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Red Cabbage Microgreens Lower Circulating Low-Density Lipoprotein (LDL), Liver Cholesterol, and Inflammatory Cytokines in Mice Fed a High-Fat Diet

Haiqiu Huang,[†,§] Xiaojing Jiang,[§] Zhenlei Xiao,[‡,⊗] Lu Yu,[§] Quynhchi Pham,[†] Jianghao Sun,[#] Pei Chen,[#] Wallace Yokoyama,[⊥] Liangli Lucy Yu,[§] Yaguang Sunny Luo,[‡] and Thomas T. Y. Wang*[,†]

[†]Diet, Genomics and Immunology Laboratory, Beltsville Human Nutrition Research Center, ARS, U.S. Department of Agriculture, Beltsville, Maryland 20705, United States

[§]Nutrition and Food Science Department, University of Maryland, College Park, Maryland 20742, United States

[‡]Food Quality Laboratory, Beltsville Area Research Center, ARS, U.S. Department of Agriculture, Beltsville, Maryland 20705, United States

[⊗]Department of Agriculture, Culinology and Hospitality Management, Southwest Minnesota State University, Marshall, Minnesota 56258, United States

[#]Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, ARS, U.S. Department of Agriculture, Beltsville, Maryland 20705, United States

[⊥]Healthy Processed Foods Research Unit, Western Regional Research Center, ARS, U.S. Department of Agriculture, Albany, California 94710, United States

S *Supporting Information*

ABSTRACT: Cardiovascular disease (CVD) is the leading cause of death in the United States, and hypercholesterolemia is a major risk factor. Population studies, as well as animal and intervention studies, support the consumption of a variety of vegetables as a means to reduce CVD risk through modulation of hypercholesterolemia. Microgreens of a variety of vegetables and herbs have been reported to be more nutrient dense compared to their mature counterparts. However, little is known about the effectiveness of microgreens in affecting lipid and cholesterol levels. The present study used a rodent diet-induced obesity (DIO) model to address this question. C57BL/6NCr mice (n = 60, male, 5 weeks old) were randomly assigned to six feeding groups: (1) low-fat diet; (2) high-fat diet; (3) low-fat diet + 1.09% red cabbage microgreens; (4) low-fat diet + 1.66% mature red cabbage; (5) high-fat diet + 1.09% red cabbage microgreens; (6) high-fat diet + 1.66% mature red cabbage. The animals were on their respective diets for 8 weeks. We found microgreen supplementation attenuated high-fat diet induced weight gain. Moreover, supplementation with microgreens significantly lowered circulating LDL levels in animals fed the high-fat diet and reduced hepatic cholesterol ester, triacylglycerol levels, and expression of inflammatory cytokines in the liver. These data suggest that microgreens can modulate weight gain and cholesterol metabolism and may protect against CVD by preventing hypercholesterolemia.

KEYWORDS: *cholesterol, inflammation, microgreen, red cabbage, triacylglycerol*

INTRODUCTION

Chronic diseases account for about 70% of deaths and >75% of total healthcare costs in the United States in 2010.[1] In 2012, close to half of the U.S. population was reported to have one or more chronic conditions,[2] and cardiovascular disease (CVD), in particular, accounted for 30% of all U.S. deaths.[3] Epidemiological studies indicate that individuals with higher intakes of fruits and vegetables tend to have a lower occurrence of CVD.[4] Therefore, increased consumption of fruits and vegetables has been recommended as a key component of a healthy diet for the prevention of chronic diseases, including CVD.[4,5] Moreover, there is growing evidence that certain groups of vegetables, such as cruciferous vegetables, may be particularly beneficial for human health.[5,6] Cruciferous vegetables, such as red cabbage, have been reported to be protective against chronic diseases such as CVD and prostate cancer.[7,8]

As part of the concerted strategy to promote health and prevent chronic diseases, the development of new foods with enhanced function, that is, functional foods, has received much attention. Microgreens, an exotic genre of edible greens (Figure 1), is one of those foods that has gained popularity in upscale markets and restaurants over the past few years.[9,10] Microgreens are tender immature plants produced from the seeds of vegetables (such as red cabbage) and herbs having two fully developed cotyledon leaves with or without the emergence of a rudimentary pair of first true leaves. Microgreens can provide a large array of intense flavors, vivid colors, and tender textures[10] and can be served as an ingredient in salads, soups, and

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Figure 1. Photographs of red cabbage microgreens and mature red cabbage.

sandwiches, to enhance their color, texture, and/or flavor. Microgreens also serve as an edible garnish to brighten up a wide variety of main dishes. More importantly, previous studies found that microgreens possess higher nutritional densities when compared to their mature counterparts.[10] For example, red cabbage microgreens contained approximately 260-fold more β-carotene, >40 times more vitamin E, and 2.4−6-fold higher vitamin C levels than previously published for mature red cabbage.[11]

Although microgreens, such as those from red cabbage, have been reported to possess more nutrients and are perceived to be "healthier", no known study has been conducted to evaluate whether consumption reduces CVD risk factors. Previous research has reported the potential health-promoting properties of red cabbage, such as antioxidant, anti-inflammation, and antibacterial effects and amelioration of dyslipidemia induced by excessive cholesterol.[12−14] On the basis of the existing literature on the protective effects of cruciferous vegetables, we hypothesized that red cabbage microgreens (RCMG) would reduce CVD risk factors in a diet-induced obesity (DIO) mouse model. The current study tested this hypothesis, and our results supported that the consumption of RCMG, at physiologically achievable levels, attenuates several high-fat diet-induced risk factors, including hypercholesterolemia and liver inflammation. Elevated levels of circulating lipid and cholesterol are known risk factors for CVD; therefore, the DIO mouse model was employed to assess the effect of RCMG to ameliorate dyslipidemia and hypercholesterolemia induced by a high-fat diet.[15−17] Additionally, possible molecular mechanisms for the health-promoting effects of RCMG were evaluated.

MATERIALS AND METHODS

Sample Preparation. Mature red cabbage samples (*Brassica oleracea* L. var. *capitata*) were obtained from a local market. Red cabbage microgreens (*B. oleracea* L. var. *capitata*) were provided by a commercial microgreens grower (Fresh Origins, San Marcos, CA, USA). Microgreens, 7 days after seeding, were harvested without roots, packaged in clamshell containers, and shipped overnight in insulated containers with ice packs. When received, all of the mature and microgreen samples were flash frozen in liquid nitrogen and lyophilized for 48−72 h (VirTis Freezemobile 35 ES Sentry 2.0 freeze-dryer, SP Scientific Corp., Warminster, PA, USA). Lyophilized samples were then ground into powder and stored in sealed bags at −20 °C before use.

