

Mulberry (*Morus alba* L.) Leaves and Their Major Flavonol Quercetin 3-(6-Malonylglucoside) Attenuate Atherosclerotic Lesion Development in LDL Receptor-Deficient Mice¹

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ABSTRACT The effects of dietary consumption of mulberry (*Morus alba* L.) leaves and their major flavonol glycoside, quercetin 3-(6-malonylglucoside) (Q3MG), on the development of atherosclerotic lesions, in relation to the susceptibility of plasma LDL to oxidative modification, was studied in LDL receptor-deficient (*LDLR*^{-/-}) mice. Male mice aged 8 wk were randomly assigned to 4 groups (control, quercetin, Q3MG, and mulberry). The control group was fed an atherogenic-diet containing 3 g cholesterol and 15 g cocoa butter/100 g. The other experimental groups were fed the same atherogenic diet supplemented with 0.05 g quercetin/100 g for the quercetin group, 0.05 g Q3MG/100 g for the Q3MG group, and 3 g dried mulberry-leaf powder/100 g for the mulberry group. The mice were fed their respective diets for 8 wk. The susceptibility of LDL to oxidative modification was significantly decreased in the Q3MG- and mulberry-treated mice, as evidenced by the 44.3 and 42.2% prolongation of the lag phase for conjugated diene formation compared with that of the control mice. The atherosclerotic lesion area in both the Q3MG- and mulberry-treated mice was significantly reduced by 52% compared with that of the controls. However, in the quercetin group, no protective effects were observed against LDL oxidation or atherosclerotic lesion formation. In conclusion, mulberry leaves attenuated the atherosclerotic lesion development in *LDLR*^{-/-} mice through enhancement of LDL resistance to oxidative modification, and these antioxidative and antiatherogenic protective effects were attributed mainly to Q3MG, the quantitatively major flavonol glycoside in mulberry leaves. *J. Nutr.* 135: 729–734, 2005.

KEY WORDS: • mulberry • quercetin 3-(6-malonylglucoside) • LDL oxidation • atherosclerosis • *LDLR*^{-/-} mice

Oxidative modification of LDL is believed to play a pivotal role in the development of atherosclerosis (1,2). Oxidized LDL is taken up by macrophages at an increased rate via the scavenger receptor (3) and subsequently leads to the formation of lipid-laden foam cells, the hallmark of early atherosclerotic fatty streak lesions (4,5). Antioxidants that prevent LDL from oxidation could attenuate the development of atherosclerosis. Epidemiologic studies indicated that dietary intake of antioxidant substances from plants is inversely associated with mortality from coronary heart disease (6,7).

Flavonoids are a large group of polyphenolic compounds ubiquitous in fruits, vegetables, and herbs; they have attracted much attention due to their potential antioxidant properties and probable role in the prevention of oxidative stress-associated diseases including atherosclerosis. Quercetin, a major representative of the flavonol group of flavonoids, was shown to have strong inhibitory effects on oxidative modification of human LDL in vitro (8,9). However, quercetin is found in

plant foods in the glycosylated form, and accumulates as glucuronide and sulfate conjugates in the blood circulation (10). Recent studies demonstrated that these conjugates also exert a substantial antioxidant effect on oxidative modification of human LDL (11,12).

In a previous study (13), we screened for antioxidant activity in 52 kinds of edible plant products in Japan using 3 [LDL oxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH)³ radical scavenging, and Folin-Ciocalteu] assays. Of these plants, mulberry (*Morus alba* L.) showed relatively high antioxidant activity, and the highest activity was observed in the LDL oxidation assay. Previous studies on the constituents of mulberry leaves identified quercetin 3-glucoside (Q3G) (isoquercitrin) and kaempferol 3-glucoside (astragalins) as the main flavonoids (14,15). We recently found that antioxidant activity of mulberry leaves can be attributed mainly to quercetin 3-(6-malonylglucoside) (Q3MG) (Fig. 1), the most abundant

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³ Abbreviations used: DPPH, 1,1-diphenyl-2-picrylhydrazyl; FC, free cholesterol; HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-C, LDL cholesterol; *LDLR*^{-/-}, LDL receptor-deficient; MDA, malondialdehyde; Q3G, quercetin 3-glucoside; Q3MG, quercetin 3-(6-malonylglucoside); TBA, thiobarbituric acid; TC, total cholesterol; TG, triglyceride.

