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Role of skeletal muscle on impaired insulin sensitivity in rats fed a sucrose-rich diet: Effect of moderate levels of dietary fish oil

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In the present study we investigated: (1) the contribution of the skeletal muscle to the mechanisms underlying the impaired glucose homeostasis and insulin sensitivity present in dyslipemic rats fed a sucrose-rich diet (SRD) over a long period of time and (2) the effect of fish oil on these parameters when there was a stable hypertriglyceridemia before the source of fat (corn oil) in the diet was replaced by isocaloric amounts of cod liver oil. Our results show an increased triglyceride content in the gastrocnemius muscle with an impaired capacity for glucose oxidation in the basal state and during euglycemic clamp. This was mainly due to a decrease of the active form of pyruvate dehydrogenase complex (PDHa) and an increase of PDH kinase activities. Hyperglycemia, normoinsulinemia, and diminished peripheral insulin sensitivity also were found. Even though there were no changes in the insulin levels, the former metabolic abnormalities were completely reversed when the source of fat was changed from corn oil to cod liver oil. The data also suggest that in the gastrocnemius muscle of rats fed a SRD over an extended period, an increased availability and oxidation of the lipid fuel, which in turn impairs the glucose oxidation, contributes to the abnormal glucose homeostasis and to the peripheral insulin insensitivity. Moreover, the parallel effect on insulin sensitivity, glucose, and lipid homeostasis attained through the manipulation of dietary fat (n-3) in the SRD suggests a role of n-3 fatty acid in the management of dyslipidemia and insulin resistance. (J. Nutr. Biochem. 11:273–280, 2000) *© Elsevier Science Inc. 2000. All rights reserved.*

Keywords: skeletal muscle; dyslipemia; insulin resistance; fish oil; (n-3) fatty acids; sucrose-rich diet

Introduction

Dietary factors play an important role in the development of insulin resistance. Substitution of dietary starch by an isocaloric amount of sucrose or fructose in normal Wistar rats for a short period of time (3–4 weeks) leads to hypertriglyceridemia, impaired glucose tolerance associated with hyperinsulinemia, and insulin resistance.[1,2] Our laboratory recently reported that when the diet was extended up to 15 to 30 weeks, we could observe a steady-state of hypertriglyceridemia, elevated plasma free fatty acids (FFA), and severe abnormal glucose homeostasis without detectable changes in the circulating insulin levels.[3]

Skeletal muscle is the major site of insulin-stimulated glucose disposal.[4] The regulation of the glucose metabolism in this tissue involves a complex interplay with other fuels, especially FFA. It is believed that insulin resistance is associated with an overabundance of lipids. Vräna et al.[5] showed that the glucose utilization was reduced, but palmitate oxidation increased, in the isolated hemidiaphragm of dyslipemic rats fed a high fructose diet for a short period of time (3–4 weeks). Moreover, an elevated triglyceride concentration within the muscle has been associated with

A preliminary report was presented at the XXXIII Annual Meeting of SAIB (Sociedad Argentina de Investigaciones Bioquímicas y Biología Molecular), November 14–17, 1997, Córdoba, Argentina.

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Received November 8, 1999; accepted February 16, 2000.

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655 Avenue of the Americas, New York, NY 10010

0955-2863/00/$–see front matter
PII S0955-2863(00)00078-4

Table 1 Composition of experimental diets*

Diet ingredients	Control (CD)		Sucrose−rich (SRD)		SRD + cod liver oil	
	% 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[†]	3.5		3.5		3.5	
Vitamin mix[‡]	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	0.0		0.0	
Sucrose	0.0		62.5	64.0	62.5	64.0
Corn oil	8.0	18.5	8.0	18.5	1.0	2.3
Cod liver oil	0.0		0.0		7.0	16.2

*Diets are based on the AIN-93 M diet.
[†]Salt mix based on salt mix AIN-93 M (in g/kg of diet): 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 selinate, 0.01025; ammonium paramolybdate, 0.00795; chromium potassium sulfate, 0.275.
[‡]Vitamin mix based on vitamin mix AIN-93 M (g/kg of diet): nicotinic acid, 3.000; Ca pantothenate, 1.600; pyridoxine-HCl, 0.700; thiamin-HCl, 0.600; riboflavin, 0.600; folic acid, 0.200; D-biotin, 0.020, vitamin B12 2,500; vitamin E (500 IU/g), 15.00; vitamin A (500,000 IU/g), 0.800; vitamin D_3 (400,000 IU), 0.250; vitamin K, 0.075.

