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Quercetin Ameliorates Metabolic Syndrome and Improves the Inflammatory Status in Obese Zucker Rats

Leonor Rivera[1], Rocío Morón[1], Manuel Sánchez[1], Antonio Zarzuelo[1] and Milagros Galisteo[1]

The aim of this study was to analyze the effects of chronic administration of high doses of quercetin on metabolic syndrome abnormalities, including obesity, dyslipidemia, hypertension, and insulin resistance. For this purpose, obese Zucker rats and their lean littermates were used. The rats received a daily dose of quercetin (2 or 10 mg/kg of body weight) or vehicle for 10 weeks. Body weight and systolic blood pressure (SBP) were recorded weekly. At the end of the treatment, plasma concentrations of triglycerides, total cholesterol, free-fatty acids (FFAs), glucose, insulin, adiponectin, and nitrate plus nitrite (NOx) were determined. Tumor necrosis factor-α (TNF-α) production, inducible nitric oxide synthase (iNOS), and endothelial nitric oxide synthase (eNOS) protein expression were analyzed in visceral adipose tissue (VAT). The raised SBP and high plasma concentrations of triglycerides, total cholesterol, FFA, and insulin found in obese Zucker rats were reduced in obese rats that received either of the doses of quercetin assayed. The higher dose also improved the inflammatory status peculiar to this model, as it increased the plasma concentration of adiponectin, reduced NOx levels in plasma, and lowered VAT TNF-α production in obese Zucker rats. Furthermore, chronic intake of the higher dose of quercetin enhanced VAT eNOS expression among obese Zucker rats, whereas it downregulated VAT iNOS expression. In conclusion, both doses of quercetin improved dyslipidemia, hypertension, and hyperinsulinemia in obese Zucker rats, but only the high dose produced antiinflammatory effects in VAT together with a reduction in body weight gain.

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INTRODUCTION

Metabolic syndrome, a clinical condition which comprises various specific abnormalities including abdominal obesity, insulin resistance, dyslipidemia, and hypertension (1), has become a major and increasingly prevalent disorder (2,3) that parallels the dramatic worldwide epidemic of type 2 diabetes and obesity. It is directly associated with an increased risk of developing cardiovascular diseases (4,5), which are the major cause of premature mortality in type 2 diabetes patients. Managing the disorders clustered in this syndrome and in the mechanisms involved in its development is of great interest to prevent or reduce the risk posed by the pathologies implicated.

The etiopathology of the metabolic syndrome has not yet been fully elucidated. It seems to be the result of a complex combination of several etiologic factors that accompany central obesity and insulin resistance (6). Recent studies have highlighted the involvement of a proinflammatory state that induces insulin resistance and leads to clinical and biochemical manifestations of the metabolic syndrome. The pathway leading to this pathology would involve an abnormal production of hormones and cytokines from the adipose tissue, namely an excessive production of proinflammatory mediators such as IL-6 and tumor necrosis factor-α (TNF-α) together with a lower secretion of the antiinflammatory adipocytokine adiponectin (7,8).

Dietary patterns which include a high intake of plant food content, such as vegetables, legumes, and fruits, have been directly associated with the management and prevention of obesity, type 2 diabetes, and other cardiovascular risk factors (9,10). Plant foods contain flavonoids, polyphenolic compounds reported to have protective effects against chronic pathologies such as coronary events, cardiovascular disease mortality, and diabetes (11–13). However, the epidemiological studies carried out to date remain inconclusive, and controversial data have been published concerning the protective effects of diet flavonoids against diabetes (14). It is difficult to ascertain whether flavonoids are really responsible for these beneficial health effects, as only limited studies have been made of their content in food, which, moreover, contains other components which can also contribute to these effects.

Among flavonoids, the flavonol quercetin (3,3′,4′,5,7-pentahydroxyflavone) (**Figure 1**) is one of the most widely distributed in human dietary sources (11). In animal models and

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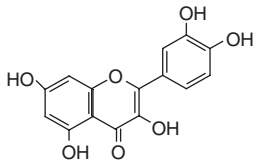

Figure 1 Structure of quercetin.

cellular lines, this molecule has been reported to have cardioprotective and antiinflammatory effects (15–17). These studies have engendered interest in the development of dietary supplements or drugs that would allow for more convenient and higher dose administration of quercetin, which might prove useful for the prevention or treatment of functional alterations clustered in the metabolic syndrome.

