Soya protein reverses dyslipidaemia and the altered capacity of insulin-stimulated glucose utilization in the skeletal muscle of sucrose-rich diet-fed rats

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The present study investigates the benefits of dietary intake of soya protein upon dyslipidaemia and insulin resistance in rats chronically (8 months) fed a sucrose-rich (63 %) diet (SRD). For this purpose, we analysed the effectiveness of soya protein isolate in improving or reversing these metabolic abnormalities. Wistar rats were fed a SRD for 4 months. By the end of this period, stable dyslipidaemia and insulin resistance were present in the animals. From months 4 to 8, half the animals continued with the SRD and the other half were fed a SRD in which the source of protein casein was substituted by soya. The control group received a diet in which the source of carbohydrate was maize starch. The results showed that: (1) soya protein normalized plasma TAG, cholesterol and NEFA levels in the SRD-fed rats. Moreover, the addition of soya protein reversed the hepatic steatosis. (2) Glucose homeostasis was normalized without changes in circulating insulin levels. Whole-body peripheral insulin sensitivity substantially improved. Besides, soya protein moderately decreases body weight gain limiting the accretion of visceral fat. (3) By shifting the source of dietary protein from casein to soya during the last 4 months of the feeding period it was possible to reverse both the diminished insulin-stimulated glucose oxidation and disposal in the skeletal muscle of SRD-fed rats. This study provides new data showing the beneficial effect of soya protein upon lipid and glucose homeostasis in the experimental model of dyslipidaemia and insulin resistance.

Soya protein: Dyslipidaemia: Insulin resistance: Skeletal muscle

Insulin resistance is a major key factor in the aetiology of the plurimetabolic syndrome cluster disease which includes type 2 diabetes, dyslipidaemia, hypertension, obesity and CVD, among other disturbances[1]. Dietary factors undoubtedly influence insulin action and play an important role in the prevention or improvement of these metabolic disorders. In recent years, there has been considerable interest in the effect of dietary soya protein on human health[2]. This is based on accumulated evidence that the addition of soya in the diet or the substitution of soya protein for animal protein has been shown to reduce plasma total and LDL- cholesterol and TAG concentrations in man and laboratory animals[3,4]. Tovar *et al.*[5] showed in Zucker obese fa/fa rats that soya protein consumption reduced the accumulation of cholesterol and TAG in the liver, preventing the development of fatty livers. The reduction in hepatic cholesterol was associated with a low expression of liver X receptor α and its target genes (e.g. 7α-hydroxylase and ATP binding cassette A1). Besides, soya protein also decreased lipogenesis through a decrease in the expression of the sterol regulatory element-binding protein-1 (SREBP1) and several of its target enzymes[5,6]. Furthermore, the reduction of hepatic lipids could also be the result of an increase in fatty acid oxidation since soya protein increases PPARα and carnitin palmityl

transferase 1 expression[5]. Dietary soya protein could also improve glucose homeostasis and insulin sensitivity[6–11]. In this vein, Taha & Wasif[12] reported that in aloxan diabetic hypercholesterolaemic rats dietary soya flour lowered the elevated plasma glucose level. Davis *et al.*[13] demonstrated that feeding SHHF (+cp) rats with a soya protein diet for 36 weeks significantly lowered body and liver weights, plasma total cholesterol and both fasting blood glucose and plasma insulin compared to rats fed casein. Moreover, in animal models of obesity, soya protein ingestion limits or reduces body fat accumulation and improves insulin resistance (for a review see Velásquez & Bhathena[14]).

Studies from our laboratory[15–18] have reported that rats chronically fed (3–8 months) with a diet rich in sucrose (SRD) developed a stable dyslipidaemia (increased plasma TAG and plasma NEFA levels), moderate hyperglycaemia and insulin resistance. This was accompanied by moderate overweight and visceral adiposity. Islets isolated from these rats showed insulin secretion patterns characterized by the absence of the first peak and enhancement of the second phase of the hormone released in response to glucose. In addition to an ectopic fat deposition in the liver, heart and pancreas tissues, an increase of the lipid storage (TAG, long-chain acyl-CoA, diacylglycerol) as well as the membrane protein