insulin resistance in an experimental animal model of high fat fed rats[6] and also in people with non-insulin-dependent diabetes mellitus.[7]

The administration of fish oil rich in n-3 fatty acids has proved to be effective in lowering plasma triglyceride and lipoprotein (especially very low density lipoprotein) concentrations in experimental animals and in normal and in hypertriglyceridemic men.[8,9] A recent work by Lombardo et al.[10] showed that the hypertriglyceridemia, elevated plasma FFA, and glucose intolerance ensuing from feeding normal rats a sucrose-rich diet (SRD) for an extended period (15–18 weeks) could be completely reversed, mediating no change in circulating insulin levels, by shifting the source of fat in the diet from corn oil to cod liver oil. However, the mechanisms underlying the effect of long-term administration of a SRD on glucose homeostasis and insulin insensitivity still remains to be fully understood. Only scarce and partial[11] information is available regarding the contribution of the skeletal muscle (the main target of peripheral insulin resistance) to lipid and glucose utilization when stable hypertriglyceridemia, increased plasma FFA, moderate hyperglycemia, and impaired glucose tolerance were induced in rats by chronically feeding a SRD (15–18 weeks). To gather additional information on the mechanism behind the development of insulin resistance in this experimental model, we studied: (1) the triglyceride metabolism, glucose oxidation [estimated as the activity of the pyruvate dehydrogenase complex (PDHc)], and glycogen storage during both the basal state and during euglycemic hyperinsulinemic clamp in gastrocnemius (as a representative of the skeletal muscle) and (2) the replacement of corn oil by cod liver oil as the source of fat in the diet, and its effects on the parameters mentioned above in the presence of a stable hypertriglyceridemia achieved by long-term feeding of a SRD.

Materials and methods

Animals and diets

Male Wistar rats initially weighing 170 to 185 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) were used in the present study. Animals were housed and initially fed rat laboratory chow (Ralston Purina, St. Louis, MO USA) and water in an animal room with controlled temperature (22 ± 1°C), humidity, and air flow conditions, and with a fixed 12-hr light/dark cycle (light 7:00 AM–7:00 PM). After a 1-week acclimatization period, rats were randomly divided into two groups: control and experimental. The experimental group was fed a semi-synthetic SRD (62.5% w/w) whereas control rats received the same semi-synthetic diet except that sucrose was replaced by starch (62.5% w/w; high starch: CD). The experimental group received the SRD for 90 days, after which animals were randomly divided in two subgroups. The first subgroup (SRD) continued on the SRD up to 120 days. The second subgroup (SRD + cod liver oil) received a SRD in which the source of fat (corn oil 8% w/w) had been replaced by cod liver oil 7% w/w 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 are given in *Table 1*. Diets were isoenergetic (all 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.[10] 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, the animals in each subgroup were randomly divided in two halves. In the first group (animals used for clamp studies) all the rats were deprived of food for 5 hr before the experiment was performed. In the second group, food

was removed at 7:00 AM (the end of the dark period) and the experiment was performed between 7:00 and 8:00 AM. The experimental protocol was approved by the Human and Animal Research Investigation Committee of the School of Biochemistry, University of Litoral (Santa Fe, Argentina).

Analytical methods

Animals were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight). Blood samples obtained from the jugular vein were rapidly centrifuged at 4°C and the plasma either assayed immediately or stored at −20°C and examined within the following 3 days. Plasma triglyceride,[12] FFA,[13] glucose,[14] and lactate[14] levels were determined by spectrophotometric methods. The gastrocnemius muscle was rapidly removed from all the animals, pulverized in a mortar precooled in liquid nitrogen, and frozen. This tissue contained both oxidative and glycolytic fibers. Insulin binding to gastrocnemius membrane reflected insulin binding to the mixture of the whole muscle of the upper and lower extremities.[15] The homogenates of frozen muscle powder were used for the determination of triglyceride,[12] glycogen,[16] and proteins[17] and the activities of PDHc[18] and pyruvate dehydrogenase kinase (PDH kinase).[19]