The main aim of this study was to examine the effects of the chronic daily administration of quercetin on the disturbances present in the metabolic syndrome, and to analyze whether the antiinflammatory properties of this molecule are involved in its effects. The doses of quercetin used are equivalent to a high intake in the human diet and to the dose used by humans as a diet supplement. For this purpose, we used obese Zucker rats, a widely used animal model of obesity and type 2 diabetes that presents many of the characteristics of human metabolic syndrome, as these animals display insulin resistance, dyslipidemia, hyperinsulinemia, and hypertension (18), while their lean littermates are insulin-sensitive, normoinsulinemic, normotensive, and present a normal lipid profile.

METHODS AND PROCEDURES

This study was carried out in accordance with the European Union guidelines for animal care and protection.

Reagents

Quercetin dihydrate and other chemicals were obtained from Sigma Chemicals (Madrid, Spain).

Animals and experimental protocol

Obese male Zucker rats and lean heterozygous littermates at the age of 13 weeks (Charles River Laboratories, Barcelona, Spain) were housed five per cage at a constant temperature (24 ± 1 °C), with a 12-h dark/light cycle and free access to tap water and food. Rats were allowed to adapt to these conditions for 2 weeks, before the beginning of the experimental protocol.

Obese and lean rats were randomly assigned to three groups, two of which received a daily dose of quercetin, either 2 or 10 mg/kg of body weight, mixed in the vehicle (1 ml of 1% methylcellulose), whereas the other group received just the vehicle. The rats were treated orally by gavage for 10 weeks. Henceforth, the obese and lean groups given the lower dose of quercetin are referred to as OQ2 (obese rats treated with 2 mg/kg/day of quercetin) and LQ2 (lean rats treated with 2 mg/kg/day of quercetin), respectively, those given the higher dose are termed OQ10 (obese rats treated with 10 mg/kg/day of quercetin) and LQ10 (lean rats treated with 10 mg/kg/day of quercetin), and the groups given the vehicle (control groups) are designated as OC (obese control rats group) and LC (lean control rats group). Administration of quercetin was stopped two days before the end of the experiments, in order to study its long-term consequences without the involvement of its acute administration effects. Over the experimental period, the rats had free access to tap water and diet, and food and water intake was measured daily.

Blood pressure measurements

Systolic blood pressure (SBP) was determined weekly, in the morning, in conscious, prewarmed, restrained rats by tail-cuff plethysmography (digital pressure meter, LE 5000; Letica, Barcelona, Spain). At least seven determinations were made in every session and the mean of the lowest three values within 5 mm Hg was taken as the SBP value.

Samples collection and storage

At the end of the experimental period, rats were fasted overnight, blood was obtained from the tail vein to analyze biochemical parameters, and animals were killed. Aorta and visceral adipose tissue (VAT) samples were excised, cleaned, and frozen until analysis. Plasma was obtained by blood centrifugation at 2000 *g* for 15 min, aliquoted and frozen.

Plasma analytical procedures

Plasma glucose, triglycerides, and total cholesterol concentrations were measured by colorimetric methods using Spinreact kits (Spinreact, Spain). Plasma free-fatty acids (FFAs) concentration was determined using a Wako NEFA C test kit (Wako Chemicals, Richmond, VA). Plasma insulin concentration was quantified using a rat insulin enzyme immunoassay kit (Amersham Biosciences, Buckinghamshire, UK). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin levels as a measure of insulin resistance (19).

The plasma concentration of the stable nitric oxide (NO) end-metabolites, nitrate plus nitrite (NOx), as markers for NO production, was measured as previously described (17). Plasma adiponectin concentration was determined using a mouse/rat adiponectin ELISA kit (B-Bridge International, Mountain View, CA).

