BRIEF COMMUNICATION: RESEARCH REPORT

CURRENT DEVELOPMENTS IN NUTRITION

Basic Science Section

Bitter Melon (*Momordica charantia* L.) Supplementation Has No Effect on Hypercholesterolemia and Atherosclerosis in Mice

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ABSTRACT

Bitter melon (BM; *Momordica charantia* L.) has been reported to ameliorate diet-induced obesity and dyslipidemia. However, the effects of BM on atherosclerosis have not been determined. This study investigated the effects of BM diet-induced atherosclerosis in LDL receptor–deficient mice. A total of 30 female mice (aged 6–8 wk) were fed a saturated fat–enriched diet. In group 1 ($n = 10$), mice were fed this diet alone, whereas mice in groups 2 and 3 ($n = 10$/group) were fed the diet supplemented with BM either 0.1% or 1% by weight. After 12 wk, body weight, plasma cholesterol, and atherosclerotic plaque areas were analyzed. No significant differences in body weight and plasma cholesterol concentrations were observed among the groups. Also, BM supplementation did not affect atherosclerosis development. In conclusion, dietary BM has no effect on plasma cholesterol concentration and atherogenesis in hypercholesterolemic mice. *Curr Dev Nutr* 2020;4:nzaa148.

Keywords: *Momordica charantia*, atherosclerosis, hypercholesterolemia, cholesterol, bitter melon

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Abbreviations used: BM, bitter melon; LDLR, low-density lipoprotein receptor.

Introduction

Atherosclerosis is one of the major cardiovascular diseases with high morbidity and mortality rates in different populations, with hypercholesterolemia and obesity as major risk factors (1, 2). Lifestyle modification and healthy dietary habits reduce the risks of predisposition to atherosclerosis and overall cardiovascular health (3). However, successful lifestyle changes are challenging. Complementary approaches and dietary adjuncts that identify potential therapeutic targets on hypercholesterolemic properties have substantial interest.

Momordica charantia L. (*M. charantia*), colloquially known as bitter melon, is a product that has been used traditionally for treatment of diabetes (4). It has been reported in both human and animal studies that *M. charantia* has beneficial effects on adiposity and lipid metabolism (5–8). In 1 study, *M. charantia* supplementation reduced plasma cholesterol and triglyceride concentrations in normolipidemic rats. Moreover, aqueous extracts of *M. charantia* fruit improved lipid profile in normolipidemic diabetic rats. Although beneficial effects of *M. charantia* on lipid metabolism in normolipidemic state have been demonstrated, there is a lack of information on anti-atherosclerosis effect in hypercholesterolemic animals.

Low-density lipoprotein receptor (*Ldlr*)–deficient mice are a commonly used animal model to study atherosclerosis (1). These mice have profoundly increased plasma cholesterol concentrations when fed a saturated fat-enriched diet. In a quest to determine the role of *M. charantia* supplementation on atherogenesis, the current study was performed to determine whether modest or substantial dietary supplementation of *M. charantia* would attenuate atherosclerosis development in $Ldlr^{-/-}$ mice.

Methods

Mice and diet

A total of 30 female $Ldlr^{-/-}$ mice (B6.129S7-Ldlr$^{tm1Her/J}$; stock no. 002207) were purchased from The Jackson Laboratory and randomly divided into 3 groups ($n = 10$ per group). One mouse in group 3 was excluded from data analysis due to premature termination because of rectal prolapse. Mice were fed a saturated fat diet (42% kcal/wt from milk fat diet no. TD.88137; Envigo), termed Western diet, in group 1 for 12 wk. The Western diet was also consumed by groups 2 and 3, with supplementation of a moderate (0.1% wt:wt; TD.170736) or high (1% wt:wt; TD.170735) amount of *M. charantia* extract, respectively ($n = 10$ per group). **Figure 1**A shows the study design. A whole plant *M. charantia* aqueous extract (4:1 wet-to-dry ratio) was purchased (Bulk Supplements)