Euglycemic clamp studies

Insulin sensitivity was measured using the euglycemic hyperinsulinemic clamp technique,[20] which had been used by our laboratory for an earlier study.[21] Briefly, after a 5-hr fasting period rats were anesthetized, a blood sample was withdrawn, and glucose,[14] insulin,[22] and lactate[14] levels were assessed. After that an infusion of highly purified porcine neutral insulin (Actrapid, Novo Nordisk Industry, Copenhagen, Denmark) at 0.8 U/Kg/hr for 2 hr was administered. Insulin was infused through one limb of a double lumen cannula connected to the left jugular vein. Blood samples for glucose assays were taken at 5- to 10-min intervals from the right jugular vein. Blood glucose concentration was measured using the glucose-oxidase method[14] (Glucomether Analyzer, Boehring Mannheim Co., Indianapolis, IN USA) within 2 min after the samples were obtained. Glycemia was maintained at an euglycemic level by injecting a 20% glucose solution at a variable rate through the limb of the double cannula. The glucose infusion began 5 min after insulin infusion had started. The glucose infusion rate (GIR) during the second hour of the clamp study was taken as the net steady-state of the whole-body glucose. In all studies, blood samples (0.3 mL) for insulin determination[22] were obtained at 60, 90, and 120 min. Samples for lactate,[14] triglyceride,[12] and FFA[13] plasma analysis were taken at 120 min. Hematocrit was measured at the start and at the end of each experiment. At the end of the clamp period, the gastrocnemius muscle was rapidly removed, frozen clamped in liquid nitrogen, and stored at −70°C for subsequent assays of triglyceride,[12] glycogen,[16] and protein[17] and the activities of PDHc[18] and PDH kinase.[19]

Extraction and assay of PDH and PDH kinase activities

Frozen gastrocnemius muscles were placed in 20 vol. (w/v) of ice-cold homogenizing buffer [50 mmol/L 2-hydroxyethyl-1-piperazinyl ethanol sulfonic acid (HEPES) buffer (pH 7.4) containing 3% Triton X-100, 2 mmol/L ethylenediamine-tetraacetic acid (EDTA), 5 mmol/L dithiothreitol, 0.5 mmol/L thiamine pyrophosphate, 2 mmol/L dichloroacetate (PDH kinase inhibitor), 50 mmol/L potassium fluoride (phosphoprotein phosphatase inhibitor), 2% bovine albumin, 0.1 mmol/L N-tosyl-L-phenylamine-chloromethyl ketone, 0.1 mg/mL trypsin inhibitor, and 0.02 mg/mL leupeptin] and homogenized with a Polytron homogenizer three times during 30 sec. After centrifugation, the supernatant was precipitated once with 9% polyethylene glycol.[18] The pellet was resuspended in the homogenizing buffer and divided to measure the active form of PDHc (PDHa) and the total PDHc (PDHt). One portion was used immediately to measure PDHa. For the measurement of PDHt, the other portion was incubated with broad specificity phosphoprotein phosphatase[23] in the presence of 10 mmol/L $MgCl_2$ at 30°C for 20 min. The activity of PDHc was spectrophotometrically determined at 30°C by measuring the reduction of NAD^+.[24] The complete assay mixture contained 30 mmol/L phosphate buffer (pH 7.4), 2.5 mmol/L NAD^+, 0.5 mmol/L coenzyme A (CoA), 0.5 mmol/L thiamine pyrophosphate, 0.5 mmol/L dithiothreitol, 5 mmol/L $MgCl_2$, 10 units pig heart dihydrolipoyl dehydrogenase, 0.5 mmol/L pyruvate, and appropriate amounts of PDHc (0.5–1.0 mg protein) in a final volume of 2 mL. The reaction was started by enzyme addition, and all assays were performed at 30°C. The PDH activity was expressed as nmoles of NADH formed per minute, per gram of wet weight tissue, and per milligram of soluble protein.

Isolation and assay of the PDH kinase was done as previously described by Popov et al.[19] Briefly, the complete reaction mixture containing 30 mmol/L HEPES, 1.5 mmol/L $MgCl_2$, 5 mmol/L dithiothreitol, 0.1 mmol/L EDTA, 0.05% (w/v) Triton X-100, 0.1 μmol/L leupeptin, 10 μg/mL trypsin inhibitor, 0.5 mmol/L adenosine triphosphate (ATP; pH 7.35), and 0.1 mg PDHc, in a final volume of 0.2 mL, was incubated at 30°C for 10 min. At various intervals (0–5 min), aliquots (20 μL) were removed and transferred into the PDH assay mixture and the residual PDH activity was spectrophotometrically measured, as previously described.[24] The apparent first-order rate constant for the ATP-dependent inactivation of the enzyme was calculated from a least-squares linear regression analysis of ln (inactivation by ATP) against time of incubation.