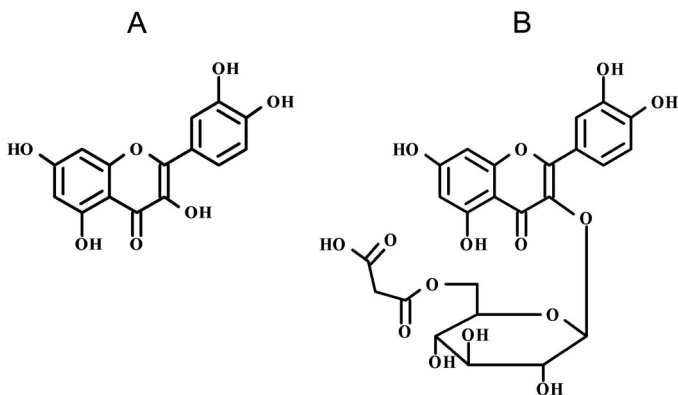


FIGURE 1 Structure of quercetin (A) and Q3MG (B).

flavonol glycoside in mulberry leaves, even greater (260 mg aglycone/100 g fresh weight) than that of onions (40–100 mg/100 g), which are considered to be one of the richest sources of quercetin (16).

Mulberry leaves, bark, and branches have long been used in Chinese medicine for healing of various health-related problems (17). Leaves of mulberry species have been widely consumed in Korea, Japan, and Chile as antihyperglycemic nutraceutical foods for patients with diabetes mellitus (18), and several studies have confirmed their hypoglycemic role (19,20). Although this antihyperglycemic role of mulberry leaves is relatively well recognized, studies concerning their *in vivo* antioxidant and antiatherogenic roles are scarce. Further, it is not clear whether such protective effects can be attributed to Q3MG because bioavailability appears to differ greatly among the various flavonoids. Thus, in the present study, we investigated the effects of dietary consumption of mulberry leaves and purified Q3MG on the development of atherosclerotic lesion, in relation to the susceptibility of plasma LDL to oxidative modification. For these purposes, we fed LDL receptor-deficient (*LDLR*^{-/-}) mice an atherogenic diet because these mice develop marked hypercholesterolemia and early-to-intermediate atherosclerotic lesions in response to 6–8 wk of consuming an atherogenic diet (21).

MATERIALS AND METHODS

Materials. Quercetin dihydrate (3,3',4',5,7-pentahydroxyflavone) and 1,1,3,3-tetraethoxypropane were obtained from Wako Pure Chemical. Mulberry leaves were harvested in Sakurae-cho, Shimane Prefecture, Japan in 2003, lyophilized and ground to powder using a vibrating sample mill (Heiko Seisakusho). The flavonol compounds of the leaves were extracted by suspending the dried powder in ethanol aqueous solution and analyzed by quantitative HPLC (LaChrom, Hitachi). The mulberry-leaf powder contained 622 mg quercetin flavonols (equivalent to 352 mg quercetin aglycone) including 328 mg Q3MG, 140 mg Q3G, 110 mg rutin, and 44 mg quercetin 3-acetylglucoside/100 g dry weight. We processed and purified Q3MG from 100 g of mulberry leaves as follows. The leaves were extracted with 1 L of 70% (v/v) ethanol solution twice and concentrated to dryness under reduced pressure. The residue was then dissolved in water and partitioned with ethyl acetate. The water layer was applied to a Diaion HP20 column chromatograph and methanol was added. The methanol-eluted fractions were concentrated to dryness under reduced pressure. The residue was dissolved in water and loaded serially into an AKTA purifier using a preparative ODS 80 Ts column (TOSOH). The peak fractions corresponding to Q3MG were pooled and concentrated to dryness under reduced pressure to obtain 160 mg of yellow powder.