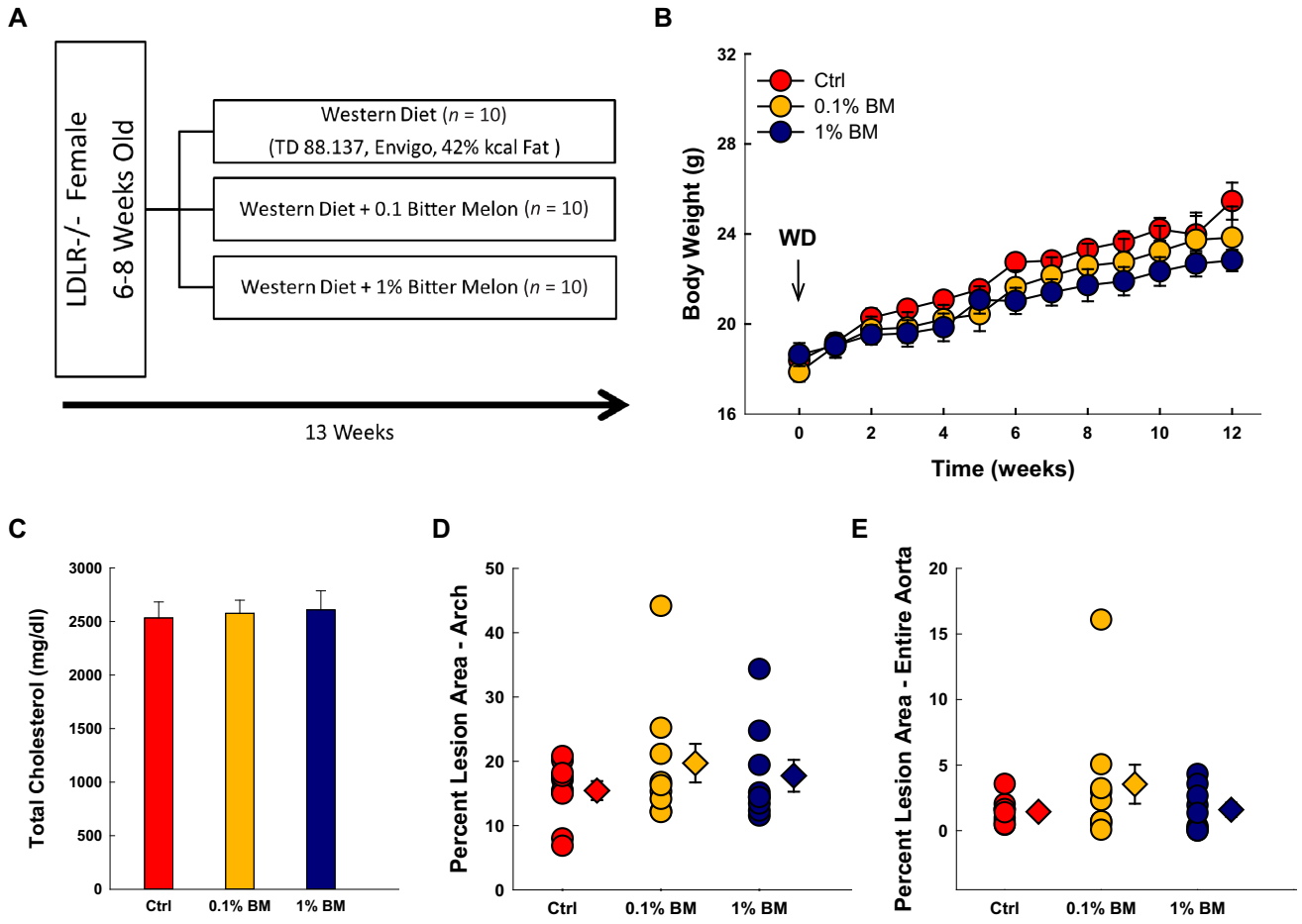

FIGURE 1 Female LDLR-deficient mice were fed a high-fat diet supplemented with *M. charantia* for 12 wk. (A) Body weights were measured weekly. (B) *M. charantia* supplementation did not show any effect on total plasma cholesterol concentrations. (C) Atherosclerotic lesion development. (D and E) Percentage of atherosclerotic lesion areas measured on the intimal surface of ascending aorta and arch (D) and entire aorta (E). Diamonds represent group means; circles represent individual mice; error bars are SEMs. BM, bitter melon; Ctrl; control; LDLR, low-density lipoprotein receptor; WD, Western diet.

and supplemented with the Western diet to provide the stated doses (customized by Envigo). The mice were maintained in a light:dark cycle of 14:10 h with free access to food and water. Mice were aged 8 wk at the initiation of the study. All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Measurement of plasma cholesterol concentrations

Plasma cholesterol concentrations were measured using an enzymatic kit (catalog no. 999–0,2601; Wako Chemicals).

Quantification of atherosclerosis

After 12 wk of Western diet feeding, mice were killed by overdose of ketamine:xylazine followed by cardiac puncture and saline perfusion.

Atherosclerosis was quantified on the intimal surface of the proximal aortic region (ascending aorta, arch, and from the aortic orifice of left subclavian artery to 3 mm below) or the entire aorta from the ascending to the iliac bifurcation by an en face method (9, 10) and in accord with the guidelines published by the American Heart Association (1) and as detailed in our description posted on protocols.io (dx.doi.org/10.17504/protocols.io.bfy8jpzw). Briefly, aortas were removed from the ascending aortic region to the iliac bifurcation and placed in formalin (10% wt:vol) overnight. Adventitial tissues were cleaned from the aortas. Then the intimal surface was exposed by a longitudinal cut, and the 3 arterial branches were cut open and pinned on a black wax surface. Images of en face aortas were taken using a Nikon digital camera (Nikon digital sight DS-Ri1) under a dissecting microscope. Data were analyzed using the Nikon NIS-Elements software (NIS-Elements AR 5.11.00).