Statistical analysis

Results were expressed as mean ± SEM. The statistical significance between groups was determined by one-way analysis of variance followed by inspection of all differences between pairs of means by Newman-Keuls' test.[25] Differences having *P*-values lower than 0.05 were considered to be statistically significant.

Reagents

Enzymes for the assays, substrate, and coenzymes 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 of reagent grade.

Results

Comparable weight gain and food intake (expressed as caloric intake in kilojoules per day) were recorded in all groups of animals throughout the experimental period. Values were as follows (mean ± SEM, n = 8): caloric intake (kJ/day) 285.3 ± 22.1 in CD, 280.0 ± 19.5 in SRD, and 279.5 ± 27.0 in SRD + cod liver oil. The weight gain (g/day) was also comparable: 1.98 ± 0.05 in CD, 2.01 ± 0.07 in SRD, and 2.00 ± 0.08 in SRD + cod liver oil.

In agreement with previous reports from our laboratory[10] and confirmed by the present findings, the high plasma levels of triglycerides, FFA, and glucose reported in rats fed the SRD for either 90 or 120 days returned to control values when cod liver oil replaced corn oil in the diet from days 90

Table 2 Triglyceride and glycogen contents and pyruvate dehydrogenase (PDH) and PDH kinase activities in the gastrocnemius muscle of rats fed on the different diets

Diet	Triglyceride (μmol/g wet weight)	Glycogen* (μmol/g wet weight)	PDHa† (% of PDHt)	PDH kinase‡ (K/min)
Control diet (CD) (days 1–120)	2.90 ± 0.31[§,a]	18.10 ± 1.70[a]	34.00 ± 3.20[a]	1.50 ± 0.09[a]
Sucrose-rich diet (SRD) (days 1–120)	5.80 ± 0.42[b]	16.60 ± 1.62[a]	19.00 ± 2.40[b]	3.00 ± 0.40[b]
SRD (days 1–120) + cod liver oil (days 90–120)	3.10 ± 0.34[a]	18.70 ± 1.73[a]	29.40 ± 1.30[a]	1.60 ± 0.14[a]

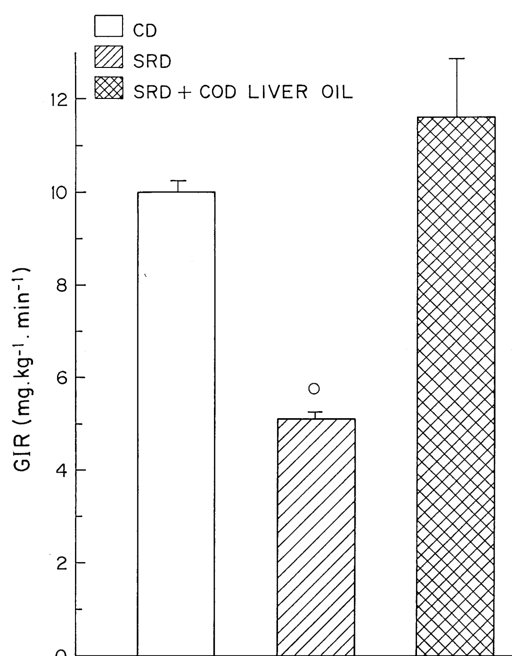
*Glycogen is expressed as μmol glucose— glycogen.
†PDHa–active form of the PDH complex, expressed as percentage of the total PDH activity PDHa:basal activity × 100/total activity).
‡PDH kinase activity was assayed as determining the adenosine triphosphate-dependent inactivation of PDH activity as a function of time (K/min) and was calculated from the first order-kinetic constant.
§Values are expressed as mean ± SEM (six animals are 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 is compared by the Newman-Keuls' test.

to 120. Values at the end of the dark period (basal state) were as follows (mean ± SEM, $n = 6$): triglycerides (mmol/L): 0.51 ± 0.03 in CD, 1.80 ± 0.11 in SRD, and 0.49 ± 0.08 in SRD + cod liver oil; FFA (mmol/L): 0.28 ± 0.03 in CD, 0.66 ± 0.02 in SRD, and 0.30 ± 0.02 in SRD + cod liver oil; glucose (mmol/L): 6.10 ± 0.07 in CD, 8.10 ± 0.10 in SRD, and 5.90 ± 0.08 in SRD + cod liver oil. Comparable plasma immunoreactive insulin (IRI) levels were recorded in all groups of animals (μU/mL): 43.1 ± 6.1 in CD, 54.3 ± 4.9 in SRD, and 47.5 ± 5.2 in SRD + cod liver oil.