Animals and diets. A breeding colony was generated from homozygous *LDLR*^{-/-} mice with a C57BL/6J × 129Sv background obtained from Jackson Laboratories. Mice were maintained under controlled environmental conditions (temperature 23 ± 2°C, relative humidity 55 ± 10%, 12-h light:dark cycle, air change 13–15 times/h) and consumed food and water *ad libitum*. At the age of 8 wk, male mice weighing 20.5 ± 0.8 g were randomly assigned to 4 (control, quercetin, Q3MG, and mulberry) groups, consisting of 10 mice each, with the exception of 8 mice in the Q3MG group because of a shortage of the purified compound. The control group was fed an atherogenic diet containing 3 g cholesterol and 15 g cocoa butter/100 g, which was prepared from a standard rodent diet obtained from Funabashi-Nojo. The diet contained 8.0% moisture, 20.8% crude protein, 4.8% crude fat, 3.2% crude fiber, 5.0% crude ash, 58.2% nonfiber carbohydrate as well as vitamins and minerals adequate to meet the nutritional needs of mice. The other experimental groups were fed the same atherogenic diet supplemented with 0.05 g quercetin dihydrate/100 g for the quercetin group, 0.05 g Q3MG/100 g for the Q3MG group, and 3 g dry mulberry-leaf powder/100 g for the mulberry group. The diets were fed for 8 wk. Food intake was recorded daily and body weight was measured once each week. This study and all procedures were approved by the Animal Care and Use Committee of Shimane University School of Medicine, Japan.

Biochemical analysis of plasma and lipoprotein fractions. After overnight food deprivation, the mice were anesthetized by an *i.p.* injection of sodium pentobarbital solution (50 mg/kg body weight). At the time of killing, blood was collected from hearts into tubes containing 1000 mg/L EDTA and centrifuged immediately at 1000 × *g* for 15 min at 4°C. Plasma was stored at -80°C before analysis. An antioxidant, BHT (4.4 mg/L), was added to plasma aliquots used for lipoprotein separation. Concentrations of total cholesterol (TC), free cholesterol (FC), HDL cholesterol (HDL-C), triglyceride (TG), and glucose were measured using enzymatic assay kits (Wako Pure Chemical; Cholesterol E test, Free cholesterol E test, HDL-cholesterol test, Triglyceride G test, and Glucose C II test); LDL cholesterol (LDL-C) was calculated by the Friedewald formula (22), and levels of non-HDL-C were estimated by the formula TC (mmol/L) - HDL-C (mmol/L) (23). Concentrations of insulin were measured using an Ultrasensitive Mouse Insulin kit (Mercodia). Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated by the following formula: fasting plasma insulin (pmol/L) × fasting plasma glucose (mmol/L)/22.5 (24). The protein concentration in the LDL fractions was determined using a Protein Assay Rapid kit (Wako Pure Chemical).



FIGURE 2 Photomicrographs of representative Sudan IV stained aortae of *LDLR*^{-/-} mice fed an atherogenic diet (control, *n* = 10) (A), or that diet supplemented with 0.05 g quercetin/100 g (*n* = 10) (B), 0.05 g Q3MG/100 g (*n* = 8) (C), or 3 g mulberry-leaf powder/100 g (*n* = 10) (D) for 8 wk.

Plasma lipoprotein separation. Plasma lipoproteins were separated for each mouse using a sequential ultracentrifugation method as described previously (25) except the initial plasma volume was reduced to 150 μL and all of the reagents were used proportionately. All fractions were stored at -80°C until analyzed. The purity of lipoprotein fractions obtained by sequential ultracentrifugation method was verified by a 2.4–15.2% polyacrylamide gradient gel electrophoresis with a Multigel-LP kit (Daiichi Pure Chemical).