TNF-α production by adipose tissue

VAT obtained from each rat was homogenized in phosphate-buffered saline. Homogenates were incubated at 37 °C for 20 min, and centrifuged at 15,000 *g* for 15 min. The levels of TNF-α in the tissue supernatants were determined using an ELISA kit (Diaclone, France) specific for rat TNF-α.

iNOS and eNOS expression in biological tissues

VAT lysates were prepared by homogenization of visceral VAT obtained from each rat in modified RIPA buffer (50 mmol/l Tris-HCl, pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% sodium dodecylsulfate, 1 mmol/l sodium ethylenediaminetetraacetate, 1 mmol/l phenylmethylsulfonyl fluoride, 5 µg/ml of aprotinin, 5 µg/ml of leupeptin). Aortic homogenates were prepared in a buffer containing 10 mmol/l N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) (pH 7.4), sucrose (0.32 mol/l), EDTA (100 µmol/l), dithiothreitol (1 mmol/l), phenylmethylsulphonyl fluoride (1 mg/ml), and leupeptin (10 µg/ml). Homogenates were centrifuged at 15,000 g for 30 min and supernatants used for assessing endothelial nitric oxide synthase (eNOS) and/or inducible nitric oxide synthase (iNOS) protein expression by western blot. Protein concentration in supernatants was determined by the bicinchoninic acid protein assay. Fifty microgram of protein of VAT and 40 µg of protein of aortic homogenates from each sample were separated by sodium dodecylsulfate–polyacrylamide (8%) gel electrophoresis in a mini-gel system (Bio-Rad Laboratories, Madrid, Spain), and then transferred electrophoretically onto nitrocellulose membranes overnight. After blocking the filters in 5% nonfat dry milk-Tris-buffered saline-0.1% Tween 20 (TBST), they were respectively incubated with a mouse anti-eNOS monoclonal antibody or with a rabbit anti-iNOS antibody (BD Transduction Laboratories, San Jose, CA), diluted at 1:2500 in 5% albumin-TBST. The filters were then washed three times for 10 min in TBST and incubated respectively with secondary peroxidase–conjugated goat antimouse or antirabbit antibody (Sigma, Barcelona, Spain), diluted at 1:2000 in

5% nonfat dry milk-TBST. Incubations were performed at room temperature for 2 h. After washing membranes, antibody binding was detected by an ECL system (PerkinElmer, LAS, Boston). Films were scanned and densitometric analysis was performed on the scanned images using Scion Image-Release Beta 4.02 software (http://www.scioncorp.com).

Statistical analysis

Results are expressed as mean ± s.e.m. of measurements. For statistical analysis, two-way ANOVA with Bonferroni's post test was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego CA; www.graphpad.com), with statistical significance set at $P < 0.05$.

RESULTS

Body weight gain and food intake

The body weight of obese Zucker rats was significantly higher ($P < 0.0001$) than that of their lean littermates when the treatment began. Chronic oral administration of the high (but not the low) dose of quercetin reduced the final body weight in both obese and lean Zucker rats (Table 1). Body weight gain, which was greater in obese than in lean rats ($P < 0.001$), was also reduced by a daily intake of the high dose of quercetin in both rat strains, compared with their control groups ($P < 0.05$) (Figure 2a).

The average daily food intake throughout the experimental period was significantly greater among the obese Zucker rats than among their lean littermates (Table 1), and quercetin did not modify this parameter in either rat strain.

Effects of quercetin on plasma parameters and on VAT

Levels of triglycerides, FFAs, and total cholesterol were higher in the OC than in the lean rats. With respect to the OC rats, the plasma concentrations of triglycerides, FFAs, and total cholesterol decreased by 29.5, 21.2, and 17.7%, respectively ($P < 0.05$ vs. OC) among the OQ2 rats, and by 33.3, 24.6, and 17.6%, respectively ($P < 0.05$ vs. OC) among the OQ10 rats. However, among the lean rats no differences were found between the rats given quercetin and their controls (Table 2). No differences were observed between the groups with respect to glucose concentration in plasma, whereas insulin was dramatically increased in the OC rats compared to the LC rats. Chronic administration of quercetin at both doses significantly reduced the hyperinsulinemia observed in OC rats ($P < 0.05$) (Table 2). As expected, the OC rats had greater insulin resistance, expressed as HOMA-IR, than did the LC rats. This parameter was significantly improved in the OQ2 and OQ10 rats ($P < 0.05$ vs. OC) (Figure 2b).