Bioactive Composition Analysis. Major bioactive components (anthocyanin, polyphenols, and glucosinolates) in the stock mature red cabbage and red cabbage microgreens to be used for the formulation of animal diets were analyzed. The extraction and analysis protocols are as follows.

Anthocyanin and Polyphenol Extraction from Microgreens and Mature Red Cabbage. Freeze-dried and powdered microgreen and mature red cabbage samples (200 mg) were extracted with 5.00 mL of methanol/water (70:30, v/v) using sonication for 60 min at room temperature. The extracts were then centrifuged at 5000*g* for 15 min (IEC Clinical Centrifuge, Damon/IEC Division, Needham, MA, USA), and the supernatant was filtered through a 17 mm (0.45 μm) PVDF syringe filter (VWR Scientific, Seattle, WA, USA). Five microliters of the filtered extract was used for UHPLC-PDA-ESI/HRMS/MS^n analysis described below.

Extraction of Glucosinolates. Glucosinolates in microgreens and mature red cabbage were isolated according to previously published methods.[18] Briefly, 100 mg of freeze-dried and powdered microgreen and mature red cabbage samples was heated at 75 °C for 1 min, mixed with 5 mL of methanol solution, and then vortexed for 20 s. Sinigrin solution (5 mM, 200 μL) was added as internal standard in one set of samples and 200 μL of methanol in the other set as control. Extraction solutions were heated with boiling methanol for 20 min and then filtered with 0.45 μm syringe filter, and supernatants were transferred to another tube for future use. Then 1 mL of extract was added to a pretreated DEAE Sephadex A25 resin mini-column; 100 μL of sulfatase solution was added and left to react overnight at ambient temperature and then eluted with two 1 mL portions of water. Five microliters of the purified extract was used for UHPLC-PDA-ESI/HRMS/MS^n analysis described below.

UHPLC-PDA-ESI/HRMS/MS^n Conditions. UHPLC-PDA-ESI/HRMS/MS^n was used to determine anthocyanin, polyphenol, and glucosinolate amounts and species. The UHPLC-HRMS system used consisted of an LTQ Orbitrap XL mass spectrometer with an Agilent 1290 UHPLC with a G4220A binary pump, a 1316C column oven, and a G4226 autosampler with temperature control and a PDA detector (ThermoFisher Scientific, San Jose, CA, USA). Anthocyanin and polyphenol separation were carried out on a Hypersil Gold AQ RP-C18 UHPLC column (200 mm × 2.1 mm i.d., 1.9 μm, ThermoFisher Scientific) with an UltraShield precolumn filter (Analytical Scientific Instruments, Richmond, CA, USA) at a flow rate of 0.3 mL/min. A gradient mobile phase was used in this study (96% A (1% formic acid in water) and 4% B (1% formic acid in ACN) at 0 min, 80% A and 20% B at 20 min, 65% A and 35% B at 40 min, 25% A and 75% B at 45 min, and 10% A and 90% B at 50−55 min) with an injection volume of 5 μL, a column temperature at 50 °C, and a sample temperature at 4 °C.

Glucosinolates separation was carried out on an RRHD Eclipseplus C18 column (150 mm × 2.1 mm i.d., 1.8 μm, Agilent) with an UltraShield precolumn filter (Analytical Scientific Instruments) at a flow rate of 0.4 mL/min. A gradient mobile phase was used in this study (98% A (1% formic acid in water) and 2% B (1:1 v/v of 1% formic acid in ACN) at 0 min, 90% A and 10% B at 10 min, 50% A and 50% B at 25 min, and 10% A and 90% B at 30 min) with an injection volume of 5 μL, a column temperature at 50 °C, and a sample temperature at 4 °C and a UV detection wavelength at 229 nm. For determination of anthocyanin, cyanidin 3-*O*-glucoside reference standard was used for determination of total anthocyanins by assuming the molar-response factor as 1.00 (UV 520 nm). Rutin (quercetin-3-*O*-rutinoside) reference standard was used for determination of other polyphenols by assuming the molar-response factor as 1.00 (UV 330 nm).[19] Identifications of anthocyanin and other polyphenols were made by comparison with the published literature by using high-resolution MS data, MS$^{2-4}$ fragmentation, and UV−vis data.[20] An LTQ Orbitrap XL mass spectrometer was used for the confirmation of each individual glucosinolates. The optimized conditions were set as follows: sheath gas at 70 (arbitrary units), auxiliary and sweep gas at 15 (arbitrary units), spray voltage at 4.8 kV, capillary temperature at 270 °C, capillary voltage at 15 V, and tube lens at 70 V. The mass range was from 100 to 1000 amu with a resolution of 30,000, FTMS AGC target at 2e5, FT-MS/MS AGC target at 1e5, isolation width of 1.5 amu, and maximum ion injection time of 500 ms. Source CID was set at 20%. The most intense ion was selected for the data-dependent scan to offer their MS^2 to MS^3 product ions, respectively, with normalization collision energy at 35%. The type and amount of desulfo-glucosinolates (desulfo-GLs) were calculated using known concentrations of standard sinigrin and recommended relative response factors with PDA signal at 229 nm (Table 1) (Supplemental Figure 4). GLs were identified on the basis of their specific accurate masses (as desulfo form) and retention times.

Animals and Diets. Male C57BL/6NCr mice, aged 5 weeks (approximately 20 g), were purchased from Charles River Laboratories (National Cancer Institute, Frederick, MD, USA). Mice were single-housed

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Table 1. PDA Response Factor of Desulfo-glucosinolate

no.	desulfo-glucosinolate	response factor
1	desulfosinigrin	1.00
2	desulfoglucoraphanin	1.07
3	desulfo-4-hydroxyglucobrassicin	0.28
4	other desulfo- hydroxyglucobrassicin	0.28
5	desulfo-glucobrassicin	0.20
6	desulfo-4-methoxyglucobrassicin	0.25
7	other desulfo methoxyglucobrassicins	0.25
8	other glucosinolates	1.00

