Quercetin Ameliorates Cardiovascular, Hepatic, and Metabolic Changes in Diet-Induced Metabolic Syndrome in Rats1–3

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3Supplemental Figures 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
4Abbreviations used: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; C, corn starch–rich diet-fed rats; CPT1, carnitine palmitoyltransferase 1; CQ, corn starch–rich diet-fed rats treated with quercetin; C, corn starch–rich diet-fed rats; HQ, high-carbohydrate, high-fat diet-fed rats treated with quercetin; LDH, lactate dehydrogenase; LV, left ventricle; NAFLD, nonalcoholic fatty liver disease; NEFA, nonesterified fatty acids; Nrf2, nuclear factor (erythroid-derived 2)-related factor-2.
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Abstract
Metabolic syndrome is a risk factor for cardiovascular disease and nonalcoholic fatty liver disease (NAFLD). We investigated the responses to the flavonol, quercetin, in male Wistar rats (8–9 wk old) divided into 4 groups. Two groups were given either a corn starch–rich (C) or high-carbohydrate, high-fat (H) diet for 16 wk; the remaining 2 groups were given either a C or H diet for 8 wk followed by supplementation with 0.8 g/kg quercetin in the food for the following 8 wk (CQ and HQ, respectively). The H diet contained ~68% carbohydrates, mainly as fructose and sucrose, and ~24% fat from beef tallow; the C diet contained ~68% carbohydrates as polysaccharides and ~0.7% fat. Compared with the C rats, the H rats had greater body weight and abdominal obesity, dyslipidemia, higher systolic blood pressure, impaired glucose tolerance, cardiovascular remodeling, and NAFLD. The H rats had lower protein expressions of nuclear factor (erythroid-derived 2)-related factor-2 (Nrf2), heme oxygenase-1 (HO-1), and carnitine palmitoyltransferase 1 (CPT1) with greater expression of NF-κB in both the heart and the liver and less expression of caspase-3 in the liver than in C rats. HQ rats had higher expression of Nrf2, HO-1, and CPT1 and lower expression of NF-κB than H rats in both the heart and the liver. HQ rats had less abdominal fat and lower systolic blood pressure along with attenuation of changes in structure and function of the heart and the liver compared with H rats, although body weight and dyslipidemia did not differ between the H and HQ rats. Thus, quercetin treatment attenuated most of the symptoms of metabolic syndrome, including abdominal obesity, cardiovascular remodeling, and NAFLD, with the most likely mechanisms being decreases in oxidative stress and inflammation. J. Nutr. 142: 1026–1032, 2012.

Introduction
Metabolic syndrome refers to the clustering of insulin resistance, hypertension, central obesity, impaired glucose tolerance, and dyslipidemia (1). Metabolic syndrome increases the risk of cardiovascular disease, nonalcoholic fatty liver disease (NAFLD)6, and diabetes (2–4). This increased prevalence of cardiovascular disease and NAFLD associated with metabolic syndrome necessitates the discovery of appropriate interventions for these complications. One of the major causes of obesity and NAFLD in Western society is a diet rich in both carbohydrates such as fructose or sucrose and saturated fats from animal sources (5,6). Excess consumption of fat and fructose in the diet leads to disturbances in fatty acid and carbohydrate metabolism (7,8). Excess fructose consumption also leads to increased lipid biosynthesis, because fructose is a lipogenic carbohydrate (9). This is accompanied by reduced fatty acid oxidation and increased storage of fat in the visceral area. Impairment of fatty acid metabolism in the liver leads to hepatic steatosis followed by NAFLD (10).

Quercetin (3,3′,4′,5-pentahydroxyflavone) is an important dietary flavonoid found in red onions, apples, berries, citrus fruits, tea, and red wine (11). Quercetin reduced systolic blood pressure in hypertensive human participants and in animal models of hypertension (12–14), reduced serum TG and cholesterol concentrations in high-fat diet-fed rabbits after 12 wk of treatment (15), and reduced body weight in obese Zucker rats without changing the mean daily food intake, also reducing plasma concentrations of TG, nonesterified fatty acids (NEFA) total...
choline, and insulin (16). High-fat, high-cholesterol, and high-sucrose diet-fed mice treated with quercetin had lower body weight, visceral fat, blood glucose, plasma insulin, plasma total cholesterol, plasma TG, plasma NEFA, and plasma TNFα concentrations with higher plasma adiponectin concentrations. These mice also had suppressed liver lipid accumulation (17). In high-fat diet-fed mice, quercetin increased energy expenditure and reduced plasma concentrations of inflammatory markers without any changes in food consumption, physical activity, body weight, or body composition (18). Recent studies have also shown the protective effects of quercetin in thioacetamide- and acrylonitrile-induced hepatotoxicity (19,20).