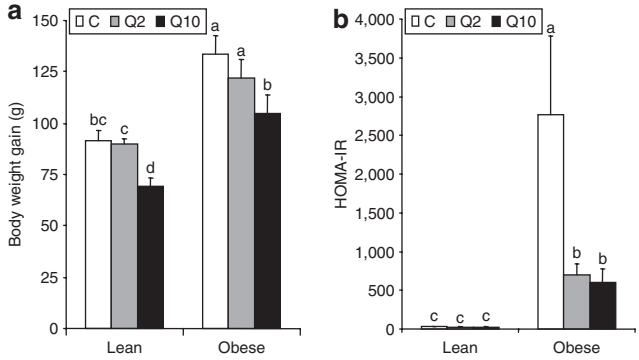

Figure 2 Body weight gain and homeostasis model assessment of insulin resistance (HOMA-IR) in lean and obese Zucker rats that received vehicle or different doses of quercetin for 10 weeks. (**a**) Weight gain and (**b**) HOMA-IR in lean and obese Zucker rats given vehicle (C), 2 mg/kg of body weight of quercetin (Q2), or 10 mg/kg of body weight of quercetin (Q10), for 10 weeks. HOMA-IR: fasting glucose (μmol/l) × fasting insulin (μU/l)/22.5. Data are expressed as means ± s.e.m. ($n = 7$). Means without a common letter differ, $P < 0.05$.

Table 1 Food intake, initial and final body weight in lean and obese Zucker rats that received vehicle, 2 or 10 mg/kg/day of quercetin for 10 weeks

Experimental groups	LC ($n = 7$)	LQ2 ($n = 7$)	LQ10 ($n = 7$)	OC ($n = 7$)	OQ2 ($n = 7$)	OQ10 ($n = 7$)
Average food intake (g/rat/day)	19.9 ± 0.2^{b}	20.4 ± 0.5^{b}	19.7 ± 0.3^{b}	22.6 ± 1.1^{a}	22.5 ± 0.9^{a}	22.3 ± 1.1^{a}
Initial body weight (g)	345.2 ± 5.7^{b}	344.0 ± 10.8^{b}	342.2 ± 4.9^{b}	401.7 ± 11.6^{a}	400.0 ± 3.7^{a}	400.0 ± 7.2^{a}
Final body weight (g)	437.0 ± 8.4^{c}	433.7 ± 9.9^{c}	411.0 ± 6.2^{d}	535.1 ± 15.5^{a}	$522.3 \pm 10.2^{a,b}$	504.8 ± 11.5^{b}

Values are expressed as mean ± s.e.m. Values within a row without a common letter differ significantly, $P < 0.05$. Experimental groups: LC, lean control rats; LQ2, lean rats given 2 mg quercetin/kg/day; LQ10, lean rats given 10 mg quercetin/kg/day; OC, obese control rats; OQ2, obese rats given 2 mg quercetin/kg/day; OQ10, obese rats given 10 mg quercetin/kg/day.

Table 2 Plasma analytical determinations in lean and obese Zucker rats that received vehicle, 2 or 10 mg/kg/day of quercetin for 10 weeks

Experimental groups	LC ($n = 7$)	LQ2 ($n = 7$)	LQ10 ($n = 7$)	OC ($n = 7$)	OQ2 ($n = 7$)	OQ10 ($n = 7$)
Triglycerides, mmol/l	0.44 ± 0.03^{c}	0.47 ± 0.02^{c}	0.49 ± 0.03^{c}	3.02 ± 0.30^{a}	2.13 ± 0.30^{b}	2.02 ± 0.16^{b}
Cholesterol, mmol/l	1.50 ± 0.11^{c}	1.48 ± 0.08^{c}	1.55 ± 0.11^{c}	4.16 ± 0.13^{a}	3.42 ± 0.20^{b}	3.43 ± 0.11^{b}
FFA, mmol/l	0.55 ± 0.05^{c}	0.55 ± 0.03^{c}	0.54 ± 0.04^{c}	0.84 ± 0.06^{a}	0.66 ± 0.03^{b}	0.63 ± 0.03^{b}
Glucose, mmol/l	10.53 ± 0.48^{a}	10.75 ± 0.99^{a}	11.02 ± 0.60^{a}	11.00 ± 0.53^{a}	10.63 ± 0.32^{a}	9.79 ± 0.63^{a}
Insulin, pmol/l	411 ± 94^{c}	384 ± 64^{c}	359 ± 94^{c}	39897 ± 15283^{a}	10555 ± 2338^{b}	9774 ± 2878^{b}

