Quercetin prevents left ventricular hypertrophy in the Apo E knockout mouse

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Hypercholesterolemia is a risk factor for the development of hypertrophic cardiomyopathy. Nevertheless, there are few studies aimed at determining the effects of dietary compounds on early or mild cardiac hypertrophy associated with dyslipidemia. Here we describe left ventricular (LV) hypertrophy in 12 week-old Apo E−/− hypercholesterolemic mice. The LV end diastolic posterior wall thickness and overall LV mass were significantly increased in Apo E−/− mice compared with wild type (WT) controls. Fractional shortening, LV end diastolic diameter, and hemodynamic parameters were unchanged from WT mice. Oral low dose quercetin (QCN; 0.1 μmol QCN/kg body weight for 6 weeks) significantly reduced total cholesterol and very low density lipoprotein in the plasma of Apo E−/− mice. QCN treatment also significantly decreased LV posterior wall thickness and LV mass in Apo E−/− mice. Myocardial geometry and function were unaffected in WT mice by QCN treatment. These data suggest that dietary polyphenolic compounds such as QCN may be effective modulators of plasma cholesterol and could prevent maladaptive myocardial remodeling.

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Introduction

While left ventricular (LV) hypertrophy is generally regarded as an adaptive response to chronic pressure or volume overload, it is also a gateway to pathogenesis and heart failure. Specifically, concentric hypertrophy is an early remodeling event that often precedes myocardial dilatation, progression to eccentric hypertrophy, and global pump failure. Several studies show that hypercholesterolemia itself is independently associated with mild LV concentric hypertrophy and increased LV mass in humans [1,2], underscoring a role for dyslipidemia in myocardial remodeling. Although some of the mechanisms contributing to these events have been elucidated, safe interventions with minimal side effects that prevent or diminish early remodeling events are scant.

Hypercholesterolemic Apo E−/− mice have substantially elevated triglycerides and increased total plasma cholesterol, and spontaneously develop atherosclerosis [3]. It has been shown that these mice exhibit increased LV mass at 6 weeks of age in the absence of hemodynamic stress [4], further suggesting that hyperlipidemia may be an early initiator of cardiac remodeling. Therefore, dietary or other therapeutic interventions that have the potential to decrease plasma lipids are of particular interest for prevention of cardiac hypertrophy. For example, the dietary polyphenol quercetin (QCN) which is present in broccoli, onions, grapes, and wine [5] has been shown to prevent hypertrophy of cultured myocytes in response to angiotensin [6] and to lower blood pressure and attenuate aortic constriction-induced cardiac hypertrophy in rats [7]. Other polyphenolic compounds such as...
resveratrol have similar effects as QCN on the cardiovascular system [8–10]. Taken together with data showing that dietary intake of polyphenols decreases plasma cholesterol in both humans and animals [11–13], polyphenols appear to have a generally beneficial effect on the cardiovascular system[10]. Nevertheless, there are relatively few reports based on the effects of orally administered polyphenols such as QCN on the heart under conditions of chronic hypercholesterolemia.

In the present study we elected to use QCN as single polyphenolic because of its previously reported beneficial effects on the cardiovascular system and its potential to serve as the basis for the development of more potent and effective therapeutics [14–17]. Therefore, we hypothesized that QCN treatment could diminish the early cardiac remodeling processes that occur during hypercholesterolemia. In this study, we examined the effects of low dose QCN on plasma cholesterol and cardiac geometry and function in Apo E−/− and wild-type (WT) mice. Our findings suggest that dietary intake of polyphenolics such as QCN may be a safe and effective means to prevent or decrease hypercholesterolemia and its associated cardiac structural changes that could lead to maladaptive remodeling and further functional defects.

Materials and methods

Reagents. All chemicals were purchased from Calbiochem (San Diego, CA), BioMol (Plymouth Meeting, PA) or Sigma-Aldrich-Fluca (St.Louis, MO). All primary antibodies were obtained from Abcam Inc (Cambridge, MA).