Thus, we characterized the effects of quercetin as a dietary intervention in a diet-induced rat model of NAFLD and cardiovascular remodeling as part of metabolic syndrome induced in rats by feeding a high-carbohydrate, high-fat diet for 16 wk (21). After treatment with quercetin, the structure and function of the cardiovascular system were characterized with echocardiography, isolated Langendorff heart preparation, vascular reactivity studies, and histopathological analysis. The structure and function of the liver were characterized with histopathological analysis and measurement of biochemical variables. Variables for obesity, dyslipidemia, and glucose tolerance were also measured. The possible mechanisms involved in the action of quercetin were characterized by the expression of proteins involved in cellular metabolism and stress regulation.

Methods

Rats, diets, and treatment with quercetin
All experimental protocols were approved by the University of Southern Queensland Animal Ethics Committee under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (8–9 wk old, 333 ± 2 g, n = 40) were obtained from the University of Queensland Biological Resources facility. Rats were randomly divided into 4 groups: corn starch–rich diet-fed rats (C; n = 10), corn starch–rich diet-fed rats treated with quercetin (CQ; 0.8 g/kg food; n = 10; MP Biomedicals), high-carbohydrate, high-fat diet-fed rats (H; n = 10), and high-carbohydrate, high-fat diet-fed rats treated with quercetin (HQ; 0.8 g/kg food; n = 10). The compositions of the diets were previously described in detail (21–23). C and H rats were fed with corn starch–rich and high-carbohydrate, high-fat diets, respectively, for 16 wk. CQ and HQ rats were fed with corn starch–rich and high-carbohydrate, high-fat diets, respectively, for the first 8 wk and the respective diets were supplemented with quercetin (0.8 g/kg food) for a further 8 wk. All the rats were individually housed under temperature-controlled, 12-h-light/dark conditions and consumed food and water ad libitum.

Physiological and metabolic variables
All rats were monitored daily for body weight and food and water intakes. Abdominal circumference and body length were measured every 4 wk using a standard measuring tape under light anesthesia with Zoletil (10 mg/kg tiletamine, 10 mg/kg zolazepam, i.p.; Virbac) (21). BMI and energy efficiency were calculated as in a previous study (21).

Oral glucose tolerance tests were performed on rats following a 12-h food deprivation as described in a previous study (21). AUC was calculated as in a previous study (24) and plasma concentrations of total cholesterol, TG, and NEFA were also measured as in a previous study (21). At the end of the protocol, abdominal fat pads (including retroperitoneal, epididymal, and omental) were separately removed, weighed, and expressed as mg/mm of tibial length.

Assessment of cardiovascular structure and function

**Systolic blood pressure measurements.** Systolic blood pressure was measured every 4 wk under light sedation with Zoletil (10 mg/kg tiletamine, 10 mg/kg zolazepam, i.p.; Virbac) as previously described (21).

**Echocardiography.** Echocardiographic examinations (Phillips iE33, 12MHz transducer) were performed to assess cardiovascular structure and function in all groups. The examination was performed at the end of the protocol as previously described (21,22).

**Isolated Langendorff heart preparation.** Following terminal anesthesia and heparin injection, plasma was collected for biochemical analyses and isolated rat hearts were perfused for measurement of left ventricular diastolic stiffness as previously described (21).

**Vascular reactivity.** Thoracic aortic rings (~4 mm in length; 3–4 rings from 10 rats/group) were used to obtain cumulative concentration–response curves for noradrenaline (contraction), sodium nitroprusside (relaxation), and acetylcholine (relaxation) as in a previous study (21).

**Histology of the heart.** Two rats from each group were exclusively used for histology. Hearts were fixed, cut, and stained as previously reported (21).

**Assessment of hepatic structure and function**

**Histology of liver.** Livers (n = 8/group) were isolated and weighed. Two rats from each group were exclusively used for histology. Liver portions were isolated from these rats and fixed, cut, and stained as in a previous study (21).

**Liver enzymes in plasma.** Plasma activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) and the plasma concentrations of albumin, total bilirubin, urea, and uric acid were determined as previously described (21).

**Western-blot analysis**
After perfusion experiments, the heart samples were weighed and immediately stored at –80°C for protein extraction (n = 4/group). Similarly, the liver samples were immediately isolated after weighing the liver (n = 4/group) and were stored at –80°C for protein extraction. These samples were homogenized and sonicated after adding cell lysis buffer, followed by centrifugation at 15,000 × g for 30 min at 4°C. Supernatants were used to measure the protein concentration in each sample by the bicinchoninic acid method (Thermo Scientific). Supernatants in equal concentrations from each group were used in Western-blot analyses to study the expression of carnitine palmitoyltransferase 1 (CPT1), nuclear factor (erythroid-derived 2)-related factor-2 (Nrf2), heme oxygenase-1 (HO-1) (antibodies from Cell Signaling Technology), and β-actin (antibody from Sigma-Aldrich) in the liver and heart. For quantitative analysis, the expression of proteins was normalized to the expression of β-actin.