Statistical analyses

SigmaPlot 14.0 (Systat Software) was used for all statistical analyses. To compare multiple groups that passed normality and equal variance tests, 1-way ANOVA with Holm–Sidak post hoc test was used. Kruskal–Wallis 1-way ANOVA on Ranks and Dunn's post hoc test was used for data that failed normality or equal variance test. $P < 0.05$ was considered statistically significant.

Results

M. charantia supplementation had no effect on body weights

M. charantia has a bitter taste. Rodents are sensitive to inclusion of extra compounds to their diet, which can consequently affect food intake. To determine the impact on the inclusion of this supplement, mice were weighed weekly during the study period. All groups had steady weight gain that was not significantly different among the 3 groups (Figure 1B).

Plasma cholesterol concentrations were not affected by *M. charantia*

Because hypercholesterolemia has a direct association with atherogenesis, plasma cholesterol concentrations were measured at the end of the study. All 3 groups had concentrations of ~2500 mg/dL that were not significantly different between groups (Figure 1C).

M. charantia had no effect on atherosclerosis

Atherosclerosis lesion areas were quantified on the intimal surface of the ascending aorta and arch region or the entire aorta. All mice had atherosclerotic lesions, but there was no significant difference between groups (Figure 1D, E).

Discussion

M. charantia has been shown to promote beneficial effects on lipid metabolism. In this study, we investigated the role of *M. charantia* supplementation on development of atherosclerosis in hypercholesterolemic mice. We hypothesized that supplementation of *M. charantia* would have a dose-dependent anti-atherosclerotic effect in female *Ldlr*$^{-/-}$ mice. However, 12 wk of *M. charantia* supplementation did not have an effect on plasma cholesterol concentrations or atherosclerosis development in this study.

M. charantia extracts have lipid-lowering effects on both diabetic and high-fat-diet–fed rats (6, 8, 11–13). Aqueous extracts of *M. charantia* not only improved glucose tolerance but also lowered plasma apoB-100 and apoB-48 concentrations in female C57BL/6 mice fed a high-fat diet (14). A 30-d administration of *M. charantia* fruit extract also decreased triglyceride and LDL cholesterol concentrations in diabetic rats (15), and *M. charantia* (0.75% wt:wt) supplementation reduced plasma cholesterol concentrations in high-fat-diet–fed rats (8). With many chemicals contained in the extract, *M. charantia*'s active hypoglycemic and hypolipidemic compounds are unknown. The known isolated compounds, such as charantin (a steroid glycoside) and a 166-residue insulin mimetic peptide, have been reported to be potential compounds responsible for such beneficial effects (16).

In agreement with results from the current study, there are inconclusive results in studies investigating the role of this traditional plant on dyslipidemia and hypercholesterolemia-induced atherosclerosis. Previous studies reported no changes in either metabolic parameters or lipid metabolism after different doses of *M. charantia* supplementation (8, 11, 17). Rats fed diets containing *M. charantia* freeze-dried powder supplemented with 1% wt:wt for 14 d had no change in plasma cholesterol concentrations (11). Also, a clinical study using encapsulated *M. charantia* for a duration of 3 mo did not report any significant difference in the lipid profile of patients (18). Another randomized, double-blind, placebo-controlled trial also reported no significant effects on mean fasting blood sugar, total cholesterol, and weight after treatment with *M. charantia* capsules (19).

Although results from our study are in contrast with some of the previously published results, these discrepancies may be attributed to different study designs used in combination with the different preparations, the range of doses, and administration modes of *M. charantia*. Our study used Western-diet–fed female *Ldlr*$^{-/-}$ mice with *M. charantia* aqueous extract supplementation. Previous studies used Sprague–Dawley rats or different genetically modified mouse models such as apolipoprotein E–deficient mice or mice homozygous for the obese spontaneous mutation, Lep^{ob}, which may lead to the disparate results (11, 13, 15, 20). In addition, our study used a whole plant *M. charantia* aqueous extract to supplement the diets, whereas previous reports used freeze-dried *M. charantia* from the fruit. Several studies have shown that atherosclerotic lesion sizes are not significantly different between male and female mice (21). We used only female mice for this long-term study because they are less aggressive when housed together and easier to handle. Moreover, confirmation of extraction and purification of *M. charantia*'s active compounds and concentration measurement of such compounds in plasma would be helpful additions for future studies. There are a variety of phytochemicals present in *M. charantia*, especially the fruit and seed, such as phenolic acids, flavonoids, quinine, and other bioactive components (22–24). Also, *M. charantia* includes pectin, a soluble fiber that has been reported to affect lipid profiles (23).

In summary, our study results indicate that supplementation of rodent diet with moderate (0.1% wt:wt) or high (1% wt:wt) doses of *M. charantia* has no effects on hypercholesterolemia and atherosclerosis development. Further studies should investigate the effective dose of supplementation, measuring the bioactive compounds as a marker of consumption.

Acknowledgments

The authors' responsibilities were as follows—SM, HSL, AD, and SPS: designed the study; SM and DAH: performed the animal experiments; SM, DAH, HSL, and AD: analyzed the data; DAH, HSL, and AD: supervised the experiments and verified the data; and all authors: wrote and revised the manuscript and read and approved the final manuscript.

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