Eur J Nutr (2013) 52:789–797
DOI 10.1007/s00394-012-0385-6

ORIGINAL CONTRIBUTION

Water-soluble rice bran enzymatic extract attenuates dyslipidemia, hypertension and insulin resistance in obese Zucker rats

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Received: 2 March 2012 / Accepted: 17 May 2012 / Published online: 4 June 2012

Abstract
Background and purpose Rice bran enzymatic extract (RBEE) has advantages compared to the original rice bran or its oils including water solubility, lack of rancidity and increased content in high nutritional proteins and nutraceutical compounds, particularly phytosterols, γ-oryzanol and tocols. Our aim was to determine the beneficial effects of RBEE in the pathogenesis of metabolic syndrome in obese Zucker rats.
Methods Obese Zucker rats and their lean littermates were fed a 1 and 5 % RBEE-supplemented diet (O1, O5, L1 and L5). Simultaneously, obese and lean Zucker rats, fed a standard diet, were used as controls (OC and LC, respectively). Body weight, food and water intake, and systolic blood pressure were weekly evaluated. After treatment, biochemical assays of serum glucose, insulin, triglycerides (TG), total cholesterol (TC), non-esterified fatty acids (NEFA), adiponectin and nitrates ($NO_{(x)}$) were determined.
Results RBEE treatment reduced circulating levels of TG and TC, whereas increased HDL-cholesterol without altering NEFA values in obese rats. The extract also induced a significant dose-dependent reduction of hypertension linked to obesity. RBEE of 5 % improved insulin resistance and subsequently reduced HOMA-IR index without altering serum glucose levels. Obese animals treated with RBEE showed partial restoration of adiponectin levels and a significant attenuation of pro-inflammatory values of $NO_{(x)}$.
Conclusion These findings evidence the nutraceutical properties of RBEE against the pathogenesis of metabolic syndrome by attenuating dyslipidemia, hypertension and insulin resistance as well as by restoring hypoadiponectinemia associated to obesity.

Keywords Rice bran · Metabolic syndrome · Dyslipidemia · Hypertension · Insulin resistance · Obese Zucker rats

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Introduction

Rice bran (RB) is a by-product of the rice milling, which derives from the outer layer of the rice grain. It is composed by the aleurone layer of the rice kernel and some part of the endosperm and germ. RB is an important source of fat, proteins and bioactive molecules with special interest as antioxidants and lipid-lowering compounds including γ-oryzanol (a mixture of ferulic acid esters of triterpene alcohols and sterols), tocols (tocopherols and tocotrienols) and unsaturated fatty acids [1, 2]. Chemical studies indicate that RB is specially rich in the phenolic compounds oryzanol and ferulic acid, which have demonstrated hypolipidemic effects—reducing total plasma cholesterol (TC) and triglyceride (TG) levels, and increasing high-density lipoprotein (HDL) levels—by mechanisms related to a strong antioxidant activity in rodents, rabbits, primates and humans [3]. In addition, proteins from RB are highly nutritional and have functional properties. Particularly, their characteristic activity as being hypoallergenic, hypocholesterolemic and anti-tumoral make them a superior

cereal protein that may find a wide range of applications (see review [4]).

Although RB shows an important composition in natural antioxidants and nutritional proteins [1, 4], its potential use as a functional food is limited by high insolubility of its protein as well as the integrity of its nutraceutical components, particularly referring to the phenolic fraction. Therefore, in the last years, oil derived from RB has been the most common form considered to study the therapeutic potential of RB. In fact, RB oil has demonstrated hypolipidemic [5–9] and hypoglycemic activities [10–13] in rodents and humans. Despite of these promising properties of RB oils, tendency to rancidity and the difficulty of administration have made necessary to obtain RB extracts with better physical and chemical properties.

Now, thanks to a novel enzymatic process it has been produced a water-soluble rice bran enzymatic extract (RBEE), which preserves functional properties and improves solubility of proteins and antioxidant components of RB [14, 15]. Besides, lipase inactivation during this enzymatic extraction avoids the problem of rancidity of RB [15]. The enzymatic treatment also increases concentrations of protein and minor functional components, especially γ-oryzanol and tocols, which are more than threefold higher compared to the original raw material RB [15, 16].

The preparation of products enriched with the antioxidant, hypolipidemic and hypoglycemic components of RB, which is the case for RBEE, may be of great interest for the treatment of chronic diseases showing oxidative stress, lipid and glucose/insulin disturbances as well as cardiovascular disorders. Metabolic syndrome has been described as a combination of several of these clinically specific risk features including obesity (central adiposity), dyslipidemia, insulin resistance, glucose intolerance, hypertension and non-alcoholic fatty liver disease and is becoming an important health problem worldwide [17, 18]. It has been established that dietary and physical activity are the first choice for improving or alleviating metabolic syndrome symptoms [19, 20]. Thus, the investigation of food components that may deal with the metabolic syndrome features is an important field to facilitate dietary-based therapies. Nowadays, dietary sources of natural antioxidants are of great interest due to the described association of the manifestations of metabolic syndrome and oxidative stress [20, 21].