**Statistical analysis**
Values are presented as mean ± SEM. Results were tested for variance using Bartlett’s test and variables that were not normally distributed were transformed (using log 10 function) prior to statistical analyses. All the groups were tested for effects of diet, treatment, and their interaction by 2-way ANOVA. When the interaction and/or the main effects were significant, means were compared using the Newman-Keuls multiple comparison post test. Mean daily quercetin intakes in CQ and HQ groups were compared with Student’s t test. P < 0.05 was considered significant. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows.

**Results**

**Physiological variables.** Body weight was higher in the H rats than in the C rats at 16 wk. Although body weight was higher in CQ than in C rats at 16 wk, it did not differ between the H and HQ rats at 16 wk (Table 1). H rats consumed less food and
null水 compared with C rats. However, total energy intake was higher in the H rats than in the C rats. HQ rats consumed more food and water than H rats, whereas CQ rats consumed less water and more food compared with C rats. HQ rats had higher energy intake compared with C and H rats, respectively (Table 1). Vascular responses, including noradrenaline-induced contraction and sodium nitroprusside-induced relaxation, were impaired in H rats compared with C rats (Fig. 1A–C). Noradrenaline-
induced contraction and acetylcholine-induced relaxation were normalized in HQ rats, whereas these responses did not differ between the C and CQ rats (Fig. 1A,C). The HQ rats had a greater sodium nitroprusside-induced relaxation response compared with H rats, whereas CQ rats had a lower response to sodium nitroprusside compared with C rats (Fig. 1B).

**Hepatic structure and function.** Liver from H rats had more infiltration of inflammatory cells (Supplemental Fig. 2C) than C rats (Supplemental Fig. 2A) along with presence of fat vacuoles (Supplemental Fig. 2G) and portal fibrosis (Supplemental Fig. 2K), which were absent in the liver from C rats (Supplemental Fig. 2E,I). The wet weight of the liver was higher in H rats than in C rats and normalized in HQ rats, whereas it was lower in CQ rats than in C rats (Table 3). Plasma activities of ALT, AST, ALP, and LDH were higher in H rats compared with C rats. Plasma activities of ALT and ALP were normalized in HQ rats, whereas they were lower in CQ rats than in C rats. Plasma AST and LDH activities did not differ between C and CQ rats. Plasma AST activities did not differ between the H and HQ rats, whereas plasma LDH activity was normalized in HQ rats (Table 3). Although plasma total bilirubin concentrations did not differ between the C and H rats, they were lower in HQ rats compared with H rats. Plasma urea and plasma uric acid concentrations were lower and higher, respectively, in HQ rats than in C rats. The HQ rats had higher plasma urea concentrations compared with H rats, although not normalized, whereas plasma uric acid concentrations were normalized in HQ rats (Table 3).

**Expression of regulatory proteins in the liver and the heart.** In the liver from H rats, the protein expression of Nrf2, HO-1, CPT1, and caspase-3 was lower whereas NF-κB expression was higher compared with C rats. In HQ rats, the expression of Nrf2, CPT1, and caspase-3 in the liver was normalized, whereas the expression of HO-1 was higher compared with H rats. NF-κB expression in the liver was lower in both CQ and HQ rats compared with C and H rats, respectively. Hepatic expression of Nrf2 and CPT1 did not differ between C and CQ rats, whereas hepatic expression of HO-1 and caspase-3 were higher in CQ rats than in C rats. Hepatic expression of NF-κB was lower in the CQ rats than in C rats (Fig. 2A,C). In the heart from H rats, expression of Nrf2, HO-1, and CPT1 was lower, whereas expression of NF-κB was higher compared with C rats. HQ rats had higher expression of Nrf2, HO-1, and CPT1, whereas the expression of NF-κB was lower in the heart compared with H rats. Cardiac expression of Nrf2, HO-1, and CPT1 did not differ between the C and CQ rats, whereas cardiac expression of NF-κB was lower in CQ rats than in C rats. Caspase-3 expression in the heart did not differ between the groups (Fig. 2B,D).

**Discussion**

Flavonoids are secondary plant metabolites that are useful, e.g., for protection of plants against fungal infection (25–27); quercetin is one of the most common flavonoids in the human diet. Because quercetin is abundant in plant-based products in the diet, it is important to determine whether quercetin can reduce human health challenges such as obesity, metabolic syndrome, and NAFLD. Hence, we have characterized the effects of quercetin in an appropriate animal model of diet-induced metabolic syndrome and associated complications (21,28). This rodent model mimics most of the complications associated with human metabolic syndrome (21).

