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Dietary rose hip exerts antiatherosclerotic effects and increases nitric oxide-mediated dilation in ApoE-null mice☆,☆☆

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Abstract

Atherosclerosis is a disease in which atheromatous plaques develop inside arteries, leading to reduced or obstructed blood flow that in turn may cause stroke and heart attack. Rose hip is the fruit of plants of the genus *Rosa*, belonging to the *Rosaceae* family, and it is rich in antioxidants with high amounts of ascorbic acid and phenolic compounds. Several studies have shown that fruits, seeds and roots of these plants exert antidiabetic, antiobesity and cholesterol-lowering effects in rodents as well as humans. The aim of this study was to elucidate the mechanisms by which rose hip lowers plasma cholesterol and to evaluate its effects on atherosclerotic plaque formation. ApoE-null mice were fed either an HFD (CTR) or HFD with rose hip supplementation (RH) for 24 weeks. At the end of the study, we found that blood pressure and atherosclerotic plaques, together with oxidized LDL, total cholesterol and fibrinogen levels were markedly reduced in the RH group. Fecal cholesterol content, liver expression of *Ldlr* and selected reverse cholesterol transport (RCT) genes such as *Abca1*, *Abcg1* and *Scarb1* were significantly increased upon RH feeding. In the aorta, the scavenger receptor *Cd36* and the proinflammatory *Il1β* genes were markedly down-regulated compared to the CTR mice. Finally, we found that RH increased nitric oxide-mediated dilation of the caudal artery. Taken together, these results suggest that rose hip is a suitable dietary supplement for preventing atherosclerotic plaques formation by modulating systemic blood pressure and the expression of RCT and inflammatory genes.

Keywords: Atherosclerosis; Reverse cholesterol transport; Diet; Apolipoprotein E; Blood pressure

1. Introduction

Atherosclerosis is a multifactorial disease and the underlying cause of myocardial infarction, stroke, unstable angina and sudden cardiac death. Collectively, these diseases represent the most common cause of death worldwide [1,2]. Atherosclerotic lesions are characterized by the accumulation of cells, connective tissues elements, lipids and debris in the subendothelial space of medium- and large-sized arteries [3,4]. Several genetic and environmental factors are associated with this pathology, including elevated levels of LDL, reduced levels of HDL, high blood pressure, diabetes, obesity and smoking [3]. Diet is another environmental factor that can exert either beneficial or detrimental effects on cardiovascular diseases depending on its content of dietary components such as fibers, cholesterol, fatty acids, vitamins and antioxidant molecules [5,6].

Accumulation of LDL-cholesterol in the intima and subsequent modification into proatherosclerotic oxidized LDL (oxLDL) is one of the precipitating events in atherosclerosis, followed by lipid uptake by macrophages, endothelial activation and release of chemokines, cytokines, proteases and vasoactive molecules [4,7]. The early lesions known as *fatty streaks* are not clinically significant but are the precursors of more advanced lesions characterized by the accumulation of debris in the lipid-rich necrotic core and recruitment of smooth muscle cells (SMC). Plaques can become increasingly complex, may undergo calcification and ulceration and can ultimately occlude the vessel due to formation of a thrombus or rupture [3].

Rose hip (RH) is the fruit of plants of the genus *Rosa*, belonging to the *Rosaceae* family widely distributed in Europe, Africa, North America and Middle East [8,9]. The fruit is rich in ascorbic acid [10], phenolic compounds [11] and carotenoids [12], and it has been shown to exert antiobesity, antiinflammatory and antioxidative effects both *in vitro* and *in vivo* [13–17]. It also has beneficial and pain-relieving

Abbreviations: RCT, reverse cholesterol transport; oxLDL, oxidized LDL; SMC, smooth muscle cells; RH, rose hip; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance test; SAA, serum amyloid A; EDD, endothelial dependent dilation; SR-B1, scavenger receptor class B member 1; TICE, transintestinal cholesterol excretion; Cch, charbachol

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properties in subjects with arthritis [18–21]. We have previously demonstrated that RH added to an HFD prevents body weight gain, increases energy expenditure and reduces plasma glucose, insulin and cholesterol levels in C57BL/6J mice [22,23]. Moreover, we also showed that 6 weeks of rose hip intake is able to lower systolic blood pressure and plasma cholesterol levels in obese subjects [9].

The aim of this study was to investigate the molecular mechanisms by which rose hip reduces plasma cholesterol and whether it exerts protective effects in the vasculature by decreasing atherosclerosis and restoring vascular function. In order to do so, we used the atherosclerosis-prone hypercholesterolemic ApoE-null mouse model.

2. Materials and methods

2.1. Experimental animal procedures

Eight-week-old female ApoE-null mice were purchased from Charles River, Italy. The animals were maintained in a temperature-controlled room with a 12:12-h light: dark cycle with free access to food and water. After 1 week of acclimatization, mice were randomly divided in two groups and fed a control HFD (CTR) or an HFD supplemented with rose hip (RH) for 6 months. The study was approved by the local animal ethics committee (Lund, Sweden; permit no. M133–14).

2.2. Diets

The two experimental diets contained 45 energy % from fat (Research diets, New Brunswick, NJ, USA) (Table 1). Rose hip powder was obtained from Orkla ASA, (Oslo, Norway) and analyzed by Eurofins Food & Agro Testing (Sweden). Based on its composition (Table 2), the macronutrient content was balanced between the two diets. Vitamin and mineral mixture compositions are described in the online supporting material (Supplemental Tables 1 and 2).

2.3. Food intake

Food intake was monitored daily for five consecutive days in single-caged mice by weighing the food. The cages were carefully inspected for food spillage.