Therefore, the potential antioxidant profile of RB and its components joined with their positive effects on lipid and glucose metabolism, led us to evaluate for the first time the effects of RB, in form of the water-soluble enzymatic extract RBEE, in the main clinical and biochemical manifestations of the metabolic syndrome developed in obese Zucker rats.

Materials and methods

Preparation and composition of RBEE

RBEE was prepared according to an enzymatic process previously described [15]. Briefly, RB was modified by enzymatic hydrolysis by using an endoprotease mixture as hydrolytic agent in a bioreactor with controlled temperature (60 °C) and pH (pH 8) and using the pH-stat method. The processing of this product follows different steps including centrifugation, filtration and concentration. The final product is brown syrup completely soluble in water. RBEE was chemically characterized by using AOAC standard protocols (Association of Official Analytical Chemists).

Chemical composition of RBEE has been previously characterized by Parrado et al. [15, 16]. Briefly, protein is the major component (38 %) in the form of peptides and free aminoacids due to the use of proteases for RB stabilization and with the aim of extract, solubilize and hydrolyze the initial insoluble proteins. The fat components present in RBEE (30 %) are mainly soluble because of protein interactions. Minor functional components of lipid fraction in RBEE include phytosterols (4,084 mg/kg), γ-oryzanol (1,260 mg/kg), tocopherols (99 mg/kg) and tocotrienols (174 mg/kg).

Animals and diets

Obese Zucker rats and their littermate controls, lean Zucker rats (8 weeks aged, Charles River Laboratories, Barcelona, Spain), were fed standard diet and water ad libitum. Obese and lean rats were divided into three groups and daily treated with either 1 % RBEE (O1 and L1) and 5 % RBEE supplementation (O5 and L5), or standard diet (SD) (OC and LC) ($n = 7$, each). Treatment with RBEE was administered during 20 weeks as a syrup form included in SD, supplemented with the concentrations indicated above. RBEE was extracted and supplied by the Enzymatic Production Technology group of the Department of Biochemistry and Molecular Biology, School of Pharmacy, University of Seville (Spain).

Body weight, food and water intake, and systolic blood pressure were weekly evaluated. Blood pressure and heart rate were measured by the pneumatic "*tail-cuff*" method with pressure meter (Niprem 645, Cibertec, Madrid, Spain). At the end of treatment, the animals were kept during 12 h fasting and were anesthetized with chloral hydrate 12 % intraperitoneally. Blood samples were collected by intracardiac puncture for biochemical assays in serum. The animals were then sacrificed, and visceral and epididymal adipose tissue (VAT and EAT, respectively), heart, pancreas, spleen, skeletal muscle and liver were

removed and weighted. The protocol for animal handling and experimentation agreed with the European Union European Community guidelines for the ethical treatment of animals (EEEC Directive of 1986; 86/609/EEC) and was approved by the Ethical Committee for Animal Research of the University of Seville.

Blood biochemical assays

Serum samples were obtained from blood by centrifugation for 20 min at 4,000 rpm and room temperature. Fasting glucose, total- and HDL-cholesterol were assessed by UV/visible spectrophotometry kits (Spin React, CIMA Diagnostics, Girona, Spain). TG and non-esterified fatty acids (NEFA) were also determined by commercial kits (WAKO Diagnostics, Richmond, VA, USA). Plasma adiponectin and insulin were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits (B-Bridge International, Otsuka, Japan; Millipore, Missouri, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) and glucose tolerance was calculated as previously described [22]. Serum levels of nitric oxide metabolites ($NO_{(x)}$) were determined by using nitrate reductase to specifically reduce nitrate to nitrite; the latter was quantified by a colorimetric assay using the Griess reagent. Absorbance was measured spectrophotometrically at 550 nm [23].

Measurement of liver TG and TC

Extraction of lipids from livers was based on the method of Folch et al. [24]. Briefly, liver tissue (150 mg) was homogenized in 3 mL chloroform:methanol 2:1 (v/v) in a Polytron disrupter. The homogenate was centrifuged at 3,500 rpm for 10 min, and the supernatant fraction was collected. After several centrifugations with different solvents, the lipidemic fraction was obtained, and TG and TC contents in the liver were measured with the commercial kits named before.

Glucose and insulin resistance tests

At 20 weeks of treatment, rats were subjected to oral glucose tolerance and insulin resistance tests. The oral glucose tolerance test was performed by oral administration of glucose (2 g/kg body weight) to experimental groups previously fasted for 14 h. Blood samples were obtained from the tail vein before and after 30, 90 and 120 min of glucose administration. Plasma glucose concentration was determined using a blood glucose commercial monitoring meter (Accutrend® GCT; Roche Diagnostics, Barcelona, Spain). For insulin resistance test, food was withdrawn 3 h before the test and the rats were injected intraperitoneally with insulin (100 IU/mL; Humulina Regular®, Lilly S.A., Spain). Blood samples were collected at the same time intervals. The area under the glucose curve from both tests was calculated using Prism GraphPad 5.01 software (San Diego, CA, USA). For glucose tolerance data, each value is the total area under glucose curve for each animal and represents glucose changes from baseline during the test. For insulin resistance test, the area increment above the glucose curve was calculated, and each value represents the area relative to the time = 0 value for each animal.