LDL oxidation. The susceptibility of LDL to oxidative modification was assessed by determining the lag phase for conjugated diene formation as described (13). Briefly, LDL was desalted using a Centricon YM-30 (Millipore) and aliquots containing 8 mg protein/L from each mouse were mixed with CuSO_4 solution (5 $\mu\text{mol/L}$ final concentration) to initiate oxidation. The appearance of conjugated dienes was then measured by monitoring continuously at 234 nm for 7 h at 37°C using a spectrophotometer UV-1700 (Shimadzu) equipped with a 16-position automated sample changer.

In addition to the lag phase, the susceptibility of LDL to oxidative modification was evaluated by the thiobarbituric acid (TBA) test. TBARS were determined spectrofluorometrically as described (26) with slight modification. Briefly, 50 μL of LDL fraction, 100 μL of 3% SDS, 400 μL of 0.1 mol/L HCl, 50 μL of 10% (wt:v) phosphotungstic acid, and 200 μL of 0.7% (wt:v) 2-TBA were combined and placed at 95°C for 30 min. Samples were cooled on ice, mixed with 500 μL of 1-butanol and centrifuged at $1800 \times g$ for 10 min at 4°C . An aliquot of the butanol phase was separated and analyzed spectrofluorometrically (excitation 510 nm and emission 555 nm) using a plate reader attachment. TBARS values were expressed as malondialdehyde (MDA) equivalents. MDA standard was prepared from 1,1,3,3-tetramethoxypropane.

Assessment of atherosclerosis in the aorta. The aortic tree of each mouse was prepared as described (27). Then the aorta was removed and fixed in 10% (v:v) neutral buffered formalin until used for staining (21). Briefly, the entire aorta and heart were rinsed in 70% (v:v) ethanol and then immersed in 0.5% (wt:v) Sudan IV in 35% ethanol:50% acetone for 15 min with continuous shaking. The aorta was destained in 80% ethanol until the background color became clear and then washed with water. Images of the aortae were captured with a digital camera mounted on a light microscope DF PLAN 1 (Olympus) (Fig. 2), processed with Adobe Photoshop 6.0FE, and analyzed using Scion Image Analysis software. Atherosclerotic lesion of the aorta was expressed as a percentage of the total surface area.

Statistical analysis. The data were analyzed statistically with SPSS statistical analysis software (Version 12.0J, SPSS). Results are expressed as means \pm SD. Because the data for TG, insulin, and HOMA-IR were significantly skewed, they were transformed logarithmically before analysis. One-way ANOVA was used to assess the

differences among 4 groups, and post hoc analyses were performed using the Bonferroni test for 2 independent variables. Unless otherwise noted, a nominal two-sided P -value < 0.05 was used to assess significance.

RESULTS

Body weight gain, liver weight, food and quercetin intakes. At the end of the study, body weight gain in the mulberry group (0.9 ± 0.2 g/wk) was lower than that in the control and quercetin groups (1.3 ± 0.3 and 1.3 ± 0.4 g/wk, $P = 0.001$). There was no difference in liver weight relative to body weight among the 4 groups. Daily food intake did not differ among the groups (4.3 ± 0.7 , 4.0 ± 0.6 , 4.5 ± 1.0 , and 4.2 ± 0.6 g/d for the control, quercetin, Q3MG, and mulberry groups, respectively). Based on food intake, the mean daily intake of quercetin was 2.0 mg/d in the quercetin group, whereas Q3MG intake was 2.2 mg/d (equivalent to 1.2 mg quercetin aglycone/d) in the Q3MG group. The mulberry treatment in our study provided 0.4 mg quercetin/d expressed as aglycone (see Materials and Methods).

Effects of quercetin, Q3MG, and mulberry on plasma biochemistry. Plasma TC, HDL-C, and non-HDL-C levels were significantly different among the groups (Table 1). Compared with the controls, HDL-C levels were lower in quercetin- ($P = 0.015$) and Q3MG-treated ($P = 0.009$) mice. Although values of FC ($P = 0.060$) and LDL-C ($P = 0.051$) also decreased noticeably in the Q3MG- and mulberry-treated mice, the differences were not significant. Plasma glucose concentration was reduced in the mulberry-treated mice compared with the controls ($P = 0.009$).