kinase C θ mass expression and impaired glucose metabolism was observed in the skeletal muscle of the SRD-fed rats[19,20]. Interestingly, this animal model exhibits many of the hallmarks present in the plurimetabolic syndrome in man. Several studies examined the effect of dietary soya protein on the SRD-induced insulin resistance and dyslipidaemia in rats. In this regard, Lavigne et al. [21] showed that in Wistar rats fed a diet containing 55 % of sucrose during 4 weeks, soya protein lowered fasting glucose and insulin concentration, and improved peripheral insulin sensitivity compared to rats fed casein. Pfeuffer & Barth[22] demonstrated that in rats fed the SRD during 7 weeks, the plasma cholesterol concentration and the rates of secretion of VLDL- cholesterol and TAG were lower when soya protein, compared to casein, was the source of dietary protein. The type of protein may improve the metabolic consequences of the consumption of a high-sucrose diet. However, to our knowledge there have been no studies focusing on the possible beneficial effects of dietary soya protein on improving or reversing both insulin insensitivity and lipid metabolism in rats fed a SRD for a long time, in which a well-established dyslipidaemia and insulin resistance was present before the source of dietary protein casein was isoenergetically replaced by soya. To test this hypothesis, the present work was designed to evaluate: (1) several aspects involved in lipid metabolism; (2) the whole-body peripheral insulin sensitivity and its possible relationship with the lipid-lowering effects of soya protein; and (3) since the skeletal muscle is the major site of insulin-stimulated glucose disposal, we analysed the glucose oxidation (estimated by the activity of the pyruvate dehydrogenase complex (PDHc)), TAG and glycogen storages and glucose-6-phosphate concentration both at the basal state and during a euglycaemic–hyperinsulinaemic clamp in the gastrocnemius muscle (as a representative of the skeletal muscle).

Materials and methods

Animals and diets

Male Wistar rats initially weighing 170–185 g and purchased from the National Institute of Pharmacology (Buenos Aires, Argentina), were maintained in the animal room under controlled temperature (22 ± 1°C), humidity and air flow condition, with a fixed 12 h light–dark cycle (light 07.00 to 19.00 hours). They were initially fed a standard non-purified diet containing by weight (g/100 g: 63 maize starch, sorghum, wheat, oats, barley), 22 protein, 3·5 fat, 6 fibre, 1 vitamin mixture and 4 salt mixtures (Ralston, Purina, St Louis, MO, USA). After 1 week of acclimation period, they were randomly divided into two groups of rats (control and experimental). The experimental group (n 78) received a purified SRD containing by weight (g/100 g): 62·5 sucrose, 18 casein-free vitamins, 7 maize oil, 7·5 cellulose, 3·5 salt mixture (AIN-93M-MX), 1 vitamin mixture (AIN-93-VX), 0·2 choline chloride, 0·3 DL-methionine[19]. The control group (n 30) received the same purified diet but with sucrose replaced by maize starch ((high-starch diet, control diet (CD)). Both groups received each diet for 4 months after which the SRD group of rats was randomly subdivided into three subgroups. The first subgroup (n 18) was immediately killed for each experiment as described later. The rats in the second subgroup (n 30) continued on the SRD up to 8 months of feeding. The third subgroup (n 30; SRD-S) received the SRD in which the source of protein (casein 18 g/100 g) had been replaced by soya protein isolate (MP Biomedicals, Solon, OH, USA) for the next 4 months. The control group was fed with the CD throughout the experimental period. Details on the composition of the diets are given in Table 1.The preparation and handling of the diets have been reported elsewhere[19]. Diets were isoenergetic, providing approximately 16·3 kJ/g food and were available ad libitum. Diets were prepared every week. The weight of each animal and the energy intake were recorded twice per week during the experimental period in all groups and subgroups of rats. At the end of the experimental period, food was removed at 07.00 hours (end of the dark period), and unless otherwise indicated experiments were performed between 07.00 and 09.00 hours. At least six rats from the three dietary groups were used in each procedure. They were anaesthetized with intraperitoneal pentobarbital Na (600 mg/kg body weight). Blood samples were obtained from the jugular vein and rapidly centrifuged; plasma was either immediately assayed

Table 1. Composition of the experimental diets (based on the AIN-93 diet)

Diet ingredients	Control diet		SRD		SRD-S§	
	g/100 g	% Energy	g/100 g	% Energy	g/100 g	% Energy
Maize starch	62·5	65	–	–	–	–
Sucrose	–	–	62·5	65	62·5	65
Casein-free vitamin	18	19	18	19	–	–
Soya protein	–	–	–	–	18	19
Maize oil	7	16	7	16	7	16
Vitamin mix‖	1		1		1	
Cellulose	7·5		7·5		7·5	
Salt mix¶	3·5		3·5		3·5	
Choline bitartrate	0·2		0·2		0·2	
DL-Methionine	0·3		0·3		0·3	

SRD, sucrose-rich diet; SRD-S, SRD with soya protein.
§ Soya protein isolate (MP Biomedicals, Solon, OH, USA). Soya protein isolate composition (g/100 g): protein, 92·0; water, 6·0; ash, 4·1; fat, 0·8; fibre, 0·25; carbohydrate, 2·85; Ca, 0·15; P, 0·8; K, 0·05; Na, 1·3. Trypsin inhibitor, 4·0–7·3 mg/g.
‖ AIN-93-VX.
¶ AIN-93M-MX.

or stored at $-20°C$. The liver, skeletal muscle (gastocnemius), epididymal and retroperitoneal adipose tissue were totally removed, weighed and immediately frozen and stored at the temperature of liquid N_2. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina.