Expression of results and statistical analysis

Data represented are mean ± SEM of $n = 7$ rats. One-way ANOVA with Tukey's comparison was used to compare data. Differences were considered significant when $P < 0.05$. A Prism GraphPad 5.01 software (San Diego, CA, USA) was used for statistical analysis.

Results

Body weight, food intake and organ weights

The body weight in obese Zucker rats was significantly higher ($P < 0.001$) than their lean littermates during the treatment (Table 1, Fig. 1). Treatment with RBEE did not modify body weights of both rat strains (Table 1, Fig. 1), excepting for L1, which final body weight attenuation was accompanied by a significant reduction in both food and caloric intake (Table 1). The average weekly food and caloric intake throughout the experimental period was also higher in obese Zucker rats than their matched lean littermates and was not altered by RBEE treatment (Table 1).

After treatment, liver and VAT relative weights (g/100 g body) resulted increased in OC versus LC ($P < 0.01$; $P < 0.001$), whereas skeletal muscle relative weight of OC was significantly reduced. RBEE treatment was able to significantly attenuate relative weights of the liver from obese animals compared to OC (Table 1). In addition, obese rats fed RBEE showed increased values of skeletal muscle relative weights, reaching similar values to those obtained in lean rats (Table 1). No changes were appreciated in heart, pancreas, spleen and EAT relative weights in both strains of animals (Table 1).

Systolic blood pressure changes

Obese Zucker rats showed moderately higher systolic blood pressure values than their lean littermates at the end of the treatment ($P < 0.001$). Administration of RBEE was able to reduce blood pressure values in O1 and O5, being

Table 1 Food and caloric intake, body weight and relative organ weights of the different groups of treatment at the end of the study. Mean ± SEM ($n = 7$)

	LC	L1	L5	OC	O1	O5
Food intake (g/wk/r)	154 ± 3[a]	127 ± 3[b]	142 ± 3[a]	175 ± 8[c]	162 ± 9[c]	161 ± 6[c]
Caloric intake (kcal)	452 ± 8[a]	375 ± 9[b]	445 ± 8[a]	508 ± 23[c]	478 ± 27[c]	506 ± 20[c]
Final body weight (g)	461 ± 11[a]	432 ± 7[b]	490 ± 11[a]	569 ± 21[c]	606 ± 27[c]	601 ± 29[c]
Organ weight (g/100 g body weight)						
Heart (g)	0.30 ± 0.01[a]	0.29 ± 0.01[a]	0.27 ± 0.01[a]	0.28 ± 0.01[a]	0.26 ± 0.01[a]	0.24 ± 0.01[a]
Pancreas (g)	0.16 ± 0.02[a]	0.22 ± 0.02[a]	0.25 ± 0.01[a]	0.13 ± 0.01[a]	0.12 ± 0.02[a]	0.16 ± 0.01[a]
Spleen (g)	0.150 ± 0.001[a]	0.130 ± 0.001[a]	0.140 ± 0.001[a]	0.17 ± 0.01[a]	0.16 ± 0.01[a]	0.150 ± 0.001[a]
Skeletal muscle (g)	1.28 ± 0.09[a]	1.66 ± 0.11[b]	1.92 ± 0.13[b]	0.79 ± 0.04[c]	1.07 ± 0.04[d]	1.17 ± 0.06[d]
Liver (g)	2.96 ± 0.14[a]	2.8 ± 0.2[a]	2.8 ± 0.2[a]	5.9 ± 0.3[b]	4.6 ± 0.2[c]	4.6 ± 0.3[c]
VAT (g)	1.61 ± 0.13[a]	1.36 ± 0.10[a]	1.50 ± 0.07[a]	2.7 ± 0.3[b]	3.10 ± 0.13[b]	3.2 ± 0.2[b]
EAT (g)	1.07 ± 0.10[a]	0.84 ± 0.07[a]	0.97 ± 0.06[a]	0.96 ± 0.06[a]	1.26 ± 0.09[a]	1.40 ± 0.10[a]

Values in a row without a common superscript letter differ significantly ($P < 0.05$)

LC lean controls fed standard diet (SD), *L1* lean fed 1 % RBEE-supplemented diet, *L5* lean fed 5 % RBEE-supplemented diet, *OC* obese controls fed SD, *O1* obese fed 1 % RBEE-supplemented diet, *O5* obese fed 5 % RBEE-supplemented diet