2.4. Glucose tolerance test

An intraperitoneal glucose tolerance test (IPGTT) was performed on 6-h-fasted mice by injecting 2 g/kg of D-(+)-glucose (SIGMA-Aldrich, St. Louis, MO, USA) followed by repetitive blood sampling from the saphenous vein in heparin-coated tubes at 0, 30, 60, 90 and 120 min. Blood glucose levels were analyzed with Accu-chek Aviva (Roche Diagnostic GmbH, Germany). Blood samples were centrifuged, and plasma was collected, snap-frozen and stored at –80 °C for insulin analysis.

Table 1
Composition of diets

	CTR	RH
Ingredients (g/kg)		
Casein	215.1	197
L-Cysteine	3.2	3.1
Corn Starch	53.8	-
Maltodextrin 10	107.5	85.5
Glucose	23.2	-
Fructose	25.2	-
Sucrose	188.2	152.5
Cellulose	104.7	-
Soybean oil	26.9	16.7
Lard	190.9	182.8
Mineral mix (S10026)	10.8	10.3
Dicalcium phosphate	14	13.4
Calcium carbonate	5.9	5.6
Potassium citrate	17.7	17
Vitamin mix	10.8	10.3
Choline bitartrate	2.1	2.0
Rose hip	-	303.8
Protein (kcal%)	18	18
Carbohydrates (kcal%)	37	37
Fat (kcal%)	45	45
Kcal/g	4.4	4.2

Table 2
Rose hip powder analysis (per 100 g)

Moisture content	8.53 g±10%
Ash	5.76 g±10%
Fat	2.98 g±10%
Protein	2.62 g±10%
Carbohydrates (calculated)	47.1 g
Fiber	33.0 g±15%
Fructose	7.93 g±15%
Glucose	7.32 g±15%
Sucrose	9. 11 g±15%
Saturated fatty acids	27.7%
Monounsaturated fatty acids	9.7%
Polyunsaturated fatty acids	51.8%
Ascorbic acid	440 mg±10%

2.5. Systolic and diastolic blood pressure measurements

Systolic and diastolic blood pressures were analyzed using the 8-channel tail-cuff noninvasive blood pressure CODA acquisition system (Kent Scientific, USA) following the manufacturer's instructions. Briefly, nonanesthetized mice were restrained in a clear acrylic tube on a warm pad for a few minutes before placing the occlusion tail-cuff around the tail. The animals were trained for 4 consecutive days before the recording sessions.

2.6. Plasma analysis

At the end of the study, blood was withdrawn by orbital puncture and collected in heparin-coated tubes from 10-h-fasted isofluorane-anesthetized mice. The blood was immediately centrifuged, and plasma was collected, snap-frozen and stored at −80 °C.

To assess plasma total cholesterol and triglycerides levels, Infinity™-Cholesterol and Infinity™-Triglycerides assays (Fisher Diagnostic, Middletown – VA, USA) were used according to the manufacturer's instructions.

ELISA assays were used to measure levels of oxLDL (Cloud-Clone Corp, USA), insulin (Mercodia, Sweden), fibrinogen, Serum Amyloid A, HDL and LDL/VLDL cholesterol, ICAM and VCAM (Abcam, UK) following the manufacturer's instruction.

2.7. Fecal cholesterol analysis

One week before the end of the study, feces were collected from mice that were individually housed in cages with grid floors for 24 h. Prior to analysis, the feces were lyophilized, weighed and pulverized by hand in a mortar. Lipids were extracted from 40 mg of feces with 500 μl of chloroform:isopropanol:NP-40 (7:11:0.1 v/v) overnight at 4 °C and then for 1 h at room temperature using an orbital shaker. After centrifugation for 5 min at 10.000× *g*, the liquid phase was removed. The fecal pellet was washed with 400 μl of chloroform:isopropanol:NP-40 (7:11:0.1 v/v) for 1 h at room temperature on an orbital shaker. The solvent was then evaporated under a stream of nitrogen, and the lipids were dissolved in assay buffer prior the cholesterol quantification using a fluorometric cholesterol quantification kit (Abcam).

2.8. Aorta en face and Sudan-IV staining

Staining and quantification of plaque area has been described elsewhere [24]. Briefly, after euthanizing the mice, the aorta was perfused with PBS through the left ventricle, dissected under a dissecting microscope and fixed in 4% paraformaldehyde for 48 h. The surrounding fat was then carefully removed, and the aorta opened longitudinally and pinned to a silicone (Sylgard 184 Silicone Elastomer kit; Dow Corning, Germany) coated dish with 0.1 mm stainless steel pins (Fine Science Tools, Germany). The intimal layer of the aorta was stained with 0.1% Sudan IV (Sigma-Aldrich, St. Louis – MO, USA) solution (dissolved in 70%-EtOH/Acetone, 1:1) for 15 min with gentle agitation, washed several times with 80% ETOH and rinsed with water. The atherosclerotic lesion area and total aorta area were measured using ImageJ software (http://imagej.nih.gov/ij/). The borders of the aorta were traced and the software calculated the aorta's surface. The plaques were highlighted by color threshold and the plaque area was also determined by the software. Finally, the plaque area/aorta surface ratio was calculated.

2.9. Wire myography

Vascular reactivity was measured in a wire myograph (610 M, DMT, Aarhus, Denmark) as described [25]. Briefly, the caudal artery was dissected in Ca^{2+}-free HEPES-buffered Krebs (135.5 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 2.9 g/L glucose, 11.6 mM HEPES, pH 7.4) and 2 mm artery segments were mounted, equilibrated for 30 min and stretched to 5 mN. Following contraction with 60 mM KCl (7 min), the arteries were relaxed (25 min) and further subjected to cumulative doses of an α1-adrenergic agonist