Analytical methods

Plasma TAG, NEFA, total cholesterol and glucose levels were determined by spectrophotometric methods as previously described[23]. The inmunoreactive insulin was measured by the method proposed by Herbert *et al.*[24]. The immunoreactive insulin assays were calibrated against rat insulin standard (Novo Nordisk, Bagsvaerd, Denmark)[24]. The TAG and total cholesterol[25] content were determined in homogenates of the frozen liver.

TAG secretion rate

The TAG secretion rate was evaluated in fasting rats (16–18 h) by blocking the removal of plasma TAG with Triton WR 1339 (600 mg/kg body weight) dissolved in 0·9 % NaCl. The TAG secretion rate was calculated from the linear increase of TAG *v.* time, following the procedure described by Lombardo *et al.*[26].

Intravenous fat tolerance test

The intravenous fat tolerance test was performed in rats fasted for 16–18 h[27] by injecting intravenous Intralipid® (Intralipid® 10 % at 0·1 ml/100 g body weight), a soyabean oil fat emulsion, as previously described[23]. The first-order rate constant (K_2) of elimination of fat emulsion from the bloodstream (fractional removal rate) was calculated by the least squares methods as described elsewhere[23].

Euglycaemic clamp studies

Whole-body peripheral insulin sensitivity was measured using the euglycaemic–hyperinsulinaemic clamp technique as previously described elsewhere[19]. Briefly, after 5 h of food deprivation twelve rats from each dietary group were anaesthetized, a blood sample was withdrawn, and glucose and insulin levels were assessed. The gastrocnemius muscle of six rats from each group was rapidly removed (starting clamp studies), frozen, clamped in liquid N_2 and stored at $-80°C$ for the determination of TAG, glycogen, glucose-6-phosphate, protein and the activity of pyruvate dehydrogenase complex (PDHc)[19,28]. In the other six rats, an infusion of highly purified porcine neutral insulin (Actrapid; Novo Nordisk, Bagsvard, Denmark) was administered at 0·8 units/(kg × h) for 2 h. Glycaemia was maintained at a euglycaemic level by infusing glucose (200 g/l) at a variable rate. The glucose infusion began 5 min after the insulin infusion started. The blood glucose concentration was measured using a Glucomether Analyzer (Boehringer Mannheim, Indianapolis, IN, USA) within 2 min of the samples being obtained. The glucose infusion rate 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[24] were obtained at 60, 90 and 120 min. Details of the methodology have been previously given[19]. At the end of the clamp period, the gastrocnemius muscle was rapidly removed, frozen in liquid N_2, and stored at $-80°C$ for subsequent analysis of metabolites and enzyme activity as described earlier.

Extraction and assays for pyruvate dehydrogenase complex activity

The extraction of PDHc from the gastrocnemius muscles was described in detail elsewhere[19,20]. The activity of PDHc was spectrophotometrically determined at 30°C by measuring the reduction of NAD^{+}[19,28]. The PDHc activity was expressed as nmol NADH formed/min per g wet weight tissue and per mg soluble protein.

Statistical analysis

Sample sizes were calculated on the basis of measurements previously made with rats fed either a CD or a SRD[15,16,18,29], considering an 80 % power. Results were expressed as means and their standard errors. Statistical comparisons were done transversely between different dietary groups. The statistical significance between groups was determined by one-way ANOVA, with one factor (diet) followed by the inspection of all differences between pairs of means by the Newman–Keuls test. Differences having *P* values lower than 0·05 were considered to be statistically significant[30]. In all cases the interclass correlation coefficients were at least 0·73.