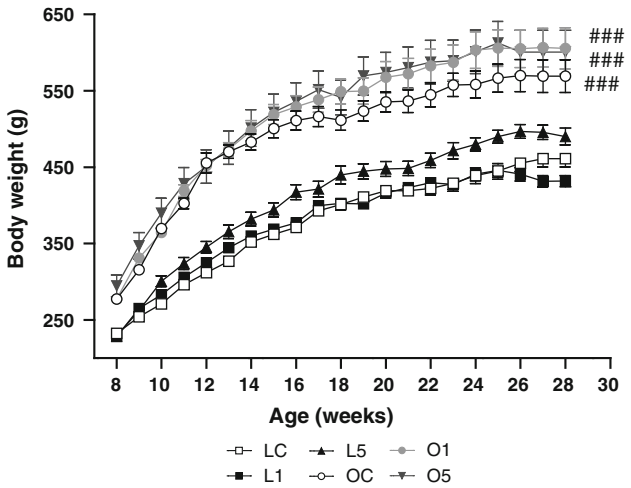

Fig. 1 Body weight evolution of Zucker rats during 20 weeks of treatment of receiving either standard diet (SD) or rice bran enzymatic extract (RBEE)-supplemented diet. Experimental groups: *LC* lean controls fed SD, *L1* lean fed 1 % RBEE-supplemented diet, *L5* lean fed 5 % RBEE-supplemented diet, *OC* obese controls fed SD, *O1* obese fed 1 % RBEE-supplemented diet, *O5* obese fed 5 % RBEE-supplemented diet. Data are mean ± SEM ($n = 7$). $^{###}P < 0.001$ versus LC

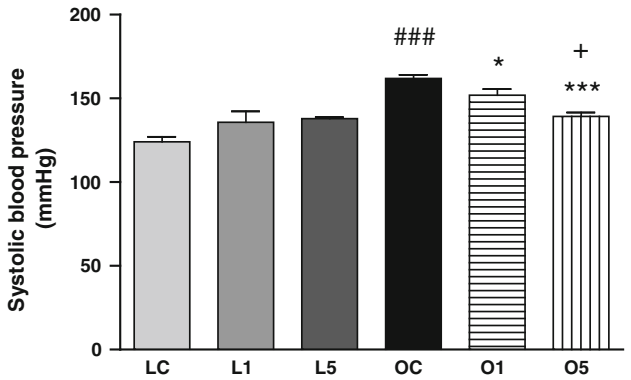

Fig. 2 Final values of systolic blood pressure after 20 weeks of treatment with either standard diet (SD) or rice bran enzymatic extract (RBEE)-supplemented diet. *LC* lean controls fed SD, *L1* lean fed 1 % RBEE-supplemented diet, *L5* lean fed 5 % RBEE-supplemented diet, *OC* obese controls fed SD, *O1* obese fed 1 % RBEE-supplemented diet, *O5* obese fed 5 % RBEE-supplemented diet. Data are mean ± SEM ($n = 7$). $^{###}P < 0.001$ versus LC; $^{*}P < 0.05$, $^{***}P < 0.001$ versus OC; $^{+}P<0.05$ versus O1

significantly most effective the high concentration ($P < 0.001$) (Fig. 2).

Lipid profile

Serum analysis showed higher concentrations of TC, HDL, TG and NEFA in obese compared with lean groups ($P < 0.001$) (Table 2). RBEE-supplemented diet produced a significant reduction of parameters such as TC, TC/HDL-C ratio and TG values, being O5 the group with the most important improvement in the lipid profile ($P < 0.001$). RBEE induced no changes in NEFA values in serum.

As shown on Table 2, ALT values were higher in obese Zucker rats than in the lean strain ($P < 0.001$ vs LC), whereas no differences were appreciated between both strains of animals regarding to AST serum levels. Neither ALT nor AST serum levels were modified by the RBEE treatment.

The hepatic lipid fraction obtained from obese rats was significantly higher than that from their lean littermates ($P < 0.001$ vs LC). Hepatic level of TG was greater in OC than LC ($P < 0.05$), whereas this value was notably attenuated by administration of 5 % RBEE in obese rats ($P < 0.01$ vs OC) (Table 2). In contrast, cholesterol levels

Table 2 Biochemical analysis after chronic treatment with RBEE. Mean ± SEM ($n = 7$)