The hypertriglyceridemia shown in the SRD group was accompanied by a significant increase of muscle (gastrocnemius) triglyceride levels without significant changes in glycogen concentration (*Table 2*). The present results show that these changes were accompanied by both a significant decrease of PDHa and increase of PDH kinase activities when compared with values obtained from age-matched control rats fed a CD. When the source of fat in the diet (corn oil) was replaced by cod liver oil for 30 days, all these parameters returned to normal values. Moreover, the total PDH activity, expressed on a wet weight basis or relative to milligrams of soluble protein, remained unchanged (data not shown).

At the end of the experimental period euglycemic hyperinsulinemic clamp studies were performed to assess the effect of dietary substitution on peripheral insulin sensitivity (insulin resistance levels). Blood glucose was clamped at 5.5 to 6.0 mmol/L. Values of 5-hr postprandial blood glucose (mmol/L) before clamp were (mean ± SEM, $n = 6$): CD 5.40 ± 0.20, SRD 7.60 ± 0.15, and SRD + cod liver oil 5.30 ± 0.18. These results were not associated with changes in plasma insulin concentration (μU/mL; mean ± SEM, $n = 6$): CD 41.5 ± 7.1; SRD 44.0 ± 6.1; SRD + cod liver oil 48.2 ± 5.5. The steady-state of blood glucose and insulin concentrations measured over the last 60 min of the clamp are illustrated in the table included in *Figure 1*. The GIR, which measures insulin action "in vivo," was significantly ($P < 0.01$) decreased in animals fed the SRD up to 120 days compared with rats fed on the CD for the same period (*Figure 1*). GIR returned to a similar value to that observed in rats fed the CD in the group of SRD rats where cod liver oil replaced corn oil from days 90 to 120 (*Figure 1*). No differences in hematocrit values were observed either within or between the groups from the beginning to the end of the clamp.

Table 3 shows that after a short period of fasting (5 hr of food withdrawal) at the beginning (0 min) of the clamp,



DIET	CD	SRD	SRD+COD LIVER OIL
GLUCOSE (mmol/L)	5.70 ± 0.24	5.90±0.29	5.98±0.06
INSULIN (μU/mL)	702±39	762±36	716±35

Figure 1 Glucose infusion rates during euglycemic-hyperinsulinemic clamp in rats fed the different diets. Values are expressed as mean ± SEM. Six animals are used in each experimental group. ○: $P < 0.05$ SRD versus CD or SRD + cod liver oil; Glucose and insulin: diet steady state of blood glucose and insulin concentration, respectively, during the last 60 min of the clamp; GIR: glucose infusion rate; CD: control diet (days 1–120); SRD: sucrose-rich diet (days 1–120); SRD + cod liver oil: SRD (days 1–120) + cod liver oil (days 90–120).

Table 3 Plasma triglyceride and free fatty acid (FFA) levels in rats fed the different diets at the beginning (0 min) and at the end (120 min) of the euglycemic hyperinsulinemic clamp

Diet	Triglyceride (mmol/L)		FFA (mmol/L)	
	Beginning	End	Beginning	End
Control diet (CD) (days 1–120)	0.58 ± 0.06*[,a]	0.15 ± 0.03[a]	0.47 ± 0.03[a]	0.15 ± 0.02[a]
Sucrose-rich diet (SRD) (days 1–120)	1.55 ± 0.02[b]	0.12 ± 0.02[a]	0.82 ± 0.02[b]	0.32 ± 0.04[b]
SRD (days 1–120) + cod liver oil (days 90–120)	0.50 ± 0.06[a]	0.13 ± 0.02[a]	0.49 ± 0.02[a]	0.16 ± 0.01[a]

*Values are expressed as mean ± SEM (six animals are 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 is compared by the Newman-Keuls' test.