in a temperature-controlled room with a 12/12 h day/night cycle. Prior to the experiment, they were fed a standard rodent chow and had free access to water for 1 week to acclimatize to the environment. For the experiment, a total of 60 mice were randomly divided into six groups (10 mice/group) and fed one of six different diets: (1) low-fat diet (LF) (10 kcal% fat diet); (2) low-fat diet supplemented with red cabbage microgreen powder (LFMG) (10 kcal% fat diet containing 10.9 g/kg diet); (3) low-fat diet supplemented with mature red cabbage powder (LFRC) (10 kcal% fat diet containing 16.6 g/kg diet); (4) high-fat diet (HF) (45 kcal% fat diet); (5) high-fat diet supplemented with red cabbage microgreen powder (HFMG) (45 kcal% fat diet containing 10.9 g/kg diet); (6) high-fat diet supplemented with mature red cabbage powder (HFRC) (45 kcal% fat diet containing 16.6 g/kg diet). The experimental diets were formulated and pelleted by Research Diets (New Brunswick, NJ, USA). Animals (10 per group) were fed the respective diets for 8 weeks with water available ad libitum. Food intake and body weight were recorded once a week. All of the low-fat diets consisted of 20% protein, 70% carbohydrate, and 10% fat on a caloric basis, and the high fat diets consisted of 20% protein, 35% carbohydrate, and 45% fat on a caloric basis. Red cabbage microgreens and mature red cabbage samples were added into diets in the form of dry powder, and the amount supplemented into diets for the mice was calculated on the basis of the equivalence of 200 g of vegetables/day/person (60 kg) using the Dose Translation Formula.[21] Detailed information on diet compositions is shown in Table 2. All animal experiments were conducted under an animal study protocol (Protocol 14-006) that was reviewed and approved by the USDA, ARS, Beltsville Area Institutional Animal Care and Use Committee (IACUC).

Plasma, Tissue, and Fecal Sample Collection. Mice were subjected to a 12 h fast with water available ad libitum. Mice were anesthetized in a CO_2 chamber, blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% w/v), and plasma was separated by centrifugation at 1300 rcf for 10 min at 4 °C. Livers were collected, and an aliquot of the tissue was immediately frozen in liquid nitrogen for later analysis; the rest was preserved in RNA Stabilization Solution (Ambion, Austin, TX, USA) and kept at −80 °C. At the end of the eighth week, 24 h fecal samples were also collected from the bottom of the cage and stored at −80 °C.

Plasma Lipoprotein Analysis. Plasma lipoprotein cholesterol concentrations were determined by size exclusion chromatography as previously described.[22] Briefly, an Agilent 1100 chromatograph was employed with a postcolumn derivatization reactor, consisting of a mixing coil (1615-50 Bodman, Aston, PA, USA) in a temperature-controlled water jacket (Aura Industrials, Staten, NY, USA). A Hewlett-Packard (Agilent, Palo Alto, CA, USA) HPLC pump 79851-A was used to deliver the cholesterol reagent (Roche Diagnostics, Indianapolis, IN, USA) at a flow rate of 0.2 mL/min. Bovine cholesterol lipoprotein standards (Sigma-Aldrich, St. Louis, MO, USA) were used to calibrate the signal on the basis of peak areas. Fifteen microliters of plasma was

Table 2. Diet Composition

	LF[a]		LF + MG[a]		LF + RC[a]		HF[a]		HF + MG[a]		HF + RC[a]	
	g%	kcal%	g%	kcal%	g%	kcal%	g%	kcal%	g%	kcal%	g%	kcal%
protein	19	20	19	20	19	20	24	20	23	20	23	20
carbohydrate	67	70	67	70	66	70	41	35	41	35	41	35
fat	4	10	4	10	4	10	24	45	23	45	23	45
total		100		100		100		100		100		100
kcal/g	3.8		3.8		3.8		4.7		4.7		4.6	
	LF		LF + MG		LF + RC		HF		HF + MG		HF + RC	
ingredient	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal
casein	200	800	200	800	200	800	200	800	200	800	200	800
L-cystine	3	12	3	12	3	12	3	12	3	12	3	12
corn starch	452.2	1809	452.2	1809	452.2	1809	72.8	291	72.8	291	72.8	291
maltodextrin	75	300	75	300	75	300	100	400	100	400	100	400
sucrose	172.8	691	172.8	691	172.8	691	172.8	691	172.8	691	172.8	691
cellulose	50	0	50	0	50	0	50	0	50	0	50	0
corn oil	25	225	25	225	25	225	25	225	25	225	25	225
lard	20	180	20	180	20	180	177.5	1598	177.5	1598	177.5	1598
mineral mix	10	0	10	0	10	0	10	0	10	0	10	0
dicalcium phosphate	13	0	13	0	13	0	13	0	13	0	13	0
calcium carbonate	5.5	0	5.5	0	5.5	0	5.5	0	5.5	0	5.5	0
potassium citrate	16.5	0	16.5	0	16.5	0	16.5	0	16.5	0	16.5	0
vitamin mix	10	40	10	40	10	40	10	40	10	40	10	40
choline bitartrate	2	0	2	0	2	0	2	0	2	0	2	0
MG powder[b]			11.63						9.46			
RC powder[b]					17.81						14.49	
total	1055	4057	1066.63	4057	1072.81	4057	858.1	4057	867.56	4057	872.59	4057

[a]All of the diets used in this animal study were formulated by Research Diet (New Brunswick, NJ, USA). LF, LF + MG, and LF + RC represent low-fat diet, low-fat diet supplemented with red cabbage microgreens powder, and low-fat diet supplemented with mature red cabbage powder, respectively. Similarly, HF, HF + MG, and HF + RC represent high-fat diet, high-fat diet supplemented with red cabbage microgreens powder, and high-fat diet supplemented with mature red cabbage powder, respectively. [b]MG powder is the powder made from lyophilized red cabbage microgreens, and RC powder is the powder made from lyophilized mature red cabbage.

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Table 3. Anthocyanins and Other Polyphenols in Microgreens and Mature Red Cabbage