Effects of quercetin, Q3MG, and mulberry on oxidative modification of LDL. The susceptibility of LDL to oxidative modification was assessed by the lag phase for conjugated diene formation and by measuring LDL-MDA concentration (Table 2). The incubation of LDL isolated from the control mice with copper-ion resulted in a lag phase of 24 min, whereas in LDL isolated from the $\text{LDLR}^{-/-}$ mice treated with Q3MG or mulberry, there was a significant prolongation of the lag phase, i.e., 44.3 and 42.2%, respectively, compared with that of the control mice. However, the lag phase in the quercetin-treated mice did not differ from that of the controls. The LDL-MDA concentration in the Q3MG group decreased compared with the control group, but the difference was not significant. MDA

TABLE 1

Plasma biochemistry of $\text{LDLR}^{-/-}$ mice fed an atherogenic diet or that diet supplemented with quercetin, Q3MG, or mulberry-leaf powder for 8 wk¹

	Diet				ANOVA P -value
	Control	0.05 g Quercetin/100 g	0.05 g Q3MG/100 g	3 g Mulberry/100 g	
	mmol/L				
Total cholesterol	25.1 \pm 4.7	25.7 \pm 7.0	18.9 \pm 5.4	18.8 \pm 8.9	0.044
Free cholesterol	7.86 \pm 1.67	8.01 \pm 2.41	6.00 \pm 1.58	5.93 \pm 2.55	0.060
LDL-C	21.6 \pm 4.5	22.7 \pm 6.5	16.1 \pm 5.1	16.0 \pm 8.7	0.051
HDL-C	1.75 \pm 0.11 ^a	1.34 \pm 0.31 ^b	1.29 \pm 0.39 ^b	1.41 \pm 0.25 ^{ab}	0.004
non-HDL-C	23.3 \pm 4.8	24.3 \pm 6.9	17.6 \pm 5.3	17.3 \pm 8.8	0.049
Triglyceride	3.77 \pm 0.92	3.48 \pm 1.40	3.15 \pm 0.86	2.87 \pm 0.88	0.208
Glucose	8.90 \pm 0.88 ^a	7.56 \pm 1.25 ^{ab}	7.47 \pm 1.19 ^{ab}	7.11 \pm 1.27 ^b	0.009
Insulin, $\times 10^{-9}$	1.78 \pm 1.15	2.02 \pm 1.50	1.57 \pm 1.11	1.22 \pm 0.61	0.436
HOMA-IR	0.10 \pm 0.07	0.09 \pm 0.07	0.08 \pm 0.06	0.06 \pm 0.03	0.210

¹ Values are means \pm SD, $n = 10$ for control, quercetin and mulberry groups, $n = 8$ for Q3MG group. Means in a row without a common letter differ, $P < 0.05$.

TABLE 2

Parameters of susceptibility of LDL to oxidative modification in LDLR^{-/-} mice fed an atherogenic diet or that diet supplemented with quercetin, Q3MG, or mulberry-leaf powder for 8 wk¹

	Diet				ANOVA P-value
	Control	0.05 g Quercetin/100 g	0.05 g Q3MG/100 g	3 g Mulberry/100 g	
Lag phase, min	24 ± 4 ^b	21 ± 4 ^b	35 ± 7 ^a	35 ± 8 ^a	0.001
MDA, μmol/L	3.5 ± 1.3 ^{ab}	4.6 ± 1.7 ^a	2.5 ± 0.9 ^b	3.4 ± 1.0 ^{ab}	0.017
MDA, nmol/mg protein	12.7 ± 2.7	17.0 ± 4.2	13.9 ± 2.1	19.1 ± 9.0	0.050

¹ Values are means ± SD, *n* = 10 for control, quercetin and mulberry groups, *n* = 8 for Q3MG group. Means in a row without a common letter differ, *P* < 0.05.

concentration adjusted to LDL protein contents did not differ among the 4 groups.