	LC	L1	L5	OC	O1	O5
Serum						
TC (mmol/L)	2.35 ± 0.10^a	2.4 ± 0.2^a	2.59 ± 0.06^a	6.0 ± 0.3^b	5.4 ± 0.2^c	5.0 ± 0.7^d
HDL-C (mmol/L)	0.88 ± 0.07^a	0.95 ± 0.11^a	0.91 ± 0.13^a	2.4 ± 0.3^b	2.9 ± 0.2^c	3.2 ± 0.3^d
TC/HDL-C	2.7 ± 0.2^a	2.7 ± 0.2^a	3.0 ± 0.3^a	3.2 ± 0.8^b	2.03 ± 0.13^c	1.7 ± 0.2^c
TG (mmol/L)	0.98 ± 0.15^a	0.66 ± 0.08^a	0.66 ± 0.07^a	4.6 ± 0.6^b	4.0 ± 0.2^c	3.30 ± 0.15^d
NEFA (mmol/L)	0.49 ± 0.04^a	0.45 ± 0.14^a	0.47 ± 0.04^a	0.94 ± 0.10^b	0.91 ± 0.03^b	0.99 ± 0.11^b
Glucose (mmol/L)	6.6 ± 0.6^a	5.2 ± 0.6^b	5.7 ± 0.3^a	8.7 ± 1.0^c	9.4 ± 0.5^c	9.0 ± 1.0^c
Insulin (ng/mL)	0.48 ± 0.12^a	0.42 ± 0.11^a	0.48 ± 0.08^a	4.5 ± 0.6^b	4.1 ± 0.5^c	2.6 ± 0.6^d
HOMA-IR	2.6 ± 0.5^a	3.8 ± 0.9^a	3.0 ± 0.4^a	46 ± 7^b	37 ± 4^b	22 ± 6^c
ALT	74.8 ± 0.5^a	73.1 ± 1.0^a	67.3 ± 0.8^a	90 ± 4^b	87 ± 2^b	93 ± 6^b
AST	141 ± 6^a	136 ± 3^a	131 ± 2^a	1 26 ± 9^a	1 24 ± 6^a	1 20 ± 8^a
Liver						
Total lipids (mg)	76 ± 2^a	81 ± 3^a	92.6 ± 0.9^b	102 ± 4^c	99 ± 3^c	102 ± 3^c
TG (mg/g)	24.8 ± 1.4^a	23 ± 3^a	38 ± 2^b	31.9 ± 0.8^c	35 ± 2^c	19.17 ± 0.04^d
TC (mg/g)	11.0 ± 0.2^a	29 ± 2^b	30 ± 3^b	9.6 ± 0.8^a	9 ± 2^a	20 ± 2^c

Values in a row without a common superscript letter differ significantly ($P < 0.05$)

LC lean controls fed SD, *L1* lean fed 1 % RBEE-supplemented diet, *L5* lean fed 5 % RBEE-supplemented diet, *OC* obese controls fed SD, *O1* obese fed 1 % RBEE-supplemented diet, *O5* obese fed 5 % RBEE-supplemented diet. *TC* total cholesterol, *HDL-C* high-density lipoprotein-cholesterol, *TG* triglycerides, *NEFA* non-esterified fatty acids, *HOMA-IR* homeostatic model assessment-insulin resistance

in liver resulted increased in both lean and obese rats treated with 5 % RBEE (Table 2).

Glucose and insulin tests

As shown in Table 2, RBEE of 5 % administration induced an attenuation in HOMA-IR values of obese rats ($P < 0.01$). This fact was according to a reduction in serum insulin levels in O5 ($P < 0.001$ vs OC and O1) while fasting glucose levels in obese groups remained unaltered. Plasma glucose concentrations that obtained in the oral glucose tolerance test at 20 weeks of treatment were slightly different between lean and obese rats (Fig. 3a). In addition, the analysis of the area under the plasma glucose curve confirmed that OC presented a deteriorated glucose tolerance compared with LC (Fig. 3c). Treatment with RBEE tended to improve glucose tolerance, but the difference versus obese control was not significant (Fig. 3c).

Insulin resistance test evidenced the development of this symptom in OC, which presented an increment of the area under the plasma glucose curve significantly lower than LC at 20 weeks of treatment ($P < 0.05$) (Fig. 3b, d). RBEE of 5 % led to a significant recovering of insulin sensibility in obese rats ($P < 0.001$ vs OC) (Fig. 3b, d).

Inflammatory factors related to obesity

Serum adiponectin values were notably reduced in OC compared to LC group ($P < 0.01$) (Fig. 4a). However, this biomarker of obesity was significantly recovered in/by treatment with RBEE ($P < 0.05$).

Serum levels of $NO_{(x)}$ were fivefold increased in obese than lean rats ($P < 0.001$) (Fig. 4b). Both concentrations of RBEE induced a significant attenuation in this parameter in obese rats ($P < 0.001$), being 5 % RBEE supplementation significantly more effective than 1 % ($P < 0.001$).

Discussion

Metabolic syndrome is a controversial clinical entity characterized by a number of cardiometabolic risk factors that include obesity, dyslipidemia, insulin resistance and hypertension. This clustering of risk factors is related to an increased risk of cardiovascular diseases and diabetes [17, 25]. Although insulin and other pharmacological strategies are able to control many aspects of diabetes, they inadequately prevent cardiovascular complications associated to metabolic syndrome [25]. Otherwise, the first strategy in the prevention of these cardiometabolic disorders consists of including in the diet food or dietary components with functional properties [19–21].