Animal treatments. Six-week old wild-type (C57BL/6, WT) or Apo E−/− (ApoemT1uc) mice were obtained from Jackson Laboratories (Bar Harbor, Maine) and fed a standard mouse pellet diet (Ralston Purina Diet) and water ad libitum for 1 week. For the following 6 weeks, QCN was administered as gelatin pellets prepared by mixing flavored gelatin solution (25% w/v) at 37 °C with QCN solution in DMSO to a final concentration of QCN 0.1% (v/v). Control groups received the pellets without QCN. All mice were maintained at constant humidity (60–70 °C), 7% dryness, and constant temperature (21 °C) throughout the study. The mice were kept in clear mesh cages to allow visual monitoring to detect any detrimental effects of QCN on the cardiovascular system. All procedures were performed prior to measurement of BP followed by 15 cycles of measurements of the systolic and diastolic pressure. Mean arterial pressure (MAP) was calculated using the equation: MAP = 1/3 Psys.

Plasma QCN analyses. After bolus gavage of QCN (equivalent to one feeding), plasma samples were acquired at various time points by retro-orbital bleeding and by cardiac puncture. They were prepared by mixing flavored gelatin solution (25% w/v) at 37 °C with QCN solution in DMSO to a final concentration of QCN 0.1% (v/v). Control groups received the pellets without QCN. All mice were maintained at constant humidity (60 ± 5%, temperature (24 ± 1 °C), and light cycle (6AM to 6PM). All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with The Guide for the Care and Use of Laboratory Animals (USDHHS, NIH publication no 86-23, 1996).

Analysis of the lipid profiles in mouse plasma. Blood was collected from anesthetized animals during sacrifice by retro-orbital bleeding. Total plasma cholesterol levels were determined colorimetrically using a commercial kit (Infinity cholesterol reagent; Sigma, St. Louis, MO). Plasma lipoprotein cholesterol profiles were analyzed by the chromatographic method described in [18].

Echocardiography and measurement of blood pressure. Animals were anesthetized using isofluorane (1–2%) and their body weight was measured. Analysis of LV parameters by two-dimensional B-mode, M-mode and PW-Doppler mode echocardiography was performed non-invasively using an echocardiography high resolution imaging system VEVO 770 equipped with a 30 MHz transducer (Visual Sonics, Toronto, Canada). To monitor arterial blood pressure (BP), the non-invasive device Codas 2 (Kent Scientific Corp.; Torrington, CT) was used. For the echocardiography measurements, cycles were performed prior to measurement of BP followed by 15 cycles of measurements of the systolic and diastolic pressure. Mean arterial pressure (MAP) was calculated using the equation: MAP = 1/3 Pdiast.

Statistical analysis of data. Results of the echocardiography measurements are presented as the mean ± standard error (SEM) for 12 animals per phenotype and treatment group. Significant differences were assessed using one-way analysis of variance (ANOVA). Intergroup comparisons were analyzed using Student-Newman-Kuels post hoc test with statistical significance set at \( p < 0.05 \).

Results

In the course of the study, WT and Apo E−/− mice were fed the control or the QCN-containing diets for 6 weeks. Daily consumption of QCN by animals during the 6 week treatment period was approximately 0.1 μmol QCN/kg body weight. For the validation of QCN absorption into the blood, solubilized gelatin pellets were fed to WT mice (N=5) by gavage and the concentration of QCN derivatives was measured in the mouse plasma by mass-spectrometry at various time points after feeding. Concentrations of the compounds in the plasma were measured by reverse-phase liquid chromatography-mass spectrometry. Data are presented as the mean ± SEM, N=5.