plasma levels of triglyceride were similar, whereas plasma FFA levels were slightly increased in all dietary groups compared with the values observed at the basal state (see second paragraph results). At the end of the clamp studies, triglyceride levels fell to similar values in all groups of animals. However, the decrease in the SRD group was 10-fold whereas it was only 4-fold in CD and SRD + cod liver oil compared with the values obtained at the beginning of the clamp studies. Levels of FFA decreased approximately three times in all groups compared with the initial values. Note, however, that the values in SRD group were still significantly different from the other two groups. Plasma lactate levels were statistically higher ($P < 0.01$) at the end of the clamp studies in the SRD group than in the other groups. Values were as follows (mean ± SEM, $n = 6$ mmol/L): CD 1.26 ± 0.25; SRD 3.20 ± 0.20; SRD + cod liver oil 1.69 ± 0.19.

Table 4 shows that triglyceride and glycogen contents in the gastrocnemius muscle at the beginning of the clamp were similar in the three dietary groups to that observed at the basal state (see *Table 2*).

At the end of the clamp, muscle triglyceride levels reached similar values in all groups of animals. An interesting finding was that in the rats fed on the CD and SRD + cod liver oil, the increases were approximately 145% compared with the values recorded at the beginning of the clamp, whereas in the SRD group the increase was only 15%. Under the euglycemic-hyperinsulinemic clamp the muscle glycogen mass increased in rats fed a CD, whereas a significant decrease in glycogen pool store was observed in the rats fed a SRD. The muscle glycogen concentration of animals fed the SRD to which cod liver oil was added from days 90 to 120 also reached values similar to those recorded in rats fed the CD. The impaired whole-body glucose utilization (low GIR) observed in the rats fed a SRD was accompanied by a significant decline of glucose oxidation (estimated from the activation state of PDHc) in the gastrocnemius muscle. PDHa was significantly lower and PDH kinase activities were higher both at the beginning and at the end of the clamp (*Table 5*). No differences in the PDHa and PDH kinase activities were observed in CD and SRD + cod liver oil fed groups. Values recorded were similar to those obtained at the beginning of the clamp.

Discussion

Skeletal muscle is the most important single tissue for insulin-mediated glucose disposal. In the gastrocnemius muscle of rats rendered dyslipemic by feeding them a SRD for long time, the present study showed: (1) an increased triglyceride content within the skeletal muscle cells, (2) an impaired capacity for glucose oxidation in the basal state and during an euglycemic, hyperinsulinemic clamp [mainly due to an increase of PDH kinase and a decrease of PDHa (active form of PDHc) activities], and (3) an impaired insulin-stimulated glycogen storage during the clamp. All

Table 4 Triglyceride and glycogen contents in gastrocnemius muscle in rats fed the different diets at the beginning (0 min) and at the end (120 min) of the euglycemic hyperinsulinemic clamp

Diet	Triglyceride (μmol/g wet weight)		Glycogen* (μmol/g wet weight)	
	Beginning	End	Beginning	End
Control diet (CD) (days 1–120)	2.70 ± 0.30[†,a]	6.60 ± 0.92[a]	17.60 ± 1.50[a]	25.80 ± 0.50[a]
Sucrose-rich diet (SRD) (days 1–120)	5.60 ± 0.35[b]	6.50 ± 0.40[a]	16.00 ± 1.70[a]	10.70 ± 0.90[b]
SRD (days 1–120) + cod liver oil (days 90–120)	3.00 ± 0.30[a]	7.10 ± 0.82[a]	17.50 ± 1.60[a]	26.00 ± 0.60[a]

*Glycogen is expressed as μmol glucose−glycogen.
[†]Values are expressed as mean ± SEM (six animals are 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 is compared by the Newman-Keuls' test.

Table 5 Pyruvate dehydrogenase (PDH) and pyruvate dehydrogenase kinase (PDH kinase) activities in gastrocnemius muscle in rats fed on the different diets at the beginning (0 min) and at the end (120 min) of the euglycemic hyperinsulinemic clamp

Diet	PDHa* (% of PDHt)		PDH kinase† (K/min)	
	Beginning	End	Beginning	End
Control diet (CD) (days 1–120)	33.50 ± 1.50‡,a	29.60 ± 1.40a	1.42 ± 0.09a	1.30 ± 0.07a
Sucrose-rich diet (SRD) (days 1–120)	19.60 ± 3.00b	22.80 ± 1.60b	2.96 ± 0.38b	2.40 ± 0.18b
SRD (days 1–120) + cod liver oil (days 90–120)	30.00 ± 2.30a	30.50 ± 2.20a	1.48 ± 0.17a	1.30 ± 0.14a