Rice, and particularly RB, is an excellent nutritional source of bioactive compounds, including high healthy value proteins and phytochemicals such as γ-oryzanol, sterols and tocols [2, 4]. Nevertheless, the use of RB as a functional food is limited by insolubility of its major components, rapid development of rancidity and possible

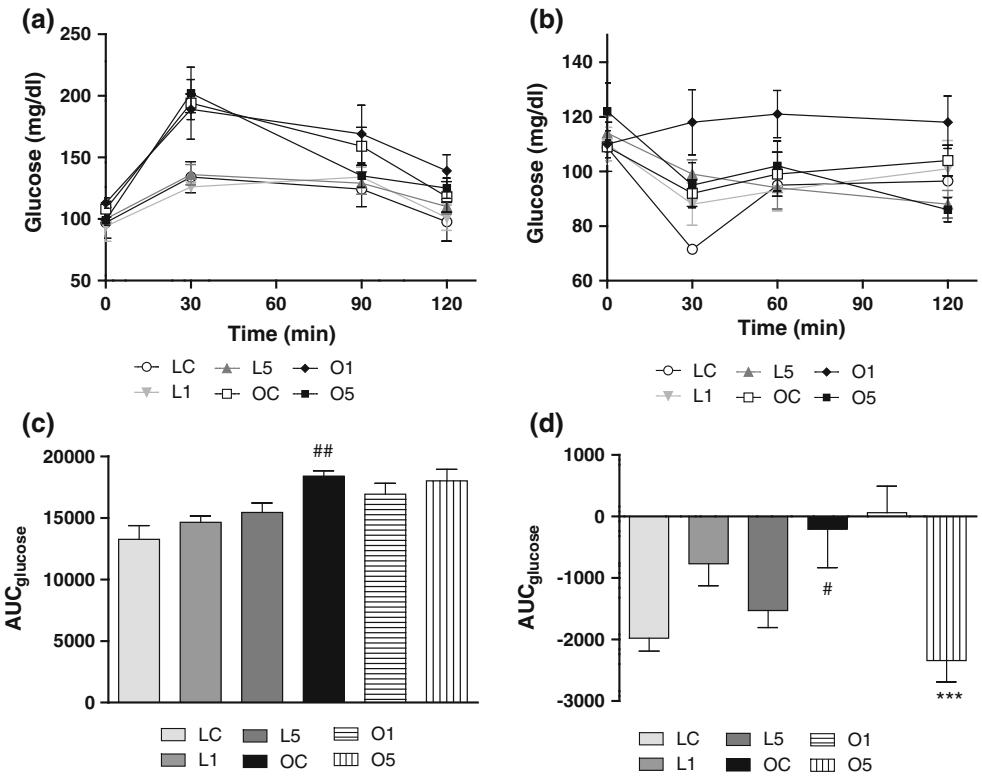

Fig. 3 Profile of serum glucose changes obtained from oral glucose tolerance (**a**) and insulin resistance tests (**b**) at 20 weeks of treatment with either standard diet (SD) or RBEE-supplemented diet. Area under curve (AUC) that results of serum glucose concentrations in the glucose tolerance test (**c**) and AUC increment obtained from insulin resistance test (**d**). *LC* lean controls fed SD, *L1* lean fed 1 % RBEE-supplemented diet, *L5* lean fed 5 % RBEE-supplemented diet, *OC* obese controls fed SD, *O1* obese fed 1 % RBEE-supplemented diet, *O5* obese fed 5 % RBEE-supplemented diet. Data are mean ± SEM ($n = 7$). $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ versus LC; $^{***}P < 0.001$ versus OC

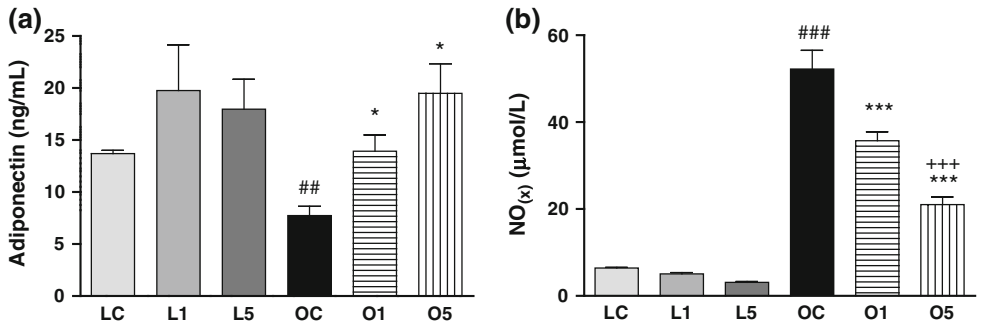

Fig. 4 Serum adiponectin (**a**) and nitrates/nitrites ($NO_{(x)}$) (**b**) values after 20 weeks of treatment with either standard diet (SD) or rice bran enzymatic extract (RBEE)-supplemented diet in lean and obese Zucker rats. *LC* lean controls fed SD, *L1* lean fed 1 % RBEE-supplemented diet, *L5* lean fed 5 % RBEE-supplemented diet, *OC* obese controls fed SD, *O1* obese fed 1 % RBEE-supplemented diet, *O5* obese fed 5 % RBEE-supplemented diet. Data are mean ± SEM ($n = 7$). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus LC; $^{*}P < 0.05$, $^{***}P < 0.001$ versus OC; $^{+++}P < 0.001$ versus O1