Results

Body weight gain and energy intake

Body weight and energy intake were carefully monitored in all groups of rats throughout the experimental period. Table 2 depicts the body weight gain and energy intake recorded in each experimental group. As previously shown[19], comparable weight-gain and energy intake were recorded in SRD and CD rats during the first 4 months on their respective diets. However, a significant increase in both parameters was observed in the rats that continued with the SRD up to 8 months. When the source of dietary protein in the SRD was replaced for the last 4 months on the diet (SRD-S), both energy intake and weight gain remained similar to those observed in the rats fed the CD. Moreover, the increased visceral adiposity (epididymal weight, g/100 g body weight) observed in the SRD-fed rats was improved when the source of protein in the diet was soya instead of casein. Values were as follows (six animals per group): 1·83 (SEM 0·11) in the CD-fed rats, 2·77 (SEM 0·14) in the SRD and 2·39 (SEM 0·09) in the SRD-S ($P < 0.001$ in SRD *v.* CD; $P < 0.05$ in the SRD *v.* SRD-S; $P < 0.01$ in SRD-S *v.* CD). A similar pattern was observed in retroperitoneal weight in each experimental group (data not shown).

Table 2. Body weight gain and energy intake of rats fed on control diet (CD), sucrose-rich diet (SRD) or the SRD with soya protein (SRD-S)§

(Mean values with their standard errors)

Diet	Initial body wt (g)		Body wt at month 4 (g)		Energy intake (initial to month 4) (kJ/d)		Diet for months 4–8	Body wt at month 4 (g)		Body wt at month 8 (g)		Energy intake (months 4–8) (kJ/d)	
	Mean	SEM	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	Mean	SEM
CD (n 30)	181·6	4·6	400·0	8·1	265·8	6·2	CD (n 30)	407·0	7·7	478·6	13·2	264·8	6·3
SRD (n 78)	185·3	4·4	415·1	8·3	263·7	3·6	SRD (n 30)	417·5	7·2	525·1**	10·7	334·7***	8·4
							SRD-S (n 30)	408·7	9·7	492·9	7·2	270·0	4·8

Mean values were significantly different from those of the CD and SRD-S: **$P < 0.01$, ***$P < 0.001$.
§ For details of procedures and diets, see the Materials and methods section and Table 1.

Plasma metabolite levels

After the 4- and 8-month feeding periods, plasma TAG, NEFA, total cholesterol and glucose levels were significantly higher in the SRD-fed rats than in the CD group ($P < 0.01$) (Table 3). The presence of soya protein in the SRD (instead of casein) for the last 4 months of the diet was able to return all the listed metabolites to the levels observed in the CD-fed rats. Interestingly, as mentioned earlier, rats fed SRD-S showed a significant decrease of body weight when compared to SRD-fed rats. Therefore, an analysis of covariance between plasma glucose levels and final body weight in SRD and SRD-S rats was performed. The results showed r 0·84, $P < 0.01$ (n 12). At the end of the experimental period, no statistically significant differences in plasma insulin levels were observed among all dietary groups.

Liver cholesterol and TAG content, TAG secretion rate and fractional removal rate of fat emulsion

The rats fed the SRD for 4 or 8 months showed a significant increase ($P < 0.001$) of TAG content and in vivo TAG secretion rate associated with a decrease of fractional removal rate (K_2, %/min) of the intravenously injected fat emulsion (Intralipid®) compared to age-matched CD-fed rats (Table 4). The substitution of casein for soya protein in the SRD for the last 4 months on the diet significantly decreased liver TAG content and liver TAG secretion rate and increased the removal rate of TAG from circulation, with values similar to those observed in the CD-fed rats (Table 4). Moreover,

the SRD-fed rats showed an increase of liver total cholesterol content that was completely normalized when the casein present in the SRD rats was replaced by soya protein during the last 4 months on the diet. Values were as follows (six animals per group): 3·51 (SEM 0·08) μmol/g wet tissue in the CD-fed rats, 4·81 (SEM 0·07) in the SRD 4 months, 4·95 (SEM 0·09) in the SRD 8 months and 3·48 (SEM 0·05) in the SRD-S ($P < 0.001$ in SRD 4 and in SRD 8 months v. CD and SRD-S).

Glucose infusion rate

In order to assess the effect of soya protein on whole-body peripheral insulin sensitivity (insulin resistance), a euglycaemic–hyperinsulinaemic clamp study was performed in rats fed CD, SRD and SRD-S, at 8 months on the diet. Blood glucose was clamped at 5·5–6·0 mm. Postprandial glucose concentration at 5 h before the clamp was as follows (six rats per group): 5·85 (SEM 0·15) mm in rats fed CD, 7·87 (SEM 0·12) in the SRD group and 6·1 (SEM 0·22) in the SRD-S group ($P < 0.001$ in SRD v. CD and SRD-S). In all dietary groups, plasma insulin levels were similar to those obtained at the end of the dark period (data not shown). Glucose infusion rate was significantly decreased ($P < 0.01$) in the SRD-fed rats compared to age-matched CD-fed rats. Moreover, although glucose infusion rate was significantly higher in SRD-S compared to the SRD-fed group ($P < 0.01$), values were still significantly different ($P < 0.05$) from those recorded in the CD-fed rats (Fig. 1). There were no differences in haematocrit from the start to the end of the clamp (data not shown).