Values are expressed as mean ± s.e.m. Values within a row without a common letter differ significantly, $P < 0.05$. Experimental groups: LC, lean control rats; LQ2, lean rats given 2 mg quercetin/kg/day; LQ10, lean rats given 10 mg quercetin/kg/day; OC, obese control rats; OQ2, obese rats given 2 mg quercetin/kg/day; OQ10, obese rats given 10 mg quercetin/kg/day.

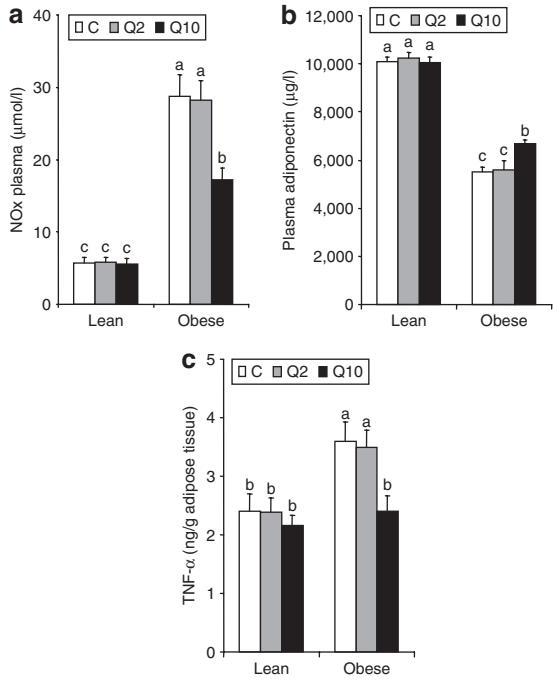

Figure 3 Inflammatory and antiinflammatory markers in plasma and visceral adipose tissue of lean and obese Zucker rats that received vehicle or different doses of quercetin for 10 weeks. (**a**) Plasma nitrate plus nitrite (NOx) and (**b**) adiponectin concentrations, and (**c**) visceral adipose tissue tumor necrosis factor-α (TNF-α) secretion, in lean and obese Zucker rats given vehicle (C), 2 mg/kg of body weight of quercetin (Q2), or 10 mg/kg of body weight of quercetin (Q10), for 10 weeks. Data are expressed as means ± s.e.m. ($n = 7$). Means without a common letter differ, $P < 0.05$.