hull contamination [26, 27]. These limitations have been counteracted by recent production of an RB extract by enzymatic hydrolysis, so called RBEE, which has important advantages over raw material or oils regarding water solubility, increased content in nutraceutical compounds and lack of rancidity [15, 16]. Recently, RBEE has demonstrated antioxidant and hypocholesterolemic activities in vivo [15, 16] that have been mainly attributed to the presence of γ-oryzanol. Also, phytosterols content has been associated to lipid-lowering action of RBEE and the presence of tocols and sulfur aminoacids to the antioxidant activity [15, 16].

In our study, we demonstrate for the first time how long-term administration of a diet supplemented in RBEE improves biochemical and metabolic disturbances associated with metabolic syndrome in obese Zucker rats by attenuating dyslipidemia, insulin resistance and hypertension as well as restoring plasmatic levels of adiponectin and $NO_{(x)}$.

In obese Zucker rats, increased adipose tissue and peripheral insulin resistance are associated with an augmented liver lipogenesis and elevated NEFA levels in serum, thus leading to ectopic depot of TG in the liver with increased circulating levels of TG and TC [28].

As expected, RBEE treatment reduced hypertriglyceridemia and hypercholesterolemia in obese Zucker rats, in a dose-dependent manner, and without altering serum NEFA values. These responses were accompanied by a significant increase of HDL-cholesterol levels. The decrease in serum and liver TG induced by RBEE, particularly at 5 %, could be related to the reduction in liver weight observed in this experimental group, since this fact cannot be a consequence of a reduction in body weight. In addition to the liver weight reduction, no evidence of hepatic steatosis was observed since levels of transaminases remained unaltered by RBEE.

The lipid-lowering property of RBEE treatment in Zucker rats agrees with previous studies using the same enzymatic extract in rats receiving high cholesterol-rich diets [16], as well as other investigations with RB oils in hypercholesterolemic rodents and humans [5–9]. The main responsible for this beneficial effect of RBEE and other RB extracts on lipid profile may be its phytosterol content. The major sterol on RBEE is 4-desmethyl sterols, β-sitosterol, which is very effective in competing with cholesterol for incorporating in mixed micelles [29]. The hypolipidemic action of the enzymatic extract may also be related to its important content of γ-oryzanol. Several mechanisms have been suggested to be involved in this beneficial effect of γ-oryzanol including a decrease in cholesterol absorption and an increased bile flow or rise in cholesterol fecal excretion [3]. Furthermore, γ-oryzanol may reduce levels of cholesterol by attenuating apolipoprotein B synthesis in humans [30]. It has also been described that triterpene alcohols from γ-oryzanol evoke hypocholesterolemic activity by themselves [31].

Moreover, this lipid-lowering action might be also related to the potential hypocholesterolemic and anti-atherogenic capacity of proteins from RB [4, 32], since solubility and bioactivity of these proteins is notably enhanced in RBEE. The beneficial actions of RB proteins against hyperlipemia and atherogenesis have also been related to the high concentration of L-arginine in the protein fraction of RBEE, which is a very effective aminoacid in this regard [15, 16].

Tocotrienols from RB, in addition to its antioxidant property, do also lower serum cholesterol by an inhibition of the regulatory enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase thus decreasing hepatic cholesterol synthesis [33, 34]. In contrast to this action, α-tocopherol has an inductor effect on this enzyme activity [34]. Since tocotrienols are also converted to tocopherols in vivo, it is necessary not to exceed a certain dose, as this could be a counter-productive. This fact could be related to the effect of the higher dose tested of RBEE on hepatic cholesterol levels. Zucker obese rats fed 5 % RBEE showed augmented levels of liver cholesterol, despite of the plasmatic cholesterol decreasing. This dose of RBEE may be excessive in terms of tocotrienols that might be converted into tocopherols thus inducing the activity of HMG-CoA reductase with the consequent increase in liver cholesterol. Although the concentrations of RBEE used in this study seem to be suitable to improve lipid profile in obese Zucker rats, the higher dose of 5 % RBEE could produce certain cholesterol accumulation in the liver of this animal model.