Table 3. Plasma TAG, NEFA, total cholesterol, glucose and insulin levels of rats fed the control diet (CD), sucrose-rich diet (SRD) or the SRD with soya protein (SRD-S)§

(Mean values with their standard errors for six animals per group)

Diet	TAG (mm)		NEFA (μm)		Total cholesterol (mm)		Glucose (mm)		Insulin (μU/ml)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CD (8 months)	0·64	0·04	310·0	10·0	2·18	0·08	6·00	0·17	70·2	5·3
SRD (4 months)	1·40**	0·09	715·0**	52·0	3·09**	0·07	7·95**	0·19	73·9	6·5
SRD (8 months)	1·35**	0·08	730·0**	80·0	3·24**	0·08	8·20**	0·19	76·3	7·7
SRD (4 months) + SRD-S (4 months)	0·64	0·07	320·0	40·0	2·08	0·15	6·72	0·10	80·4	7·4

Mean values were significantly different from those of the CD and SRD-S: **$P < 0.01$.
§ For details of procedures and diets, see the Materials and methods section and Table 1.

Table 4. Liver TAG content, TAG secretion rate (TGSR) and fractional removal rate of fat emulsion (K_2) of rats fed a control diet (CD), sucrose-rich diet (SRD) or the SRD with soya protein (SRD-S)§

(Mean values with their standard errors for six animals per group)

Diet	Liver TAG (μmol/g wet tissue)		TGSR (nmol/min per 100 g body wt)		K_2 (%/min)	
	Mean	SEM	Mean	SEM	Mean	SEM
CD (8 months)	12·80	0·53	152·0	6·4	8·60	1·30
SRD (4 months)	20·42†††	0·91	207·2†††	4·5	5·10*	0·45
SRD (8 months)	22·35†††	1·61	211·0†††	5·1	4·30**	0·40
SRD (4 months) + SRD-S (4 months)	10·33	0·65	157·1	2·6	8·90‡	0·60

Mean values were significantly different from those of the CD: *$P < 0.05$, **$P < 0.01$.
Mean values were significantly different from those of the CD and SRD-S: †††$P < 0.001$.
Mean values were significantly different from those of the SRD (4 months) and SRD (8 months): ‡$P < 0.001$.
§ For details of procedures and diets, see the Materials and methods section and Table 1.

Muscle TAG, glucose-6-phosphate and glycogen content, and pyruvate dehydrogenase complex activity at the start of euglycaemic–hyperinsulinaemic clamp studies

At the start of the clamp (0 min), the SRD-fed rats showed a significant increase of gastrocnemius muscle TAG levels without changes in glycogen and glucose-6-phosphate content and

Fig. 1. Glucose infusion rate (GIR) during the euglycaemic–hyperinsulinaemic clamp in rats fed control diet (CD), sucrose-rich diet (SRD) or SRD with soya protein (SRD-S) at the end of the experimental period. Values are means with their standard errors depicted by vertical bars (six animals per group). [a,b,c] Mean values with unlike letters were significantly different ($P < 0.05$).
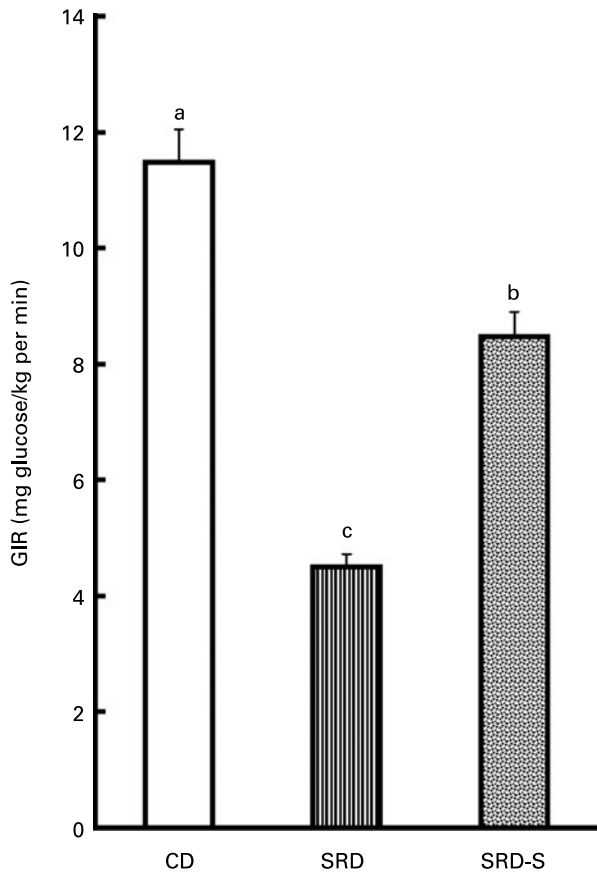
a significant decrease of activity of PDHa compared to CD-fed rats (Table 5). When the source of protein in the SRD (casein) was replaced by soya protein for the last 4 months of the diet, no changes in glycogen and glucose-6-phosphate levels were recorded among the groups. Furthermore, TAG content and the PDHa activity returned to control values. No differences were observed in total PDHc activity in gastrocnemius muscle of rats fed on the different diets expressed both as either mU/g wet tissue or mU/mg protein (data not shown). A similar pattern was observed in all dietary groups at the end of the dark period (data not shown).