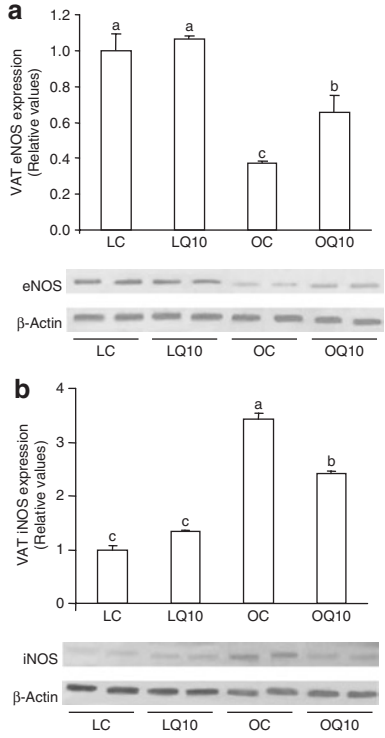

Figure 4 eNOS and iNOS protein expression in visceral adipose tissue of lean and obese Zucker rats that received vehicle or 10 mg/kg/day of quercetin for 10 weeks. (**a**) Endothelial nitric oxide synthase (eNOS) and (**b**) inducible nitric oxide synthase (iNOS) protein expression in visceral adipose tissue (VAT) of lean and obese Zucker rats given vehicle or quercetin (10 mg/kg of body weight) for 10 weeks. Experimental groups: LC, lean control rats; LQ10, lean rats given 10 mg quercetin/kg/day; OC, obese control rats; OQ10, obese rats given 10 mg quercetin/kg/day. Relative values are expressed as means ± s.e.m. ($n = 5$), and they indicate the values from densitometric analysis normalized to β-actin, relative to LC measurements, which were assigned a value of 1.0. Means without a common letter differ, $P < 0.05$.

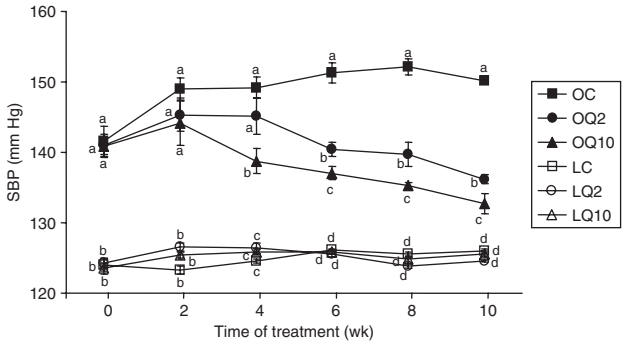

Figure 5 Evolution of systolic blood pressure (SBP), measured by tail-cuff plethysmography, in lean and obese Zucker rats given vehicle or quercetin (2 or 10 mg/kg of body weight), for 10 weeks. Experimental groups: LC, lean control rats; LQ2, lean rats given 2 mg quercetin/kg/day; LQ10, lean rats given 10 mg quercetin/kg/day; OC, obese control rats; OQ2, obese rats given 2 mg quercetin/kg/day; OQ10, obese rats given 10 mg quercetin/kg/day. Values are expressed as means ± s.e.m. ($n = 7$). Means at a time without a common letter differ, $P < 0.05$.

Plasma NOx levels were fivefold increased in the OC rats ($P < 0.0001$ vs. LC rats) (**Figure 3a**). In the OQ10 rats, this parameter was 40.2% lower than among the OC group ($P < 0.001$), whereas no effect was observed in the OQ2 rats. The adiponectin plasma concentration was lower among the OC rats than among the LC rats ($P < 0.0001$). A daily intake of the high (but not the low) dose of quercetin for 10 weeks significantly increased adiponectin concentration in plasma ($P < 0.001$ vs. OC rats) (**Figure 3b**). No differences in this parameter were observed among the lean rats groups.

TNF-α production by VAT was higher in obese Zucker rats than in the lean rats ($P < 0.05$) (**Figure 3c**). The daily administration of 10 mg/kg of body weight of quercetin for 10 weeks reduced the production of this inflammatory cytokine in obese rats, whereas no differences were observed at the lower dose of 2 mg/kg of body weight.

As only the dose of 10 mg/kg of body weight of quercetin seems to produce antiinflammatory effects, as shown by augmented plasma adiponectin concentration and reduced NOx plasma concentration and VAT TNF-α production, we analyzed eNOS and iNOS expression in the VAT of rats treated with the higher dose of quercetin. eNOS protein expression was slightly downregulated (**Figure 4a**), whereas that of iNOS was enhanced in the VAT of obese Zucker rats (**Figure 4b**). Chronic administration of the higher dose of quercetin significantly increased eNOS and downregulated iNOS expression in the VAT of obese Zucker rats, but produced no effects on the expression of either protein among the lean rats.

