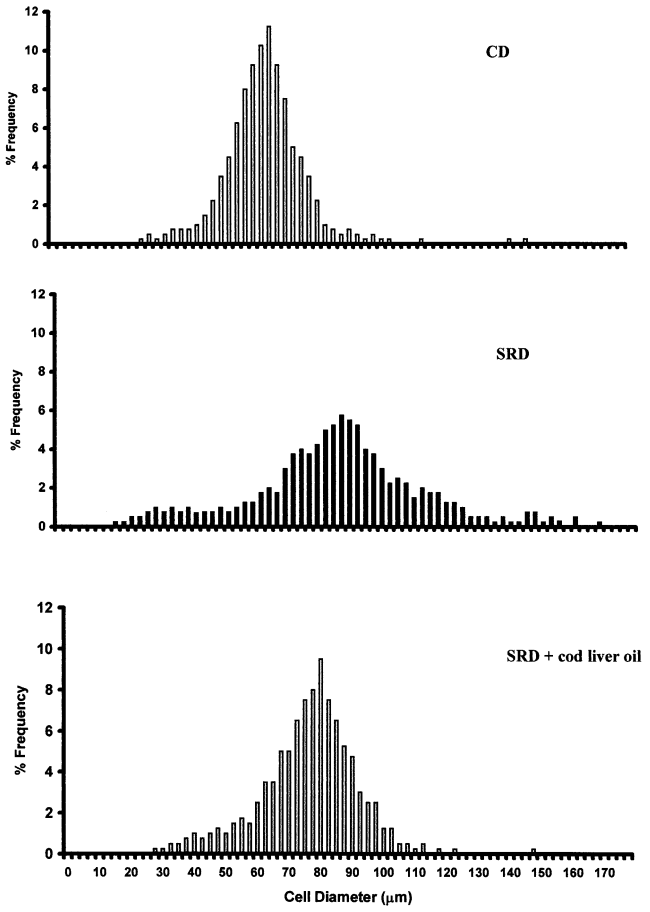


Fig. 2. Representative histograms distribution of adipocytes cells mean diameters isolated from the epididymal depots of rats fed a control diet (CD : days 1–120), sucrose-rich diet (SRD: days 1–120) or SRD (days 1–120) + cod liver oil (days 90–120). The histograms were constructed by sizing at intervals of 2.5 μm, 100 adipocytes from each individual rat. Eight animals were included in each experimental group. Columns represent the mean of the cell measured (percentage) which falls within a given size indicated.