Muscle TAG, glucose-6-phosphate and glycogen content, and pyruvate dehydrogenase complex activity at the end of euglycaemic–hyperinsulinaemic clamp studies

At the end of the clamp study, muscle TAG contents reached values similar in all three dietary groups. Interestingly, this increase was up to 70–80 % in both the CD and the SRD-S but only 6 % in the SRD-fed rats compared to the basal state (Fig. 2(A)). Insulin stimulated muscle glycogen and glucose-6-phosphate deposition in the CD-fed rats. However, no significant changes in these metabolites were observed in the SRD-fed group. When the source of protein in the SRD (casein) was replaced by soya in the last 4 months of the experimental period both glucose-6-phosphate and glycogen content increased, reaching values similar to those observed in the CD-fed rats (Fig. 2(B, C)). No changes in PDHa activity were observed under insulin stimulation among all three dietary groups at the end of the clamp (data not shown).

Discussion

In the present study, soya protein was administered instead of casein as a dietary source of protein to analyse the benefits of this nutritional intervention on a non-genetic model of stable dyslipidaemia and insulin insensitivity induced in normal rats by feeding them with a SRD for a long period (8 months). The new major findings of the present work are the following: (1) soya protein markedly reduced the *in vivo* hepatic TAG secretion rate and enhanced its removal from the circulation leading to a normalization of plasma TAG levels. Besides, both plasma cholesterol and NEFA levels returned to normal values when soya protein replaced the casein present in the

Table 5. Gastrocnemius TAG, glucose-6-phosphate and glycogen content, and pyruvate dehydrogenase complex (PDHa) activity in rats fed a control diet (CD), sucrose-rich diet (SRD) or SRD with soya protein (SRD-S)§

(Mean values with their standard errors for six animals per group)

| Diet	TAG (μmol/g wet tissue)		Glycogen (μmol/g wet tissue)		Glucose-6-phosphate (μmol/g wet tissue)		PDHa activity (% of total PDHa)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CD (8 months)	3·84	0·36	4·70	0·18	0·44	0·05	37·6	2·3
SRD (8 months)	7·25**	0·81	5·20	0·40	0·40	0·05	19·2***	1·5
SRD (4 months) + SRD-S (4 months)	4·12	0·55	4·14	0·43	0·50	0·02	38·6	1·1

Mean values were significantly different from those of the CD and SRD-S: **$P<0.01$, ***$P<0.001$.
§ For details of procedures and diets, see Materials and Methods section and Table 1.

SRD during the last 4 months of the experimental period. Moreover, the addition of soya protein reversed the hepatic steatosis present in the rats fed a SRD. (2) Glucose homeostasis returned to normal values despite the fact that no significant changes occurred in the circulating insulin levels. Whole-body peripheral insulin sensitivity substantially improved, reaching values approaching those of the CD group. Besides, soya protein moderately decreased body weight gain, limiting the accretion of visceral fat. (3) By shifting the source of dietary protein from casein to soya during the last 4 months of the feeding period, it was possible to reverse both the diminished capacity of insulin-stimulated glucose oxidation and disposal in the skeletal muscle of SRD-fed rats.