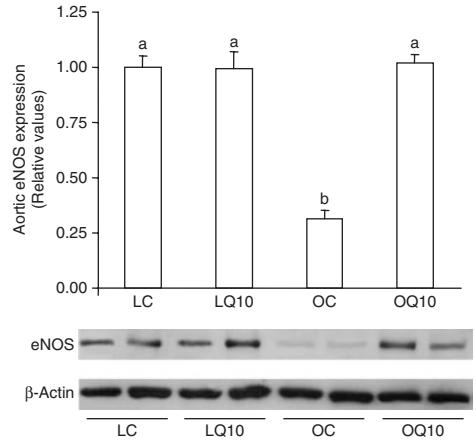

Figure 6 Endothelial nitric oxide synthase (eNOS) protein expression in aortic rings of lean and obese rat given vehicle or quercetin (10 mg/kg of body weight) for 10 weeks. Experimental groups: LC, lean control rats; LQ10, lean rats given 10 mg quercetin/kg/day; OC, obese control rats; OQ10, obese rats given 10 mg quercetin/kg/day. Relative values are expressed as means ± s.e.m. ($n = 5$), and they indicate the values from densitometric analysis normalized to β-actin, relative to LC measurements, which were assigned a value of 1.0. Means without a common letter differ, $P < 0.05$.

Systolic blood pressure

At the beginning of the experimental period, the blood pressure of the obese Zucker rats was moderately but significantly higher than that of the lean Zucker rats ($P < 0.0001$) (Figure 5). SBP increased very slightly among the OC rats during the experiment, but decreased among the OQ2 and OQ10 rats. The administration of quercetin did not affect SBP among the lean rats. In the case of the OQ10 rats, the decrease was significant ($P < 0.01$) from the third week of administration, whereas in OQ2 this reduction was observed from the fifth week. By the end of the experimental period, SBP in the OQ2 and OQ10 rats had fallen by 57.7 and 72.2%, respectively, with respect to the OC rats ($P < 0.001$) (Figure 5).

eNOS expression in aortic rings

eNOS protein expression was significantly lower ($P < 0.001$) in the aortic rings of the OC rats compared to those of the LC rats (Figure 6). The aortic expression of this enzyme in the OQ10 rats ($P < 0.001$ vs. OC) was similar to that found in the lean Zucker rats.

DISCUSSION

This study describes for the first time how the daily oral administration of high doses of the flavonoid quercetin, ranging between the equivalent of the dose obtained from a high intake in the human diet to that derived when it is taken as a diet supplement, to obese Zucker rats for 10 weeks, reduces dyslipidemia, insulin resistance, and hypertension (these factors being incorporated into the experimental model). When the higher dose of quercetin is administered, such effects are accompanied by a reduction in the proinflammatory status characteristic of the model, as shown by the decreased production of TNF-α by the VAT, the reduced plasma NOx levels, and the increased concentration of adiponectin. This administration of the higher dose of quercetin, as well as reducing the proinflammatory status, also decreases the animal's weight gain.

Recent studies of metabolic syndrome have documented the involvement of an important inflammatory component in its etiopathology. Obese and type 2 diabetes Zucker rats and human subjects are characterized by adipose tissue overproduction of proinflammatory cytokines such as TNF-α and by decreased plasma concentration of antiinflammatory adipocytokines such as adiponectin (20–22). This inflammatory status is also evidenced by elevated plasma levels of NOx (23,24; present results), which reflects an overproduction of NO by iNOS, because in such metabolic disorders iNOS expression in fat or muscle is induced by proinflammatory cytokines (25,26). Under normal conditions, the low amounts of NO produced by eNOS play an important role in the regulation of metabolic homeostasis, because mice lacking the eNOS gene develop metabolic syndrome (27). Furthermore, eNOS expression is downregulated by TNF-α in the adipose tissue of obese Zucker rats (28). On the contrary, iNOS induction is associated with the production of massive amounts of NO that may be implicated in the development of insulin resistance and associated cardiovascular complications. Hence, treatments that decrease eNOS downregulation or inhibit iNOS induction mediated by proinflammatory cytokines are expected to ameliorate the alterations found in metabolic syndrome (29). Our present results show that the chronic administration of the higher dose of quercetin reduces adipose tissue TNF-α production and plasma NOx concentration in obese Zucker rats, while increasing plasma adiponectin concentration, resulting in an important antiinflammatory effect. Moreover, this dose of quercetin produces an enhancement of eNOS and the downregulation of iNOS expression in the adipose tissue of obese Zucker rats, effects that could be connected with the reduction in TNF-α as this cytokine upregulates iNOS and inhibits eNOS expression in fat (25,28). Flavonoids have antiinflammatory effects and thus they may protect against diabetes. A recent report indicates that the administration of quercetin reduces the plasma levels of NO among streptozotocin-treated rats (24). In fact, quercetin and its glycoside quercitrin have previously been found to have antiinflammatory effects both in *in vitro* and in experimental models of inflammatory diseases (16,30). Quercetin downregulates iNOS expression, NO and TNF-α release in LPS-activated macrophages, an effect that has been associated with the inhibition of the NF-κB pathway (16).