Effects of quercetin, Q3MG, and mulberry on aortic atherosclerotic lesion. The extent of atherosclerosis in the aorta was evaluated by computer-assisted image analysis. Atherosclerotic lesions were found in the aortae, mainly in the aortic arches, of all mice groups (Fig. 2). Compared with that of the controls, the atherosclerotic lesion area in both the Q3MG- and mulberry-treated mice was significantly reduced by 52% (Fig. 3). However, the atherosclerotic lesion area in the quercetin-treated mice did not differ from that of the control group.

DISCUSSION

In this study, we demonstrated with LDLR^{-/-} mice that dietary supplementation of mulberry leaves effectively inhibited the progression of atherosclerotic lesions, and that this antiatherogenic effect was related to the enhanced resistance of plasma LDL to oxidative modification. Moreover, we clarified that the antiatherogenic and antioxidative protective effects of mulberry leaves could be attributed mainly to the major flavonol glycoside, Q3MG.

We observed that mulberry and Q3MG treatments retarded the onset of plasma LDL oxidation, as evidenced by the prolongation of the lag phase for conjugated diene formation. However, the treatments did not inhibit the formation of the end product of the LDL oxidation, as measured by the TBARS

level. By way of explanation, we surmise the following. Oxidation of LDL is a free radical-driven lipid peroxidation process, which can be divided chronologically into a lag phase, a propagation phase, and a decomposition phase (28). During the course of the lag phase, the PUFAs in LDL are protected from oxidation by the lipophilic antioxidants, particularly α-tocopherol, which is the most abundant antioxidant in LDL (29). Similarly, flavonoids were shown to protect LDL from oxidation at the initial stage of lipid peroxidation by acting as free radical scavengers (30,31). In our previous study (13), mulberry demonstrated potent free radical scavenging activity as determined by the DPPH radical scavenging assay. Quercetin and other flavonoids were shown to bind to the surface of LDL particles via the formation of an ether bond (32), limiting the access by oxidants and their initial attack on the surface. These mechanisms are likely responsible for the delayed onset of LDL oxidation observed in the mice consuming quercetin glucoside-rich mulberry leaves or pure Q3MG. Moreover, in these 2 groups, the prolongation of the lag phase for conjugated diene formation was associated with a reduction in atherosclerotic lesion formation. Studies in humans also demonstrated the lag phase to be independently associated with the severity of coronary atherosclerosis (33).

During the propagation phase (the next course of lipid peroxidation), depletion of the intrinsic antioxidants and LDL-bound flavonoids leads to rapid oxidation of PUFAs, forming lipid peroxides, followed by the decomposition phase characterized by the formation of aldehydes including MDA. However, we observed no differences in TBARS formation among the 4 experiment groups. Some studies reported that the reduction of atherosclerotic lesion formation after dietary supplementation of flavonoids is associated with the inhibition of formation of both conjugated diene and TBARS (32,34), although the same protective effect against atherosclerosis may be solely the result of inhibition of the former (32). Results of our study suggest that inhibition of conjugated diene formation at the initial stage of lipid peroxidation (delay of the onset of LDL oxidation) plays an important role in the attenuation of early atherosclerotic lesion formation.

The lack of relation between the values of the lag phase and LDL-MDA points out the limitations of measuring the susceptibility of LDL to oxidation *ex vivo*. Although measurement of MDA is one of the most widely used analytical methods of lipid peroxidation, it has been criticized as unspecific and prone to artifacts during sample processing (35). The measurement of MDA by TBA-test reflects only the formation and decomposition of certain lipid peroxidation products. In addition, MDA is not derived exclusively from PUFAs; under certain environmental circumstances, amino acids, carbohydrates, peptides, and proteins can also release intermediates