Confirming previous studies[15–17], an increase of the VLDL-TAG secretion rate and a defective removal of TAG from the circulation led to a steady state of hypertriglyceridaemia in rats chronically (up to 8 months) fed a SRD. Diets rich in sucrose or fructose induced the activities of key enzymes involved in lipogenesis through an activation of the gene expression of SREBP-1 and decreasing the activities of the enzymes related to fatty acid oxidation by down-regulation of PPARα resulting in an increase of both VLDL-TAG secretion and liver TAG storage. Besides, Soria et al.[29] demonstrated that in long-term SRD-fed rats there is an increased release of NEFA from adipose tissue to non-adipose tissue that in turn could enhance hepatic sterification of NEFA to TAG and also contribute to the development of liver steatosis. In the present study on rats fed a SRD, in which during the last 4 months of the feeding period casein was replaced by soya protein, it is shown that the substitution for this macronutrient plays a key role in the changes observed in lipid metabolism and insulin sensitivity. Both the VLDL-TAG secretion rate and liver TAG and cholesterol depot were completely normalized, possibly through the mechanism that included opposite changes in SREBP1 and PPARα expression leading to a significant decrease of hepatic de novo lipogenesis[5]. In this regard, there is evidence that soya protein prevents the overexpression of SREBP1 and several of its target genes such as fatty acid synthase, malic enzyme, stearoyl-CoA desaturase 1 and Δ5 and Δ6 desaturases
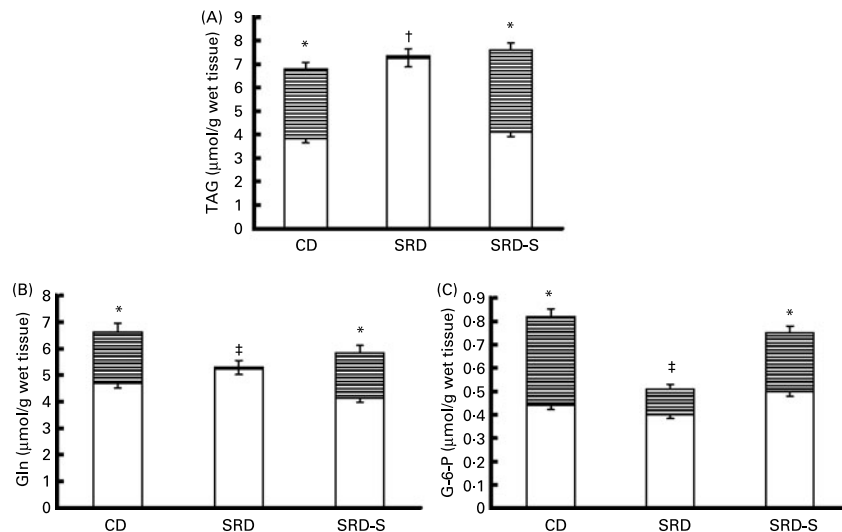
Fig. 2. TAG (A), glycogen (Gln; B) and glucose-6-phosphate (G-6-P; C) levels in gastrocnemius muscle at the start (0 min; □) and at the end (120 min; ▤) of the clamp studies in rats fed control diet (CD), sucrose-rich diet (SRD) or SRD with soya protein (SRD-S) at the end of the experimental period. Values are means, at the start and at the end of the clamp, with their standard errors depicted by vertical bars (six animals per group). Mean values were significantly different from those of CD or SRD-S rats at the start v. the end of the clamp: *$P<0.05$. Mean values were significantly different from those of SRD rats at the start v. either CD or SRD-S rats at the start of the clamp: †$P<0.05$. Mean values were significantly different from those of SRD rats at the end v. either CD or SRD-S rats at the end of the clamp: ‡$P<0.05$.

decreasing lipid accumulation[5]. Furthermore, plasma TAG levels and clearance reached values similar to those recorded in CD-fed rats. Besides, the addition of soya protein decreased the excessive availability of plasma NEFA. This, in turn, contributed to decrease the synthesis and secretion of VLDL-TAG from the liver, limiting the formation of LDL particles and, therefore, reducing plasma TAG and cholesterol levels. In addition to the reduction of lipogenesis, Tovar *et al.*[5] showed that in rats fed soya protein the reduction of serum cholesterol was associated with a stimulation of bile acid synthesis possibly through the induction of the enzyme cholesterol 7α-hydroxylase 1. Interestingly, in line with the present results, *in vitro* studies by Sugano *et al.*[31] showed a decrease of hepatic secretion of TAG and cholesterol in rats fed soya protein in a SRD during a short period of time (few weeks) when compared to those fed casein. Comparable data were recorded by Pfeuffer & Barth[32] in rats at different ages (10 or 25 weeks old). Thus, in the SRD-fed rats, the reversion of dyslipidaemia suggests that the principal action of soya protein on hepatic lipid metabolism involves a shift from lipid synthesis and storage to lipid oxidation and, therefore, both mechanisms contribute to the lipid-lowering effect of soya protein. However, we cannot discard the possibility that other mechanisms such as changes in insulin to glucagon ratio, amino acid composition, a rise in thyroid hormone levels as well as the presence of other components in the soya protein including lipids, fibre and isoflavones could also contribute to the hypolipidaemic effect on blood and liver in the SRD-fed rats[7,14,33–37].