*PDHa: active form of PDH complex, expressed as percentage of total PDH activity (PDHa: basal activity × 100/total activity).
†PDH kinase activity was assayed as determining the adenosine triphosphate-dependent inactivation of PDH activity as a function of time (K/min) and was calculated from the first-order-kinetic constant.
‡Values are expressed as mean ± SEM (six animals are 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 is compared by the Newman-Keuls' test.

this is accompanied by a sharp increase of plasma FFA, mild hyperglycemia in the presence of normoinsulinemia, and a sustained impairment of whole-body peripheral glucose utilization. These findings support the role of the skeletal muscle in the insulin resistance that develops in this animal model. Moreover, the metabolic abnormalities mentioned above were completely reversed when cod liver oil replaced corn oil as the source of fat in the diet, even though no changes were recorded in the circulating insulin levels.

Intracellular triglyceride is an important energy source for the skeletal muscle. However, Storlien et al.[26] recently demonstrated in rats that insulin action in skeletal muscle deteriorated in situations where triglyceride supply was high. This was shown in both rats fed for 3 weeks a high-fat diet (59% of calories as fat) where mainly skeletal muscle triglyceride increased and in rats fed for 3 weeks a high-fructose or sucrose diet where the circulating triglyceride was increased.[26]

In the present study using a high sucrose diet but extending the administration of the diet up to 15 to 18 weeks, we were able to further this observation by showing that the hypertriglyceridemia was also accompanied by a substantial increase of triglyceride stores in the gastrocnemius muscle. The difference between our data (increased triglyceride both in plasma and skeletal muscle) and that of Storlien et al. (high fructose or sucrose diet) could be explained by the different metabolic and hormonal milieu present in rats fed a SRD for a short (3–4 weeks) or a long (15–30 weeks) period, which might affect the skeletal muscle fuel utilization. For instance, although hypertriglyceridemia is a common feature in both periods, a large increase of plasma FFA is only recorded after 15 to 30 weeks on a SRD. Moreover, normoglycemia and hyperinsulinemia present after a short time of feeding evolve to moderate hyperglycemia and normoinsulinemia after a long time on the SRD.[1,3]

The increase of muscle triglyceride stores was accompanied by both a low PDHa and a significant increase of PDH kinase activities in the basal state. These results suggest that an increased availability and oxidation of fatty acids, which increase the mitochondrial acetyl-CoA/CoASH and NADH/NAD^+ ratios stimulating PDH kinase, would be the mechanism responsible for impaired glucose oxidation in the gastrocnemius of rats chronically fed with a SRD. Indeed, an increased availability of FFA provided both through the circulating triglyceride and FFA as well as from the dynamic triglyceride store within the muscle are significantly increased in SRD-fed rats. In addition, it has been recently postulated that long chain acyl CoA concentration plays a role in the control of malonyl CoA levels in tissues including skeletal muscle. Both these CoA derivatives were proposed as important regulators of fat and glucose metabolism in this tissue. Malonyl CoA seems to be a component of a fuel sensing and signaling mechanism that responds to changes in the fuel milieu and energy expenditure of the muscle cells.[27] Changes in the level of this metabolite could also contribute to the abnormal fuel utilization recorder in skeletal muscle ensuing by long-term SRD.

Insulin stimulates glucose uptake, glycolysis, and glycogen synthesis in the skeletal muscle. In the present study the euglycemic clamp was performed at supraphysiologic doses of insulin. Therefore, it could be expected to result in a near maximal rate of insulin-stimulated glucose uptake. Under these conditions, the significant decrease of glucose required to maintain the euglycemic clamp in rats fed a SRD compared with age-matched controls fed a CD indicates a marked whole-body insulin insensitivity. As expected, the gastrocnemius of CD fed rats at the end of the clamp showed a significant increase of triglycerides and glycogen.[28] These parameters overtook the values observed at the beginning of the clamp. A different picture was observed in the gastrocnemius of rats fed a SRD. In these animals, even in spite of the higher availability of circulating triglyceride and FFA, muscle triglyceride store was unchanged at the end of the clamp. Moreover, the low PDHc and the increase of PDH kinase activities suggest that the gastrocnemius muscle continues to preferentially use fatty acids as a source of energy and that the impaired glucose oxidation observed at the basal state was still present at the end of the clamp. In addition, glycogen mass was significantly lower at the end of the clamp compared with the values recorded at the beginning of the clamp.