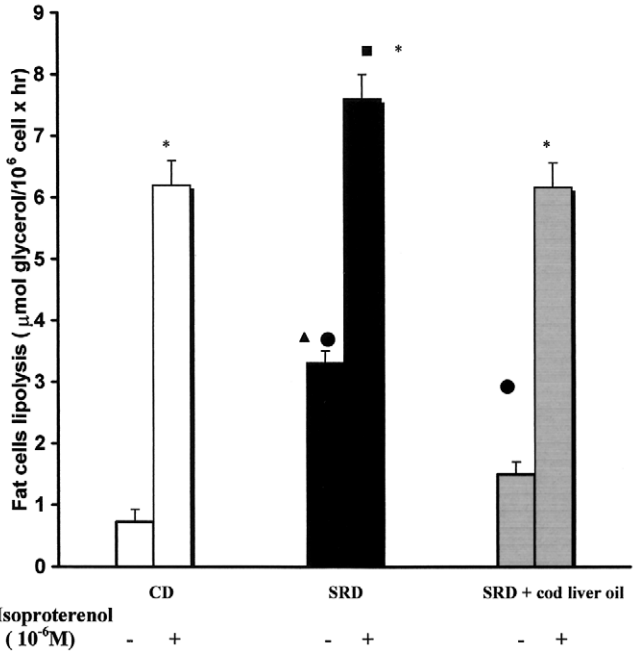


Fig. 3. Basal and isoproterenol stimulated lipolysis in isolated adipocytes from epididymal fat tissue of rats fed a control diet (CD: days1–120), sucrose-rich diet (SRD days 1–120), or SRD (days 1–120) + cod liver oil (days 90–120). Values are expressed as mean ± SEM. Six animals were included in each experimental group. Lipolysis was estimated as the glycerol release from the isolated adipocytes. For further details on the methodology see Materials and Methods. ▲ $p < 0.05$ SRD basal vs CD or SRD + cod liver oil basal. ● $p < 0.05$ SRD basal or SRD + cod liver oil basal vs CD basal. * $p < 0.05$ isoproterenol (10^{-6} M) vs their respective basal groups. ■ $p < 0.05$ SRD isoproterenol (10^{-6} M) vs CD isoproterenol (10^{-6} M) or SRD +cod liver oil isoproterenol (10^{-6} M).

On the other hand retroperitoneal fat tissue weight that was increased in the SRD fed rats, significantly decreased after fish oil administration. Values were as follows: mean ± SEM, n = 6, Total weight (g) 5.20 ± 0.29 in CD, 9.02 ± 0.44 in SRD and 6.90 ± 0.35 in SRD + cod liver oil; relative weight (g/100g of body weight): 1.30 ± 0.07 in CD, 2.15 ± 0.10 in SRD and 1.70 ± 0.08 in SRD + cod liver oil; $p < 0.05$ CD vs SRD and SRD + cod liver oil and $p < 0.05$ SRD vs SRD + cod liver oil.

3.4. Adipose cell size distribution

Fig. 2 shows the histograms of adipose cell size distribution (at 2.5 μm intervals) at the end of the experimental period. In the SRD group there was a clear differentiation in the cell size distribution with a significant increase (37%) of the mean cell diameter compared to the CD fed animals. The addition of cod liver oil to the SRD under the present experimental conditions resulted in a significant reduction of the cell size diameter. In this group cell size distribution approached that recorded in the CD group (Fig. 2).

3.5. Basal and stimulated lipolysis of the fat cells

As shown in Fig. 3 the basal lipolysis in enlarged fat cells of SRD fed rats was almost four-fold increased as compared to age matched controls fed a CD. When the source of fat -corn oil- was replaced by cod liver oil in the SRD, basal lipolysis significantly decreased although values remained slightly higher than the control. The isoproterenol stimulated rate of lipolysis was significantly greater than the basal rate ($p < 0.05$) in all dietary groups. The SRD group exhibited a stimulated rate of lipolysis significantly higher than those recorded in the adipocytes of rats fed both a CD or an SRD in which cod liver oil was added. However, the relative stimulation, fold increase over the basal, was lower in the SRD group (2.5 fold) than in the other dietary groups (6.5 and 4.0 fold in CD and SRD plus cod liver oil, respectively).