Furthermore, the chronic administration of quercetin enhances aortic eNOS expression in obese Zucker rats, an effect which would be connected with the reduction in the SBP values of obese Zucker rats with respect to those of lean rats. Different studies have previously described quercetin as an efficient antihypertensive agent in several experimental models of hypertension in rats when administered at the dose of 10 mg/kg of body weight (17,31,32). Our present results show that a lower dose of quercetin (2 mg/kg of body weight) also reduces moderate hypertension. This antihypertensive effect has been

related to the beneficial properties of quercetin on endothelial dysfunction and, although these mechanisms are not yet fully explained, the antioxidant properties of this flavonol seem to be involved in this effect. In the model of obese Zucker rats, as well as in subjects with insulin resistance, FFAs are involved both in insulin resistance and in the inhibition of aortic eNOS activity through an oxidative mechanism (33–35). Our present results show that the chronic daily administration of the higher dose of quercetin restores aortic expression of eNOS in obese Zucker rats, an effect which could explain the improved endothelial function and the antihypertensive effects of this flavonoid in the insulin resistance model described. Previously, our group has shown that the chronic intake of the same dose of quercetin enhances aortic eNOS activity in the SHR model, as an essential mechanism for its antihypertensive effects (32).

Quercetin and its glycoside have been shown to have hypocholesterolemic actions in other experimental models (36–38). The hypocholesterolemic mechanisms of polyphenols have been attributed to their antioxidant action resulting in the inhibition of LDL oxidation, but there is increasing evidence that these compounds act by other mechanisms, including the alteration of hepatic cholesterol absorption, triglyceride assembly and secretion, or by beneficial effects on inflammation (39). Be that as it may, the present results suggest that the antiinflammatory effects of quercetin are independent of the hypocholesterolemic action *in vivo*, as the lower dose of quercetin produces a similar hypocholesterolemic and hypotriglyceridemic effect to that observed when the higher dose is used. Flavonoids have also been described as modulators of lipid homeostasis in the adipose tissue and liver, through the inhibition of phosphodiesterases (40).

In this study, the chronic administration of the high dose of quercetin significantly reduced final body weight and body weight gain of obese and lean Zucker rats compared with the controls. This effect does not seem to be related to a lower dietary intake, as quercetin did not modify food intake. In the case of the obese rats, it could be connected with the antiinflammatory effects produced by this dose of quercetin on adipose tissue. Indeed, most human studies in this respect have established a relationship between weight loss and an increase in adiponectin levels (21), and the present results also suggest that an increase in plasma adiponectin concentration parallels a decrease in body weight among obese Zucker rats.

In conclusion, the present results show for the first time that the chronic daily administration of both 2 and 10 mg/kg of body weight of quercetin reduces insulin resistance, dyslipidemia, and hypertension in the experimental model of metabolic syndrome of obese Zucker rats. The higher dose of quercetin also reduces weight gain, while at the same time there is an important improvement in the inflammatory status present in this model, as shown by the increase in plasma adiponectin and the reduction in plasma NOx concentration and VAT TNF-α production, together with a downregulation of fat iNOS expression and an improvement of eNOS expression in VAT. Although further studies are required to explain the mechanisms involved, our findings reinforce dietary recommendations for diabetes treatment, encouraging a high intake of plant foods, rich in bioactive agents such as quercetin, and also the use of supplements of this flavonoid, as it reduces cardiovascular risk factors such as hypertension or dyslipidemia, as well as insulin resistance in this experimental model of metabolic syndrome.

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DISCLOSURE

The authors declared no conflict of interest.

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