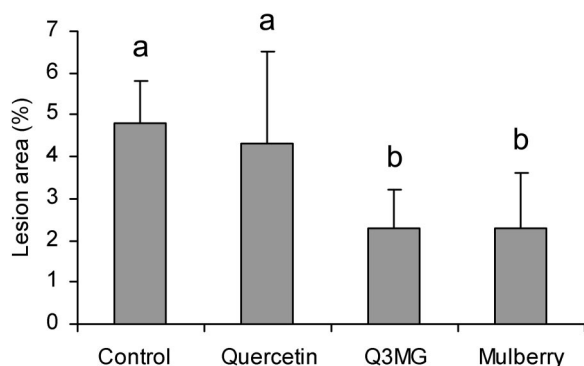


FIGURE 3 Percentage of total aortic atherosclerotic lesion areas in LDLR^{-/-} mice fed either an atherogenic diet or that diet supplemented with 0.05 g quercetin/100 g, 0.05 g Q3MG/100 g, or 3 g mulberry-leaf powder/100 g for 8 wk. Lesions were stained with Sudan IV. Data are means ± SD, *n* = 10 for control, quercetin and mulberry groups; *n* = 8 for Q3MG group. Means without a common letter differ, *P* < 0.05.

that can form a MDA-TBA adduct under the acid-heating conditions of the test (36). Due to these limitations of the TBARS assay, we considered the inhibition of LDL oxidation observed in the "lag phase" to be a more reliable indicator. Continuous monitoring of the formation of conjugated dienes through measurement of the increase in absorbance at 234 nm, which reflects the initiation stage of lipid peroxidation, reveals the exact oxidation lag time (28).

In this study, quercetin, a well-known *in vitro* antioxidant, had no protective effects against LDL oxidation and atherosclerotic lesion formation even though daily quercetin intake in the quercetin group was higher (2 mg/d) than that of the Q3MG and mulberry (1.2 and 0.4 mg/d, respectively) groups, indicating that the concentration in the diet is not material. Differences in the absorption processes of quercetin glucosides and its aglycone may be responsible for their different effects on LDL oxidation and atherosclerotic lesion formation. Absorption of onion quercetin glucosides, 45% of which is quercetin-4'-glucoside (37), was far superior to that of its aglycone (38). Moreover, bioavailability of Q3G was shown to be as high as that of quercetin-4'-glucoside (39). In a study by Hollman et al. (38), glucosides were transported into enterocytes by the intestinal glucose carrier-sodium dependent glucose transporter in the small intestine, and subsequently hydrolyzed by intracellular cytosolic β -glucosidase (40). Another pathway involves lactase phlorizin hydrolase, a glucosidase of the small intestine brush border membrane, which catalyzes extracellular hydrolysis of certain glucosides, followed by diffusion of the aglycone across the brush border (41). Q3G and Q3MG are not substrates for cytosolic β -glucosidase, but they were absorbed after hydrolysis by lactase phlorizin hydrolase (42,43). Once absorbed, quercetin glucosides produce a rapid, higher plasma peak level and have a very slow elimination half-life (44). Thus, the repeated daily supplementation of pure Q3MG and quercetin-glucoside-rich mulberry leaves likely led to a buildup of a sufficient concentration to protect LDL from oxidation. The retardation of the onset of the LDL oxidation and prevention of atherosclerosis progression in the mulberry group despite a lower daily intake of quercetin vis a vis the Q3MG group indicates the possible role of some other flavonoids and biological active constituents present in mulberry leaves.

In this study, the mulberry treatment inhibited the increase in body weight of mice compared with the controls. Adding dried mulberry-leaf powder to the atherogenic diet reduced overall energy density (energy content in a given weight of a food, e.g., kcal/g or kJ/g) due to its fiber content such as cellulose, hemicelluloses and lignin, all present in plant cell walls (45). Consumption of the same amount of less energy-dense food may have led to the reduction of body weight change in the mulberry group. Plasma glucose levels after food deprivation were also reduced in the mulberry-treated mice. It was suggested that this effect is related to polyhydroxylated alkaloids, including 1-deoxynojirimycin, which exerts potent inhibitory activity against α -glucosidase, to fagomine, which potentiates glucose-induced insulin release, and to the increase in tissue uptake of glucose by the leaves (19,20,46).

In conclusion, dietary consumption of mulberry leaves attenuated atherosclerotic lesion development in *LDLR*^{-/-} mice through enhancement of LDL resistance to oxidative modification, and these antioxidative and antiatherogenic protective effects were attributed mainly to Q3MG, the quantitatively major flavonol glycoside in mulberry leaves.

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