(A) Anthocyanins	
tentative identification	μmol/g
Red Cabbage Microgreens	
cyanidin 3-diglucoside-5-glucoside	0.41
cyanidin 3-(sinapoyl)-diglucoside-5-glucosides	1.13
cyanidin 3-(glucosyl)(sinapoyl)(*p*-coumaroyl)sophoroside-5-glucoside	0.49
cyanidin 3-(glucosyl)(sinapoyl)(feruloyl)sophoroside-5-glucoside	0.57
cyanidin 3-diferuloylsophoroside-5-glucoside	0.59
cyanidin 3-(coumaroyl)sophoroside-5-glucoside	0.57
cyanidin 3-(feruloyl)sophoroside-5-glucoside	1.68
cyanidin 3-diferuloylsophoroside-5-glucoside	2.06
cyanidin 3-(sinapoyl)(feruloyl)sophoroside-5-glucoside	1.30
cyanidin 3-(sinapoyl)(sinapoyl)sophoroside-5-glucoside	3.65
total	12.44
Mature Red Cabbage	
cyanidin 3-diglucoside-5-glucoside	2.42
cyanidin 3-(sinapoyl)-diglucoside-5-glucosides	0.63
cyanidin 3-(glucosyl) (sinapoyl)(*p*-coumaroyl)sophoroside-5-glucoside	0.82
cyanidin-3-(glucosyl)(sinapoyl)(feruloyl)sophoroside-5-glucoside	1.54
cyanidin 3-(coumaroyl)sophoroside-5-glucoside	0.83
cyanidin 3-diferuloylsophoroside-5-glucoside	1.27
cyanidin 3-(feruloyl)sophoroside-5-glucoside	22.96
cyanidin 3-(sinapoyl)(feruloyl)sophoroside-5-glucoside	0.74
cyanidin 3-(sinapoyl)(sinapoyl)sophoroside-5-glucoside	2.13
total	33.36
(B) Other Polyphenols	
tentative identification	μmol/g
Red Cabbage Microgreens	
citric acid	0.27
3-caffeoylquinic acid	2.40
caffeoyl glucose	1.62
hydroxyferuloylglucose	1.59
kaempferol 3-diglucoside	2.80
sinapoyl-hexose	27.80
disinapoylgentiobiose	12.00
1,2,2′-trisinapoylgentiobiose	8.36
unknown	1.72
total	58.57
Mature Red Cabbage	
citric acid	0.36
3-caffeoylquinic acid	1.92
sinapoyl-hexose	0.99
kaempferol acyl-glucoside	11.89
kaempferol 3-sinapoylsophoroside-7-glucoside	0.17
kaempferol 3-hydroxyferuloylsophorotrioside-7-glucoside	0.69
kaempferol 3-sinapoylferuloylsophoroside-7-glucoside	0.48
kaempferol 3-disinapoyldiglucoside-7-glucoside	0.07
1,2,2′-trisinapoylgentiobiose	0.27
unknown	0.39
total	17.22

injected via an Agilent 1100 autosampler onto a Superose 6HR HPLC column (Pharmacia LKB Biotechnology, Piscataway, NJ, USA). The lipoproteins were eluted with a pH 7.0 buffer solution containing 0.15 M sodium chloride and 0.02% sodium azide at a flow rate of 0.5 mL/min. Plasma lipoprotein concentration was calculated on the basis of a standard curve.

Hepatic Lipid Extraction. The lipid extraction method was modified from the Folch method.[23,24] Frozen liver (10 mg) was minced and transferred to a test tube, and 600 μL of chloroform/methanol (2:1, v/v) was then added, followed by homogenization at 6500 rpm for 10 s. Samples were then centrifuged at 14,000 rpm for 10 min. After centrifugation, 300 μL of the organic layer was transferred into a clean tube

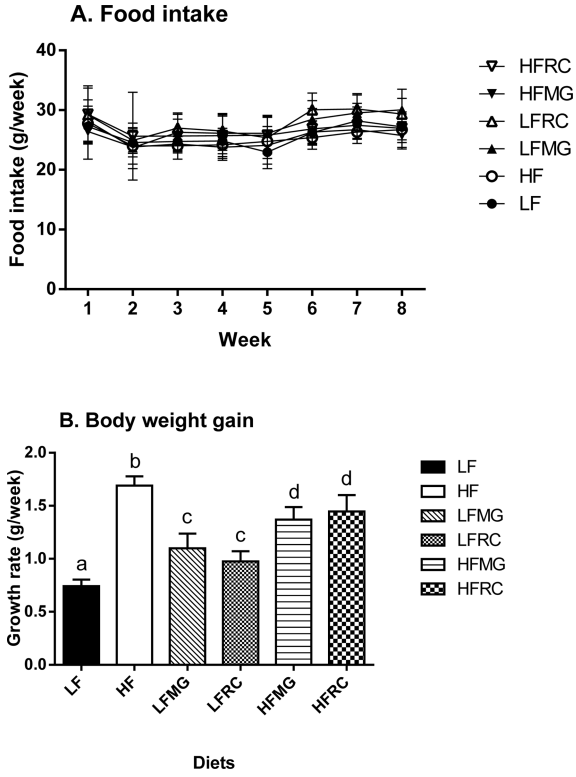

Figure 2. Food intake (A, g/week) and body weight gain (B, g/week). Animals were divided into six diet groups and fed the respective diets for 8 weeks. Food intake and body weight gain were assessed as described under Materials and Methods. The diet groups were LF, HF, LFMG, LFRC, HFMG, and HFRC. Results are expressed as the mean ± SE (n = 10/group). Bars with different letters indicate significant difference at $p \leq 0.05$.

and vacuum-dried for 20 min. The dried lipid was weighed and redissolved in 200 μL of 1× reaction buffer (5× reaction buffer: 0.5 M potassium phosphate, pH 7.4, 0.25 M NaCl, 25 mM cholic acid, 0.5% Triton X-100) provided with the Amplex Red cholesterol assay kit (Invitrogen, Eugene, OR, USA) and used for triacylglycerol and cholesterol analysis as described below.

Triacylglycerol, Total Cholesterol, and Free Cholesterol in the Liver. Hepatic triacylglycerol (TG) was enzymatically determined using commercial kits (Triglyceride-SL, Sekisui Diagnostics PEI Inc., Charlottetown, Canada). Total cholesterol and free cholesterol were quantified by a fluorometric method using the Amplex red cholesterol assay kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols.

Fecal Cholesterol Extraction and Analysis. Fecal cholesterol was extracted using a modified protocol. Fecal samples were collected during the 24 h period on day 56 after initiation of the experiment. The samples were freeze-dried and weighed. A dried fecal sample (~10 mg) was homogenized in 200 μL of chloroform/methanol (v/v 2:1) and vortexed for 2 min. The mixture was then centrifuged for 5 min at 14,000 rpm in a microcentrifuge tube. The organic phase was transferred into a new clean tube and then vacuum-dried. The samples were redissolved in 200 μL of 1× reaction buffer (5× reaction buffer: 0.5 M potassium phosphate, pH 7.4, 0.25 M NaCl, 25 mM cholic acid, 0.5% Triton X-100). The fecal cholesterol content was quantified by a fluorometric method using a commercial kit (Amplex red cholesterol assay kit, Invitrogen, NY, USA) following the manufacturer's protocol.