Table 1

| Time after gavage, (min) | QCN (nmol/L) | Met-QCN (nmol/L) |
|-------------------------|--------------|------------------|
| 30                      | 111.47 ± 12.03 | 23.58 ± 5.61     |
| 60                      | 65.90 ± 6.81  | 10.33 ± 2.35     |
| 90                      | 22.86 ± 1.25  | 2.69 ± 0.42      |

Liquefied quercetin pellets were administered by gavage, and the mouse plasma was collected at different time points after feeding. Concentrations of the compounds in the plasma were measured by reverse-phase liquid chromatography-mass spectrometry. Data are presented as the mean ± SEM, N=5.
times (Table 1). Thirty min after the consumption of solubilized QCN-containing pellets, plasma QCN levels reached readily detectable levels that decreased linearly with time ($R^2=0.99$; Table 1). QCN was not detectable (<0.1 nmol/L) in untreated animals. The methylated form of QCN, Met-QCN, which is a metabolite of free QCN, had similar pharmacokinetics, decreasing with time in the plasma (Table 1). These data confirm that oral intake of QCN increases systemic QCN levels for a significant period of time.

Separation of the total cholesterol from the plasma of WT and Apo E$^{-/-}$ mice, fed the control or QCN-diet, by fractions, was performed using FPLC. The resulting chromatograms are presented as Fig. 1A. In agreement with previous reports and as expected [3], Apo E$^{-/-}$ mice displayed severe hypercholesterolemia: the amount of total cholesterol in Apo E$^{-/-}$ animals was 6-fold higher than WT. A QCN-dependent decrease in VLDL (peak a) was observed in the total plasma cholesterol of Apo E$^{-/-}$ mice. The difference between the chromatograms of WT and Apo E$^{-/-}$ was attributed to intermediate lipoprotein fractions: IDL-LDL fraction (peak b) in Apo E$^{-/-}$ animals and LDL-HDL1 fraction (peak c) in WT mice. Specifically, VLDL and IDL-LDL fractions were significantly increased in Apo E$^{-/-}$ animals with respect to WT controls. Moreover, high density lipoprotein (HDL) was decreased in Apo E$^{-/-}$ mice compared with WT mice. Dietary QCN treatment for six weeks decreased circulating VLDL in Apo E$^{-/-}$ animals by 30% but did not affect levels of IDL-LDL and HDL (Fig. 1A and B). Treatment with QCN had no effect on plasma cholesterol fractions in WT mice.

Apo E$^{-/-}$ mouse echocardiographic measurements showed no significant changes in LV end diastolic diameter (LVEDD), but demonstrated significant increases in LV posterior wall diameter (LVPWd) and decreased LVEDD/LVPWd ratios when compared with WT controls (Figs. 2 and 3). Moreover, overall LV masses were also increased in Apo E$^{-/-}$ mice (Table 2). LV fractional shortening and systolic, diastolic, and mean arterial blood pressures were not significantly different when compared with WT controls (Table 2). Therefore, unlike Apo E$^{-/-}$ mice at 24 weeks of age and older which have relatively high arterial blood pressures [19,20], the 12 week-old Apo E$^{-/-}$ mice showed no indication of derangements of vascular tone or blood pressure. Taken together, these data suggest that hypercholesterolemia alone could promote a mild myocardial concentric hypertrophy in the absence of hypertension.

Body weight was increased in the Apo E$^{-/-}$ group relative to controls and was not altered by QCN treatment (Table 2). Treatment with QCN, however, significantly decreased LVPWd in Apo E$^{-/-}$ mice and restored LVEDD/LVPWd ratios to control values (Figs. 2 and 3). Furthermore, QCN treatment prevented the increases in LV mass that were observed in Apo E$^{-/-}$ mice fed vehicle alone. QCN did not affect heart rate or fractional shortening in Apo E$^{-/-}$ mice or WT controls (Table 2). Taken together, these data demonstrate that Apo E$^{-/-}$ mice display an early, mild concentric hypertrophy that is independent of hemodynamic stress and is ameliorated by QCN treatment.