Relative impairment of insulin-stimulated glycogen synthesis associated with muscle lipid accumulation[29] could be

due to decreased activation of the enzyme glycogen synthase. Recently Klimes et al.[30] reported an impaired response of glycogen synthase activity to insulin "in vitro" in soleus muscle of rats fed a SRD for a short period of time (3–4 weeks). They also observed a significant inverse correlation between insulin-stimulated glycogen synthase activity and muscle triglyceride content. At present, we are unaware of any publication concerning glycogen synthase activity—either basal or insulin-stimulated conditions—in skeletal muscle of rats fed a SRD for an extended period. However, the high triglyceride store and the lack of increase of the net glycogen mass during the euglycemic clamp might suggest an impairment of insulin-stimulated glycogen synthase activity in the gastrocnemius muscle of the SRD fed rats.

Insulin promotes glucose transport in skeletal muscle. Insulin deficiency from untreated streptozotocin-induced diabetic rats adversely affected the glucose transport system in this tissue, reducing both basal and maximum insulin-stimulated glucose transport and the number of transporter molecules.[31] An impaired glucose transport, possibly due to reduced amounts of Glut4, was observed in rats fed a high-fat diet.[32] In addition, Glut4 protein levels in muscle remained unchanged in an insulin resistant state such as db/db mice or hyperinsulinemic hypertensive rats.[33,34] Therefore, we cannot discard the possibility that altered glucose transporters and/or their activity in the gastrocnemius and/or skeletal muscles in general might also contribute to the impaired insulin sensitivity observed in the SRD fed rats.

Previous reports from our laboratory,[10] confirmed by the present findings, indicated that changes in the fat composition (from corn oil to cod liver oil) of the diet in rats fed a SRD over an extended period lead to a normalization of plasma triglyceride, FFA, and glucose levels without changes in plasma insulin concentration. In addition, in this work it was shown that: (1) the whole body peripheral glucose utilization returned to normal by the administration of cod liver oil and (2) in the gastrocnemius muscle oxidative and storage components of insulin-stimulated glucose disposal achieved normal values when shifting the source of fat from corn oil to cod liver oil in the SRD. This was accompanied by normalization of triglyceride stores within the muscle cells.

The mechanisms involved in the effects of fish oil on insulin action are still unclear. However, the normalization of circulating plasma triglyceride and FFA as well as tissue triglyceride contents achieved by the administration of cod liver oil could contribute to the restoration of glucose homeostasis and insulin sensitivity in the skeletal muscle. On the other hand, consumption of n-3 fatty acids may induce changes in structure and fluidity of cells membranes that could in turn affect insulin action.[35] Possible mechanisms include modulation of the focal protein-lipid interactions (e.g., affecting insulin receptors affinities and/or translocation and intrinsic activity of the glucose transporters) or yielding diacylglycerol with specific conformations that make them particularly potent intracellular second messengers of insulin action.[36–39] These in turn suggest that mechanisms other than competition for oxidative substrates could also play a role in the restoration of glucose metabolism and oxidation in the gastrocnemius muscle as well as in the whole-body glucose peripheral utilization in rats fed a SRD over an extended period.

Finally, our data suggest that in the gastrocnemius muscle of rats fed a SRD over an extended period an increased availability and oxidation of lipid fuel, which in turn impairs glucose oxidation, and decreased insulin-stimulated glycogen stores contribute to the abnormal glucose homeostasis and to the peripheral insulin insensitivity. Furthermore, in the presence of a stable hypertriglyceridemia the parallel effect on insulin sensitivity, glucose, and lipid homeostasis attained through the manipulation of dietary fat (n-3) in the SRD suggests a role of n-3 fatty acid in the management of these metabolic abnormalities.

Acknowledgments

This investigation was carried out with the support of Conicet (Consejo Nacional de Investigaciones Científicas y Tecnológicas), Agencia Nacional de Promoción Científica y Tecnológica SECYT-Conicet, and Universidad Nacional del Litoral; by grants # PID 4129, PICT # 05-0921, CAI+D, 12/B121/96; and with financial aid from the A.J. Roemmer Foundation for Biochemistry Research, Argentina.

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