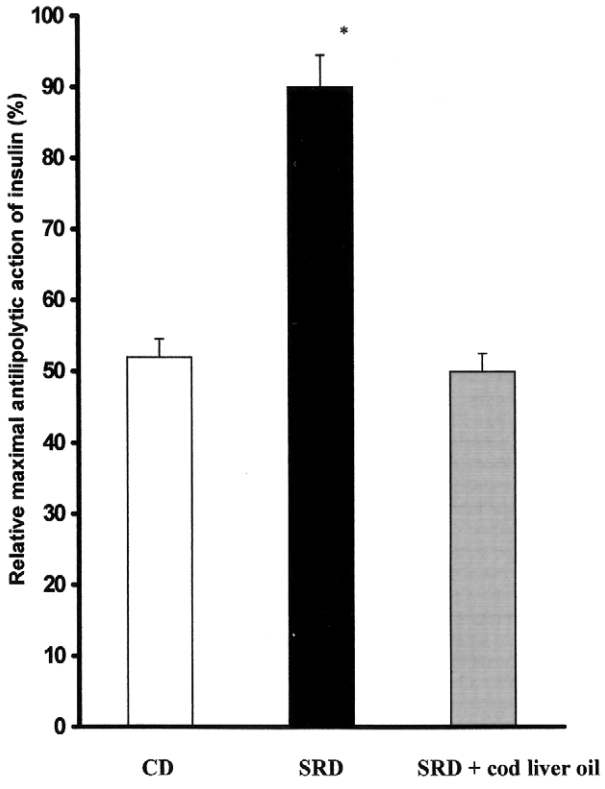


Fig. 4. Relative maximal antilipolytic action of insulin in isolated epididymal adipocytes of rats fed a control diet (CD: days 1–120), sucrose-rich diet (SRD days 1–120), or SRD (days 1–120) + cod liver oil (days 90–120). Values are expressed as mean ± SEM. Six animals were included in each experimental group. Adipocytes were incubated in triplicate with 10^{-7} M isoproterenol in the presence or absence of 2 nM of insulin. The results are expressed as the ratio between the value of the insulin inhibited-lipolysis and that of the isoproterenol-stimulated lipolysis in the absence of the insulin. * $p < 0.05$ SRD vs CD or SRD + cod liver oil.

3.6. Insulin-mediated inhibition of lipolysis

Fig. 4 shows the insulin-mediated suppression of isoproterenol-stimulated lipolysis. Compared to the CD fed rats, animals fed an SRD showed a decrease adipocyte sensitivity to the antilipolytic action of insulin. The hormone failed to inhibit β-agonist stimulated lipolysis in isolated epididymal fat cells. Otherwise, the addition of cod liver oil to the SRD from day 90 to 120 completely restored the sensitivity of adipocyte to the antilipolytic action of insulin.

4. Discussion

The present work focusses on the beneficial effect of fish oil to reverse the preexistent morphological and metabolic changes in the epididymal fat tissue of rats rendered dyslipemic and insulin resistant by feeding them with a sucrose-rich diet for a long time (up to 120 days). The major new findings of this study are the following: i) Dietary fish oil markedly reduced the fat pads mass, the hypertrophy of fat cells and improved the altered cell size distribution. ii) The presence of fish oil in the diet corrected the inhibitory effect of the high sucrose diet upon the antilipolytic action of insulin, reduced the “in vitro” enhanced basal lipolysis and normalized isoproterenol -stimulated lipolysis. Fat pads lipoprotein lipase activity decreased reaching values similar to those observed in age matched controls fed a control diet (CD). These effects were not accompanied by any change in rat body weight. Furthermore, in agreement with our previous results [14] the addition of fish oil was able to reverse the whole body peripheral insulin resistance, the abnormal glucose homeostasis, the elevated plasma triglyceride and free fatty acids without detectable changes in plasma insulin levels. All the changes mentioned above were obtained by shifting during four weeks the source of fat in the sucrose-rich diet from corn oil to cod liver oil.

Adipose tissue deposition is the result of the difference between energy intake and energy expenditure. Although the present work is mainly focused in the epididymal tissue our data show a decrease in both epididymal and retroperitoneal fat pads weight after the addition of cod liver oil to the SRD. The reduction of retroperitoneal tissue weight would indicate an important reduction of body lipid. On this regard, Newby et al. [31] demonstrated in Wistar rats a marked association between the growth of the retroperitoneal fat tissue and the accretion of body lipid. Rats fed a dietary fish oil showed food intake and body weight gain similar to both CD and SRD fed groups. Since carcass analyses were not performed in the present study, we are unable to discern if fish oil favors an increase of lean mass or body water or reduce energy expenditure per unit of lean mass.