**Discussion**

Polyphenolic compounds have several purported beneficial cardiovascular effects and have been shown to decrease oxidative stress, reduce or prevent growth of atherosclerotic plaques, inhibit platelet aggregation, decrease blood pressure, improve vascular reactivity, and decrease plasma lipids and lipoproteins [21]. In general, QCN has a beneficial effect on the cardiovascular system, and has been shown to prevent cardiac remodeling in severe models of cardiac hypertrophy [7]. Although other studies have shown that Apo E$^{-/-}$ mice even at 6 weeks of age demonstrate increased myocardial mass [4], the study described herein is the first to demonstrate a concentric hypertrophy that develops seemingly due to hypercholesterolemia alone. This finding is particularly important because hypercholesterolemia is a major contributor to the mild concentric hypertrophy that occurs in humans [1,2], emphasizing a need for therapeutic interventions to prevent or diminish the early myocardial structural changes that could potentially lead to hypertrophic cardiomyopathy. The development of left ventricular hypertrophy is progressive and one of the commonest risk factors for cardiovascular mortality. Importantly, the risk for developing cardiac hypertrophy is associated with the metabolic syndrome, increased LDL to HDL ratio and unfavorable fatty acid profile suggesting that intervention with low dose QCN could be potentially beneficial [2,22]. Recently, it has been suggested that polyphenolics may have benefit in lessening the pathological effects of the metabolic syndrome [23]. Interestingly, we found that low dose dietary QCN treatment prevents hypertrophy of the LV posterior wall in Apo E$^{-/-}$ mice.
The molecular mechanisms by which QCN prevents hypertrophy in this mouse model are unknown. Since hypercholesterolemia alone has been suggested to play a causative role in the etiology of cardiac hypertrophy [1,2], we suggest that the QCN-dependent decrease in cardiac hypertrophy in this study can be ascribed to the decrease in plasma lipids. Recent studies suggest that QCN
may modulate the inflammatory response of pro-atherogenic macrophages and decrease atherosclerotic lesions [14,16]. Several polyphenolic compounds, including those found in grapes [12], wine [24], chocolate [25], and olive oil [26], decrease blood lipids and seem to have a general beneficial effect on the cardiovascular system. Indeed, we observed a 25% decrease in total plasma cholesterol and a 30% decrease in plasma VLDL in Apo E−/− mice fed QCN (Fig. 1). As the most pro-atherogenic lipoprotein, VLDL specifically has been shown to promote transformation of macrophages to foam cells [27] and to induce synthesis of the pro-inflammatory cytokines TNF-α and interleukin-1β [28,29]. The VLDL in the Apo E−/− mouse model appears to be aggressively pro-atherogenic, possibly because of preferential presence of Apo B48 over Apo B100 in its structure [30,31]

Table 2

| Animal group | Wild Type | Wild Type +QCN | Apo E−/− | Apo E−/− +QCN |
|--------------|-----------|----------------|----------|---------------|
| Body weight, (g) | 22.35 ± 0.31 | 23.12 ± 0.37 | 25.44 ± 0.64* | 25.31 ± 0.63* |
| Heart rate, BPM | 439 ± 13 | 442 ± 14 | 449 ± 13 | 453 ± 9 |
| Systolic BP | 146 ± 3 | 152 ± 3 | 160 ± 6 | 150 ± 7 |
| Diastolic BP | 115 ± 3 | 116 ± 3 | 126 ± 6 | 114 ± 7 |
| Mean arterial BP | 125 ± 3 | 128 ± 3 | 137 ± 6 | 126 ± 7 |
| LV mass | 81.41 ± 3.72 | 87.0 ± 3.74 | 92.42 ± 3.95* | 88.14 ± 3.42 |
| LV FS | 0.30 ± 0.02 | 0.28 ± 0.02 | 0.28 ± 0.01 | 0.28 ± 0.01 |

*p < 0.05 (n = 12) compared to WT (W; one-way ANOVA).

Fig. 3. Group data of echocardiographic measurements taken from hearts of WT and ApoE−/− mice fed control and QCN-supplemented diets: M-mode images of the LV of WT and ApoE−/− mice on control and QCN diet were obtained using echocardiography. Parameters of LV geometry were measured directly using the resulting images. Panel A: LVEDD. Panel B: LVPWd. Panel C: ratio between LVEDD and LVPWd. N = 12 in each group. Data are presented as the mean ± SEM. *p < 0.05 compared to WT-controls (one-way ANOVA).

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