Fecal Bile Acid Extraction and Analysis. Fecal samples collected during the 24 h period on day 56 after initiation of the experiment were lyophilized, pulverized, and weighed. Twenty milligrams of fecal powder was hydrolyzed in 0.5 mL of 2 M KOH at 50 °C for 5 h. The cooled mixture was then extracted with two 3 mL portions of diethyl ether to remove nonsaponifiable components. Sodium chloride (0.5 mL, 20%) and subsequently hydrochloric acid (0.1 mL, 12 M) were then added to

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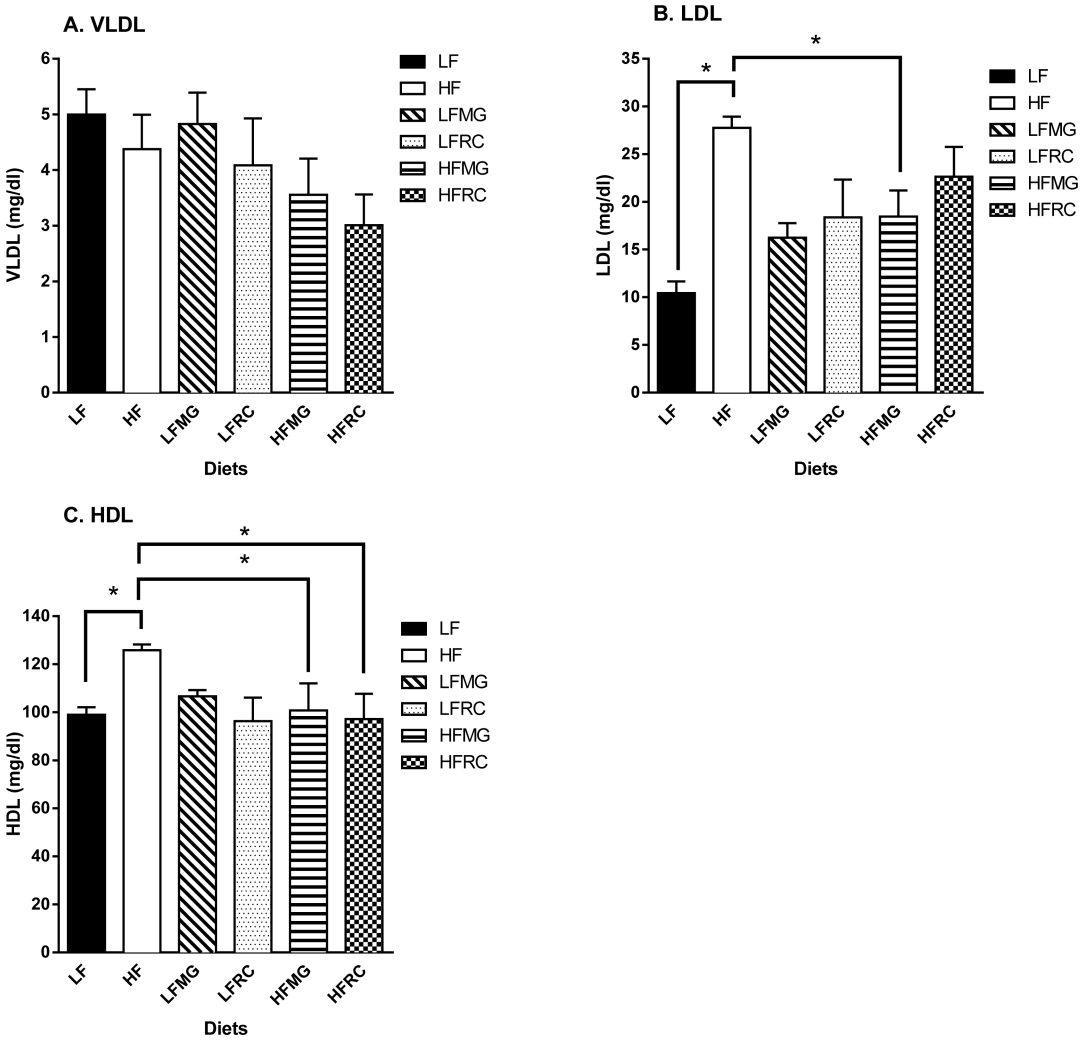

Figure 3. Plasma lipoproteins levels. Animals were divided into six diet groups and fed the respective diets for 8 weeks. Plasma HDL (C), LDL (B), and VLDL (A) levels were measured as described under Materials and Methods. The diet groups were LF, HF, LFMG, LFRC, HFMG, and HFRC. Results are expressed as the mean ± SE (n = 10/group). (∗) Significant difference between respective groups at $p \leq 0.05$.

the mixture. The acidified mixture was extracted with two portions of 6 mL of diethyl ether, evaporated under nitrogen, and redissolved in 0.5 mL of pure ethanol. Bile acid concentration was measured using a commercial kit (Total Bile Acid Assays, Cell Biolabs, Inc., San Diego, CA, USA) following the manufacturer's protocol.

Total RNA Isolation, cDNA Synthesis, and Gene Expression Analysis from Liver and Adipose Tissue. To determine changes in the gene expression, livers were cut into 0.1−0.2 g pieces and homogenized using a Precellys 24 by Bertin Technologies (Villeurbanne, France). The RNeasy Mini Kit from Qiagen (Valencia, CA, USA) was used for total RNA isolation for the liver. An AffinityScript cDNA Synthesis Kit from Agilent Technologies (Santa Clara, CA, USA) was used to reverse transcribe complementary DNA. Real-time PCR was performed using the TaqMan method as previously reported according to the manufacturer's protocol (Life Technology). Mouse TaqMan gene expression assays used in this study were purchased from Life Technology and included acetyl-CoA acetyltransferase (ACAT) 1 and 3, cytochrome p450 7A1 (CYP7A1), diacylglycerol *O*-acyltransferase 1 (DGAT1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), lecithin-cholesterol acyltransferase (LCAT), LDL receptor (LDLR), sterol *O*-acyltransferase 1 (SOAT1), and TATA-binding protein (TBP).

Statistical Analysis. All end point assays for each sample were conducted in triplicate, and the average was used for group analysis. Data for each treatment group are presented as the mean ± SEM. Statistics analysis was performed using GraphPad Prism 6 (2012, GraphPad Software, La Jolla, CA, USA). A significant level of differences in the means was detected using one-way ANOVA follow by Fisher's PLSD post hoc test. Statistical significance was defined at $p \leq 0.05$.