Obesity is a chronic condition characterized by excess fat deposition in the abdomen, including retroperitoneal, epididymal, and omental fat pads. Excess fat deposition increases morbidity and mortality through health complications, including oxidative stress, chronic low-grade inflammation, dyslipidemia, type 2 diabetes, cardiovascular disease, NAFLD, and some cancers (29–36). In this study, we have targeted NAFLD, obesity, and cardiovascular disease with quercetin using an appropriate rat model of metabolic syndrome (21). Using the same model, we showed that rutin, a glycoside of quercetin,
Our recent study with chia seeds has demonstrated a link between lipid trafficking away from abdomen and attenuation of metabolic syndrome in a diet-induced rat model. The hepatic and cardiac expression of NF-κB was downregulated, clearly indicating the presence of inflammation and oxidative stress in both the liver and the heart. This, in turn, gives rise to proteins and enzymes, such as HO-1, which reduce the cellular stress (39). This suggests that the activators of Nrf2 system can protect organ systems. Similarly, the role of NF-κB has been established in the activation of inflammation (40).

In obesity, oxidative stress and inflammation induce organ dysfunction (31). Our results showed that the high-carbohydrate, high-fat diet upregulated the hepatic and cardiac expression of NF-κB, whereas the hepatic and cardiac expression of Nrf2 was downregulated, clearly indicating the presence of inflammation and oxidative stress in both the liver and the heart. The hepatic and cardiac expression of NF-κB was down-regulated by quercetin, confirming its antiinflammatory role. Similarly, quercetin supplementation upregulated the expression of Nrf2, resulting in activation of antioxidant response elements, followed by upregulation of HO-1, and hence the reduction of oxidative stress. Thus, attenuation of hepatic and cardiac changes by quercetin could be mediated through its antioxidative and antiinflammatory actions.

Quercetin also upregulated the expression of CPT1, a regulator of fatty acid oxidation, in the liver and the heart. This change could attenuate NAFLD, thereby leading to attenuation of steatosis through higher fatty acid oxidation in the liver. Caspase-3 expression was also higher in the liver, but not in the heart, with quercetin as with rutin (24). Greater expression of caspase-3 indicates higher levels of apoptosis, possibly leading to the removal of steatotic cells from the liver. These results explain the role of quercetin in the attenuation of hepatic changes in metabolic syndrome.

In this study, 0.8 g/kg food of quercetin was used to provide a daily dose of ~50 mg/kg body weight. This dose corresponds to ~1 g/d quercetin in a 70-kg human based on scaling equation (41) or ~0.6 g/d based on body surface area comparisons between rats and humans (42). Although the mean daily human intake of quercetin is not known, the total intake of polyphenols is ~1 g/d, with two-thirds being flavonoids, including quercetin and rutin (43). This suggests that the dose of quercetin used in this study is realistic in humans.

In conclusion, quercetin was effective against the symptoms of metabolic syndrome in a diet-induced rat model. The trafficking of fat away from the abdomen did not lower body weight and body composition (18). This study also showed that the lipid components in plasma were higher with quercetin treatment consistent with the trafficking of fat by the circulation. The lack of difference in the body weights of quercetin-treated and untreated rats may suggest that the abdominal fat has been moved to other fat storage areas or has been converted to muscle mass.

Although dyslipidemia was not attenuated with quercetin, the other symptoms of metabolic syndrome were attenuated, including systolic blood pressure, glucose tolerance, and visceral obesity. Quercetin abolished hepatic steatosis, prevented the infiltration of inflammatory cells in the liver, and reduced the portal fibrosis along with improvements in liver function. Along with these changes were cardioprotective effects, including reduced collagen deposition, less infiltration of inflammatory cells, inhibition of cardiomyocyte hypertrophy, reduced ventricular stiffness, lower ventricular dimensions, and a return toward normal ventricular function.

The presence of inflammation and oxidative stress leads to cellular injury leading to organ dysfunction (37,38). One of the major defense systems against stress-related injury is the Nrf2 system (39). Nrf2 is the transcription factor present in inactive forms in the cell. Once activated, Nrf2 translocates to the nucleus and activates the antioxidant response elements (39). This, in turn, gives rise to proteins and enzymes, such as HO-1, which reduce the cellular stress (39). This suggests that the activators of Nrf2 system can protect organ systems. Similarly, the role of NF-κB has been established in the activation of inflammation (40).
weight and blood lipids, while the cardiovascular and liver complications of metabolic syndrome were attenuated. Quercetin supplementation attenuated the changes in expression of markers for oxidative stress and inflammation in the liver and the heart such as Nrf2, HO-1, and NF-κB along with higher fatty acid oxidation. Livers had greater expression of caspase-3, an apoptotic marker, indicating the attenuation of steatosis. Thus, quercetin can be considered as a nutraceutical with potential for the treatment of metabolic syndrome; clinical trials of this relatively safe natural compound should be undertaken.

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Literature Cited