The increased activities of lipoprotein lipase (LPL) and G-6-PDH found in the fat pad of SRD fed rats returned to normal values when cod liver oil replaced corn oil in the diet. An increase of LPL activity in epididymal adipose tissue was also observed by Dehaies [32] in rats fed an SRD. Moreover, no relationship between plasma insulin levels and LPL activity was recorded, suggesting that the level of insulin following intake of sucrose does not determine per se the extent of stimulation of white adipose tissue LPL by this nutrient [32]. Recently Raclot et al. [33] have shown that feeding n-3 PUFAs, mainly 22:6 n-3 decreased mRNA levels of several enzymes including FAS and LPL in retroperitoneal adipose tissue of rats, and Haug et al. [34] reported a reduction of adipose tissue LPL activity by fish oil feeding. Besides, a preferential oxidation and mobilization of n-3 PUFA in adipose tissue was observed by Jandacek et al. [35]. It is well known that hepatic lipogenesis is greatly enhanced by dietary sucrose. N-3 PUFA inhibits hepatic “de novo” lipogenesis in rodent by suppressing the mRNAs encoding several proteins involved in both lipogenesis and glucose metabolism such as acetyl-CoA carboxilase, FAS, G-6-PDH and L-PK [36,37]. The decrease of these enzyme activities by fish oil could be one of the mechanisms involved in the reversal of the hypertriglyceridemia induced

by long-term sucrose feeding. The mechanisms mentioned above could contribute to reduce the triglyceride content within the adipocytes after the administration of cod liver oil. Furthermore, in this dietary group adipocytes cell volume decreases and the abnormal cell size distribution tend to normalize without changes in total cell number population. On this regard, it has been shown that n-3 polyunsaturated fatty acids (EPA, DHA) bind and activate PPARγ expression in white adipose tissue. PPARγ activation, a key transcription factor involved in adipogenesis, results in a continuous remodeling of the adipose tissue [38]. A reduction of the adipose tissue mass and the relative expression of leptin mRNA as well as a decrease of both plasma levels of triglyceride and leptin was shown by Sebokova et al. [39] in rats fed during 3 weeks with a high sucrose or fat diets supplemented with fish oil. However, a difference with the present results is that in this study the main effect of fish oil was to prevent but not to reverse the described metabolic changes.

The next step of the present work was to examine the effect of fish oil on both basal and stimulated lipolysis and the antilipolytic effect of insulin in isolated adipocyte from epididymal tissue. At the end of the experimental period, the increase basal lipolysis recorded in the SRD fed group was markedly improved after the administration of fish oil, although it still remained high compared to CD fed rats. Isoproterenol stimulated lipolysis was significantly higher in adipocytes of rats fed an SRD, while the relative stimulation of glycerol release was lower than in the other dietary group. The addition of the fish oil in the SRD normalizes the stimulated lipolysis reaching values similar to those recorded in the CD group. It is known that enlarged adipocytes increases the HSL activity, e.g. basal lipolysis is elevated in fat cells of obese rats [40]. Moreover, it has been recently shown that lipolysis in fat cells reflects no only the HSL catalytic activity but also that the translocation of HSL to the surface of endogenous lipid droplets may play a role in the response to lipolytic agents [41]. Although in the present study HSL activity was not evaluated, we cannot discard the possibility that the observed decrease of relative stimulation of lipolysis in the SRD fed rats could take place through the mechanisms mentioned above. Regarding the effect of fish oil Rustan et al [42] showed a decrease of basal and stimulated lipolysis from epididymal and perirenal adipocytes of rats fed a high fat (lard) diet supplemented with n-3 fatty acids for 7 weeks compared to rats fed a lard diet for the same period of time. On the other hand, Parrish et al. [43] recorded a high lipolytic response to adrenergic stimulation in fat pads of rats fed a fish oil diet for a short period of time compared to a lard diet. An increase in the lipolytic response to noradrenaline in adipocytes from rats fed fish oil compared to sunflower oil was also observed within a relatively short time (1 week) on the diet. This suggests a general effect of n-3 PUFA in modulating the lipolytic cascade pathway [44]. Our study does not provide data concerning the mechanisms or cellular processes responsible for the normalization of the enhanced lipolysis observed in the adipocytes of SRD fed rats after fish oil administration. However, it has been shown that either sucrose or fructose diets induce an elevation of blood pressure maybe through a mechanism that includes the activation of the sympathetic nervous system [45]. The latter may contribute to the increased lipolytic activity. On the other hand, Huang et al. [46] showed that 3 weeks of fish oil supplementation significantly ameliorated the hypertension induced in fructose fed rats. This in turn could influence adipose tissue lipolysis. Besides, the significant reduction of the adipocyte cell sizes after the fish oil feeding could also contribute to the observed decreased lipolysis.