Table 4. Desulfo-glucosinolates in Microgreens and Mature Red Cabbage

no.		red cabbage microgreens (μmol/g dry weight)	mature red cabbage (μmol/g dry weight)
1	progoitrin	6.87	2.29
2	glucoraphanin	4.80	0.88
3	sinigrin	6.12	1.53
4	gluconapin	1.31	1.33
5	4-hydroxyglucobrassicin	1.96	0.39
6	glucoerucin	1.16	0.37
7	glucobrassicin	1.15	1.26
8	4-methoxylglucobrassicin	0.51	0.26
9	neoglucobrassicin	0.15	nd
	total	17.15	8.30

RESULTS

Comparison of Phytochemical Composition in Mature Red Cabbage (RC) and Red Cabbage Microgreens (MG). Major purported bioactive components, anthocyanins, polyphenols, and glucosinolates, in mature red cabbage and red cabbage

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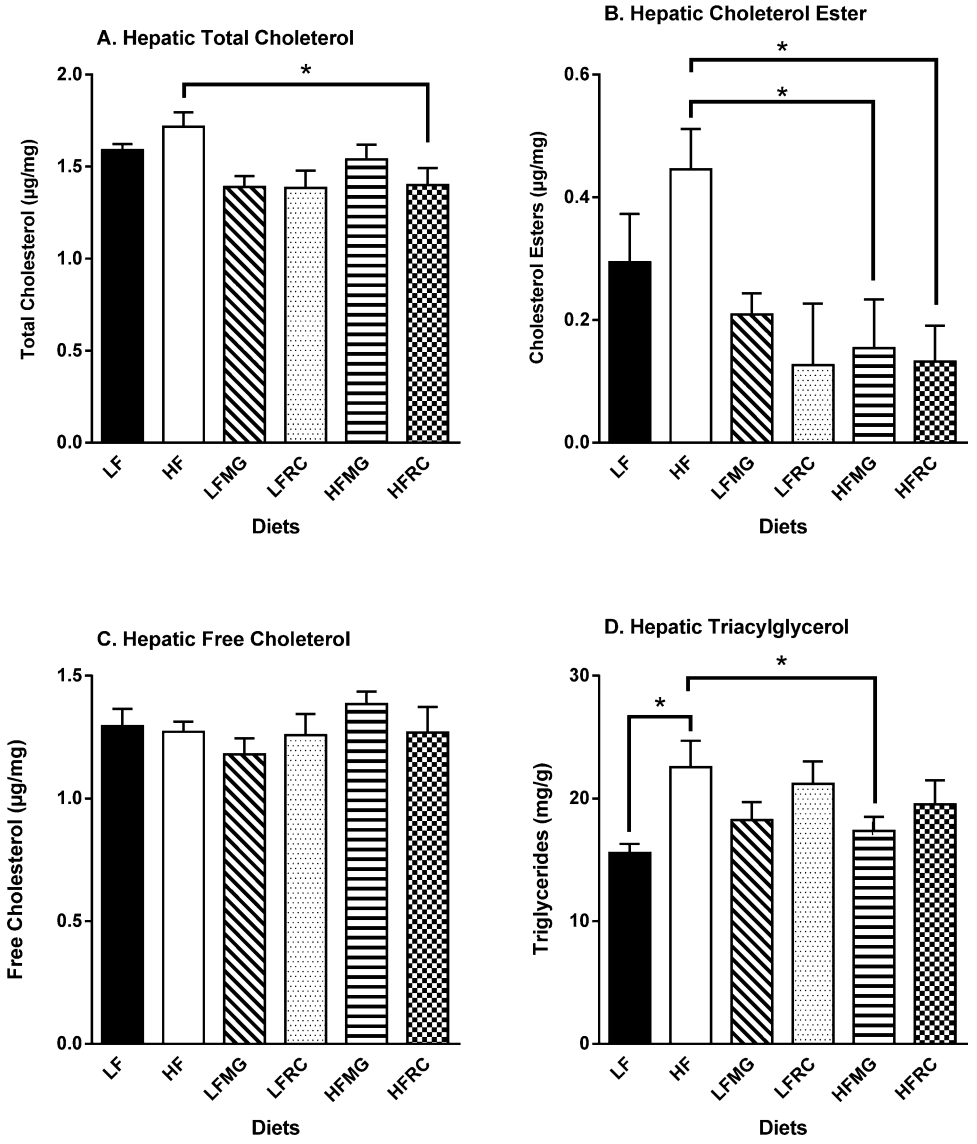

Figure 4. Liver cholesterol and triacylglycerol levels. Animals were divided into six diet group and the fed respective diets for 8 weeks. Liver cholesterol (A, total cholesterol; B, cholesterol ester; C, free cholesterol) and triacylglycerol levels (D) were measured as described under Materials and Methods. The diet groups were LF, HF, LFMG, LFRC, HFMG, and HFRC. Results are expressed as the mean ± SE (n = 10). (*) Significant difference between the respective groups at $p \leq 0.05$.

microgreens were compared.[20−26] Total anthocyanin concentration was determined to be 12.44 μmol/g dry weight in MG and 33.36 μmol/g dry weight in RC. Cyanidin 3-(sinapoyl)(sinapoyl)-sophoroside-5-glucoside was the most abundant anthocyanin in MG, followed by cyanidin 3-diferuloylsophoroside-5-glucoside and cyanidin 3-(feruloyl)sophoroside-5-glucoside. By contrast, the major RC anthocyanin is cyanidin 3-(feruloyl)sophoroside-5-glucoside (Table 3A). Concentrations of other polyphenols range from 58.57 μmol/g dry weight in MG to 17.22 μmol/g dry weight in RC. Sinapoyl-hexose, disinapoylgentiobiose, and 1,2,2′-trisinapoylgentiobiose are the top three polyphenols in MG. Kaempferol acyl-glucoside was the major polyphenol in RC (Table 3B) (Supplemental Figures 1−3). Progoitrin and sinigrin were the most abundant GLs in both MG and RC. However, MG contains 3 and 4 times more progoitrin and sinigrin per gram dry weight than does RC, respectively. Overall, MG (17.15 μmol/g) contained 2-fold more GLs than RC (8.30 μmol/g) (Table 4).

Effects of Red Cabbage Microgreen on Food Intake and Body Weight Gain. There were no significant differences in food intake among the diet treatments (Figure 2A). For all diet groups, body weights continued to increase through the end of the experiment (week 8). Body weights were significantly increased in animals fed the HF diet, compared to the LF diet group, beginning at week 5. Weight gain in animals fed HFMG and HFRC was significantly less than that of HF-fed animals (Figure 2B). Interestingly, the rate of body weight gain appeared to be higher for LFMG and LFRC when compared to the LF control.

Effect of Red Cabbage Microgreens on Plasma Lipoprotein Levels. Compared to the animals on the LF diet, low-density lipoprotein (LDL) (166%),and high-density lipoprotein (HDL) (27%) were significantly higher in the HF diet fed group (Figure 3). Animals on the HFMG diet showed significantly lower LDL than the HF diet group, whereas HFRC had significantly lower HDL than the HF group. There were no differences in VLDL between the groups. Animals fed the LFRC diet also had significantly elevated LDL (56%) when compared to those of the LF diet group.