Previous studies using different animal models of obesity demonstrated that dietary soya protein diminished body weight and visceral fat[14]. In the present work, visceral adiposity was significantly reduced as well as the increases of weight gain and energy intake recorded in long-term SRD-fed rats when soya protein replaced casein as a source of dietary protein. Besides, all these parameters reached values similar to those recorded in the CD-fed animals. The present results do not provide data concerning the mechanism involved in the improvement of visceral adiposity and body weight after soya protein administration in the SRD-fed rats. Moreover, since the animals were fed *ad libitum*, it is worth considering the risk of uncontrollable bias associated with body weight changes between the groups. On the other hand, in agreement with the present data, Torre-Villalvazo *et al.*[38] demonstrated that normal Wistar rats fed a soya protein in a high-fat diet gained less weight than those fed casein, in part due to an increase of their thermogenic capacity mediated by mitochondrial uncoupling protein 1. Iritani *et al.*[39] also recorded a lower body gain weight in Wistar fatty rats fed a soya protein during a short period of time compared to those fed casein. In these rats, an increase of triiodothyronine production seemed to play a role in the reduction of body weight. Furthermore, Tovar *et al.*[5] showed that dietary soya protein reduced the SREBP1 expression in adipocytes of ZDF fa/fa rats preventing their hypertrophy.

As previously shown elsewhere[15] and now confirmed in the present study, long-term SRD feeding to rats develops abnormal glucose homeostasis and whole-body insulin insensitivity without changes in basal plasma insulin levels. Insulin resistance is strongly correlated with an increase of both plasma TAG and NEFA levels and muscle TAG storage. In this regard, dyslipidaemia and a substantial increase in TAG concentration within the gastrocnemius muscle were shown in rats fed a long-term SRD. Skeletal muscle is the most important tissue for insulin-mediated glucose disposal. In the present work, the euglycaemic clamp was performed at supraphysiological doses of insulin. Therefore, it could be expected to result in a near maximal rate of insulin-stimulated glucose uptake. Under this experimental condition in the SRD-fed rats, the decreased capacity of glucose oxidation in the basal state and during the euglycaemic clamp, mainly due to a significant reduction of the PDHa activity, was completely normalized when soya replaced casein as a source of protein. Dietary soya protein reversed the impaired insulin-stimulated glycogen storage (glycogen concentrations reached values similar to those of the CD group), as well as hyperglycaemia, and substantially improved the whole-body peripheral insulin resistance. Additionally, as mentioned earlier, plasma NEFA levels returned to normal values, indicating a reduced spillover of this metabolite from adipose tissue to non-adipose tissue including skeletal muscle, mainly by increased insulin sensitivity. Thus, the present data suggest that the hypolipidaemic effect of soya protein decreases the availability and oxidation of lipid fuel within the skeletal muscle of SRD-fed rats and could in turn restore glucose oxidation and contribute to increase peripheral insulin sensitivity[40]. In this vein, Lavigne *et al.*[21], during a euglycaemic–hyperinsulinaemic clamp, have recently shown that both glucose disposal and plasma glucose disappearance rates were higher in rats fed soya protein in a SRD for a short period of time (4 weeks) compared to those fed casein. Furthermore, in these rats soya protein decreased plasma TAG levels. However, we cannot discard the possibility that other proposed mechanisms by which soya protein may improve glucose homeostasis and insulin action could contribute to the present findings. Among these mechanisms, there is experimental evidence suggesting that soya protein improves insulin resistance and lipid levels by activating PPAR[7,8]. Iritani *et al.*[9] reported that dietary soya protein in a saturated high-fat diet improved insulin sensitivity by increasing insulin receptor mRNA level in liver and adipose tissue, while plasma glucose levels were not significantly affected. Moreover, differences in the amino acid composition of dietary proteins have also been suggested to mediate protein-dependent changes in glucose and insulin dynamics[10,11].

In brief, the present study provides new data showing that in SRD-fed rats, in the presence of a stable dyslipidaemia and insulin resistance, the parallel effect on insulin sensitivity, glucose and lipid homeostasis and visceral adiposity attained through the manipulation of dietary soya protein suggests a role for this macronutrient in the management of these metabolic abnormalities.

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