Obesity is a well-established risk factor for the development of hypertension, since there is a strong correlation between overweight and hypertension [35]. In fact, the increase in blood pressure developed by fatty animals was higher than that found in their littermates lean rats. Obesity-related hypertension is associated with both a decrease in adiponectin concentration and the development of insulin resistance, which are in turn related [36]. The reasons for the association of insulin resistance and essential hypertension can be sought in at least four general types of mechanisms: Na^+ retention, sympathetic nervous system overactivity, disturbed membrane ion transport and proliferation of vascular smooth muscle cells [36]. Also, hypertension related to obesity has been related to vascular and endothelial dysfunction due to decreased endothelial NO release by down-regulation of endothelial NO synthase as well as increased oxidative stress leading to an augmented breakdown of endothelial NO, independently of NO metabolites produced by inducible NOS (iNOS) in fat or muscle [37]. Additional mechanisms such as increased generation of angiotensin II and endothelin-1 seem to contribute to arterial hypertension in obese Zucker rats [37]. According to our results, RBEE had a concentration-dependent positive effect on systolic blood pressure, insulin resistance and adiponectin levels. That is, RBEE-supplemented diet of 5 % induced a stronger reduction in systolic blood pressure of obese treated-rats than RBEE of 1 %. At the same way, in the insulin resistance test, serum glucose was recovered to normal levels in O5, as well as in the case of serum adiponectin values. These findings may be related to the high content of RBEE in γ-oryzanol, since it has been recently reported that this compound is able to recover mice-induced hypoadiponectinemia thus improving insulin sensibility [38]. On the other hand, there are several studies that confirm the capacity of RB to reduce blood pressure in genetic models of hypertensive rats, being ferulic acid the main responsible for this action [39, 40]. Besides, single administration of ferulic acid to spontaneously hypertensive rats induced an attenuation of blood pressure by inhibiting plasmatic activity of angiotensin-1-converting enzyme activity [41].

We also found that RBEE ameliorated insulin resistance in obese Zucker rats. Although the extract did not significantly change fasting glucose, insulin resistance test data

strongly indicate the beneficial effect of RBEE on this regard. Qureshi and coworkers demonstrated that fractions from stabilized RB extract rich in tocopherols/tocotrienols can effectively control blood glucose levels and insulin resistance in diabetic humans [10, 34]. They propose a synergistic effect of several RB bioactive components, particularly tocols and γ-oryzanol, which has potent antioxidant activity thus ameliorating complications of diabetes such as glycation, glycoxidation and hyperlipemia as well as exerting indirect effects on glucose absorption, utilization or excretion [10, 12, 34]. Also, proteins derived from the enzymatic treatment of RB might be involved in the beneficial effect of RBEE in glucose and insulin metabolism, since these proteins may attenuate formation of advanced glycosylation end products, which are increased under a diabetic state [10].

On the other hand, the attenuation induced by RBEE on insulin resistance of obese rats may be partially related to an increased level of adiponectin in treated obese animals. Obese animals and humans are featured by a dysregulated production of hormones and adipocytokines secreted by adipose tissue, namely decreased adiponectin and overproduction of pro-inflammatory mediators such as iNOS and tumor necrosis factor (TNF)-α, leading to obesity-related insulin resistance and inflammation [42]. In our study, RBEE restored plasmatic adiponectin levels in obese Zucker rats, thus providing protection against the pathogenesis of metabolic syndrome. Regarding to the pro-inflammatory state associated with obesity in addition to the hypoadiponectinemia, in the present investigation, plasmatic values of $NO_{(x)}$ have been evaluated as a biomarker of iNOS induction in obese rats. RBEE diet improved the imbalanced production of $NO_{(x)}$ in obese animals, thus indicating the potential role of this enzymatic extract on systemic inflammation, which is a key mechanism in the pathogenesis of metabolic syndrome features [17]. The main constituent of RBEE involved in the potential action of the enzymatic extract on hypoadiponectinemia and inflammation related to obesity may be the content on γ-oryzanol, since recent studies has evidenced the beneficial effect of this component restoring adiponectin levels in obese mice [38] as well as its steryl ferulates on inflammatory markers involved in metabolic syndrome [43].

In summary, this investigation demonstrates that chronic administration of a novel water-soluble enzymatic extract of rice bran could be a suitable treatment for improving or alleviating metabolic syndrome-associated risk factors. This RBEE has shown physical and chemical advantages over raw material or RB oils regarding water solubility, lack of rancidity, and increased content in nutraceutical compounds such as γ-oryzanol, sterols and tocotrienols, thus leading to an important bioactivity in vivo. In this study, an RBEE-supplemented diet had positive effects in obesity-derived hyperlipidemia, hypertension and hyperinsulinemia. In addition, obese animals treated with RBEE showed a significant restoration of adiponectin levels and an attenuation of pro-inflammatory values of $NO_{(x)}$ that seem to be associated with obesity. All of these activities could be mainly attributed to the presence of γ-oryzanol and ferulic acid in RBEE and/or a synergistic effect of its nutraceutical compounds. Also, solubility of high nutritional proteins in the enzymatic extract could be involved in the beneficial effects.

Further studies are in progress to better clarify the mechanisms implicated in these beneficial actions and thus to reinforce the potential of RBEE as a functional food, with special emphasis in the pathogenesis of the metabolic syndrome and associated complications.

Acknowledgments This research was supported by the Spanish Ministry of Science and Technology (AGL2009-1159). Justo M.L. is a recipient of a FPU fellowship from the Spanish Government.

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