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Effects of Red Cabbage Microgreens on Liver Cholesterol and Lipid Content. There were no differences in hepatic cholesterol content between LF- and HF-fed animals (Figure 4A–C). HF diet fed animals had significantly higher hepatic TG than did LF-fed animals (Figure 4D). HFRC-fed animals had significantly lower total hepatic cholesterol than did HF-fed animals, with a trend for lower total hepatic cholesterol in the HFMG versus the HF (p = 0.098) (Figure 4A). Similarly, LFMG and LFRC also had a trend of slightly lower total hepatic cholesterol than LF with p values of 0.096 and 0.073, respectively (Figure 4A). Both HFMG and HFRC group had significantly lower hepatic cholesterol ester levels than did HF-fed animals (Figure 4B). No differences were observed in hepatic free cholesterols among any of the diet groups (Figure 4C). Additionally, HFMG but not HFRC had significantly lower hepatic TG levels than those of HF diet animals (Figure 4D). However, the LFRC group has significantly higher TG than the LF group (Figure 4D).

Effects of Red Cabbage Microgreens on Fecal Bile Acid and Cholesterol Content. Compared to the LF diet group, animals fed the HF diet excreted more bile acids in their feces (55%) (Figure 5A). However, neither MG nor RC supplements affected bile acid level in their respective LF and HF diet groups (Figure 5A). The HF diet group excreted significantly more cholesterol than animals fed the LF diet (177%) (Figure 5B). HFRC-fed animals showed reduced total fecal cholesterol (32%) when compared to animals in the HF diet group (Figure 5B).

Effects of Red Cabbage Microgreens on Genes Associated with Cholesterol and Triacylglycerol Metabolism in the Liver. Transcriptional regulation of cholesterol and triacylglycerol metabolism-related genes was examined as it correlates to the biochemical analyses to elucidate potential mechanisms. Feeding an HF diet significantly up-regulated bile acid synthesis enzyme CYP7A1, which is the rate-limiting step for cholesterol removal,[27,28] compared to the LF diet group (Figure 6A). No differences were observed on the expression of CYP7A1 in any of the MG- or RC-supplemented diet groups with the HF or LF control diets. We also did not observe an effect of MG or RC supplementation on the cholesterol pathway enzyme, HMGCR, or LDLR (data not shown). An animal fed the HF diet had increased expression of ACAT1 and ACAT3 (Figure 6B,C), enzymes involved in fatty acid oxidation. HFMG- and HFRC-fed animals had significantly reduced expressions of ACAT1 and ACAT3 (Figure 6B,C). No effects were observed for ACAT2 in any of the diet groups (data not shown). HFMG-fed animals also had significantly lower levels of SOAT1 mRNA, an enzyme involved in cholesterol ester synthesis, than the HF-fed animals (Figure 6D). HFMG fed animals had significantly lower mRNA levels for a gene necessary for storage of triacylglycerols, DGAT1, than did the HF-fed animals (Figure 6E). Both MG and RC supplementation in the HF diet significantly lowered mRNA levels of the lipoprotein synthetic enzyme, LCAT (Figure 6F).

Effects of Red Cabbage Microgreens on Inflammatory Markers in the Liver. Consuming HF diets can lead to hepatic inflammation.[29,30] Therefore, expression of hepatic inflammatory markers was also analyzed. CRP and TNF-α expression levels were shown to be elevated in the livers of the HF diet group compared to those of the LF diet group (Figure 6G,H). Animals supplemented with MG and RC had significantly lower CRP and TNF-α mRNA levels (Figure 6G,H). IL-6 mRNA was also analyzed but was not detectable in any group under the same assay condition.

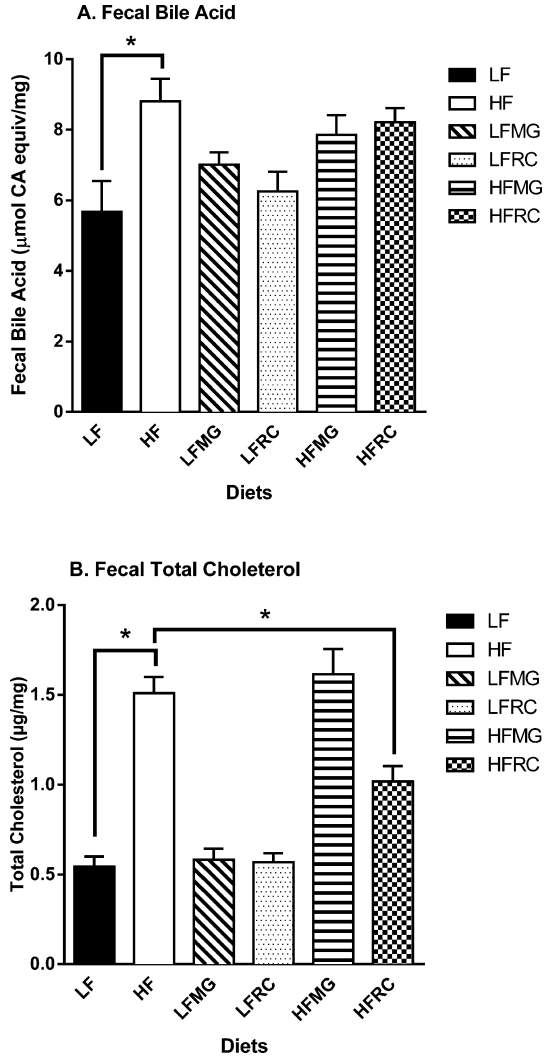

Figure 5. Fecal bile acid and cholesterol levels. Animals were divided into six diet groups and fed the respective diets for 8 weeks. Fecal samples were collected on the day of sacrifice, and fecal bile acid (A) and cholesterol levels (B) were measured as described under Materials and Methods. The diet groups were LF, HF, LFMG, LFRC, HFMG, and HFRC. Results are expressed as the mean ± SE (n = 10/group). (*) Significant difference between the respective groups at $p \leq 0.05$.

DISCUSSION

This is the first study evaluating the potential health-promoting properties of RCMG in a rodent model. We observed a 1.09% RCMG supplementation in a high-fat diet for 8 weeks attenuated body weight compared to the HF diet control. Elevated circulating LDL is a known risk factor for CVD.[15,31] Supplementation of RCMG improved lipoprotein profiles in blood by lowering LDL levels induced by the high-fat diet and thus may reduce the risk of CVD. High fat diet induced hypercholesterolemia and chronic inflammation are also associated with the development of several chronic diseases, such as CVD, obesity, and cancer.[32−34] Animals fed RCMG-supplemented diets showed reductions in hepatic cholesterol ester, triacylglycerol levels, and expression of inflammatory cytokines (CRP and TNF-α). Hence, the improvements in CVD risk parameters from microgreens supplementation are consistent with the general health-promoting effects for RCMG.