The administration of dietary fish oil was able to reverse the impaired antilipolytic action of insulin present in the fat pads of rats fed an SRD for a long term. On the other hand, Vrana et al. [47] showed the correcting effect of dietary n-3 fatty acids on insulin action in the adipocytes of rats fed a sucrose rich-fish oil diet for 3 weeks. Besides, stimulation of glucose transport and glucose oxidation was also observed by Luo et al. [12] in rats fed during 6 weeks with a sucrose rich diet in which fish oil (30%) was added.

The adipose tissue only accounts for a small fraction of the total glucose disposal in the intact body. However, it is a key tissue to supply fatty acid for the whole body utilization. Plasma free fatty acids exert an important modulator effect on insulin action [10]. The present results show that the effect of dietary fish oil upon morphological and metabolic changes of epididymal tissue was accompanied by reversing the preexistent whole body peripheral insulin insensitivity recorded in rats chronically fed an SRD. The normalization of glucose homeostasis and insulin resistance were in agreement with previous works of Lombardo et al. [14] and D'Alessandro et al. [15] using a similar experimental approach. However, Podolin et al. [8] recently demonstrated that dietary menhaden oil (6% of calories) was unable to reverse the preexisting sucrose induced insulin resistance. Differences in the amount of fish oil present in the diet (16 vs 6% of calories), as well as in the polyunsaturated-saturated ratio between the two fish oils (1.23 for cod liver vs 0.88 for menhaden oil), may contribute to the differences in the effectiveness of fish oil in reversing insulin insensitivity.

The mechanisms involved to explain the role of fish oil in the reversion of the preexisting metabolic and morphological changes in the fat pads of rats fed a high sucrose diet are still unknown. One explanation may be found on the hypolipidemic role of fish oil that could contribute to the restoration of glucose homeostasis and insulin sensitivity in insulin target tissues (e.g. adipose, skeletal muscle). Moreover, the consumption of n-3 fatty acids rapidly induces changes in the fatty acid composition of membrane phospholipids from both fat pads [12] and skeletal muscle of rats [48]. This may induce changes in structure and fluidity of cell membranes that could in turn affect insulin action [49].

Finally, the current study provides new information in-

dicating that in the presence of a stable dyslipidemia and insulin resistant environment, dietary fish oil is able to reverse the morphological and metabolic changes preexistent in fat pads of rats chronically fed an SRD. Moreover, the data clearly suggests that the reduction of fat pad adiposity, cell hypertrophy and lipolysis as well as the normalization of insulin action in epididymal and whole body peripheral tissue was due to fish oil administration. Although care must be taken when extrapolating results from rats to human beings, the dyslipemic rats fed a 16% of total calories as dietary fish oil constitute a useful animal model to study diet-regulated insulin action.

Acknowledgments

This investigation was carried out with the support of Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ministerio de Salud de la Nación and Universidad Nacional del Litoral, by Grants #: PICT #05–06960, PID #4129, Beca Ramón Carrillo- Arturo Oñativia/2000, CAI+D 12B/121/96.

The authors thank Norma Ferraris for her technical assistance. Andrea Rossi is a recipient of a fellowship "Ramón Carrillo-Arturo Oñativia" from the Department of Health, Argentina.

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