Compared to the mature RC counterpart, the RCMG shared similarity as well as differences in biological effects. Both RCMG

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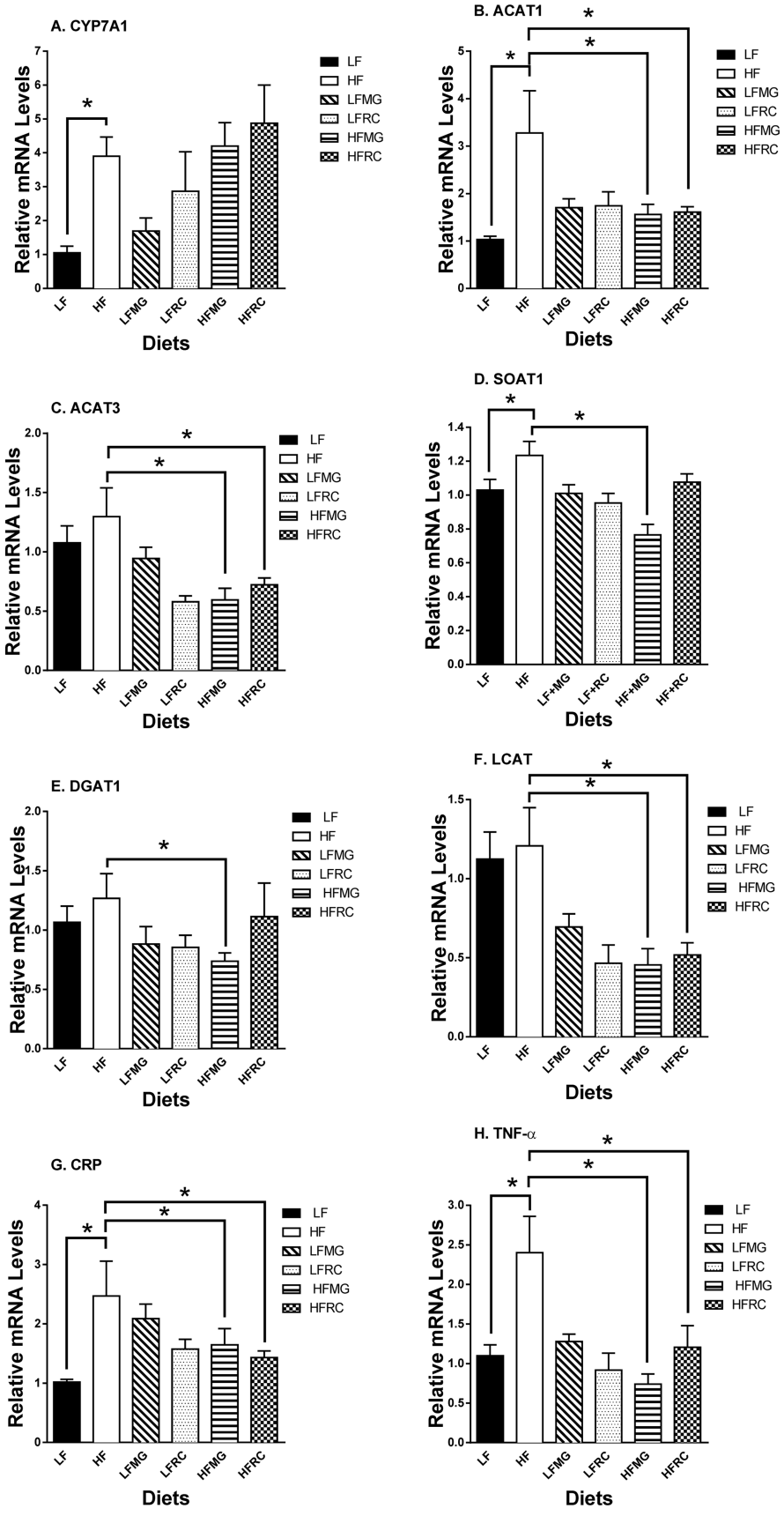

Figure 6. Cholesterol, triacylglycerol metabolism, and inflammation-related gene expression levels in the liver: (A) CYP7A1; (B) ACAT1; (C) ACAT3; (D) SOAT1; (E) DGAT1; (F) LCAT; (G) CRP; (H) TNF-α. Animals were divided into six diet groups and fed the respective diets for 8 weeks. Liver samples were obtained at the end of 8 weeks. RNA was isolated and expression of marker genes was assessed using RT-PCR as described under Materials and Methods. The diet groups were LF, HF, LFMG, LFRC, HFMG, and HFRC. Results are expressed as the mean ± SE (n = 10/group). (*) Significant difference between the respective groups at $p \leq 0.05$.

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and RC lowered body weight gain, hepatic cholesterol, and expression of inflammatory markers induced by HF diets. HFRC- but not HFMG-fed animals had significantly lower fecal cholesterol excretion; however, the exact mechanism was not clear. Intestinal cholesterol excretion or gut microbiota may play a role in the altered fecal cholesterol excretion, and further study is warranted to elucidate the underlying mechanism. HFMG- but not HFRC-fed animals had significantly lower plasma LDL and hepatic TG levels than did the HF-fed animals. These results suggest that RC and RCMG share general health-promoting effects, although the underlying mechanisms may differ. This notion is further supported, at the molecular level, by the differential effects on gene expression of cholesterol and triacylglycerol metabolism enzymes in the liver. RCMG but not RC affected the cholesterol ester synthesis enzyme SOAT1, which is consistent with the cholesterol ester lowering effect of RCMG observed in the HF-fed animals. The triacylglycerol synthesis enzyme DGAT1[35,36] was down-regulated by RCMG but not RC. DGAT1 catalyzes the terminal and only committed step in triacylglycerol synthesis by using diacylglycerol and fatty acyl CoA as substrates.[35] Down-regulation of DGAT1 is consistent with the triacylglycerol-lowering effects in the liver of RCMG but not RC.

ACAT1, a mitochondrial enzyme that catalyzes the reversible conversion of two molecules of acetyl-CoA to acetoacetyl-CoA, is involved in the fatty oxidation/degradation pathway.[37,38] Consuming an HF diet induces this enzyme and is consistent with the animal's response to the HF diet by increasing fatty acid oxidation. Both RCMG and RC supplementation led to attenuation of HF diet induced increases in ACAT1. The result may be a reflection of the overall effect of RCMG and RC on overall lipid metabolisms altered by the HF diet. How RCMG or RC may directly regulate ACAT1 or reduce the effect of HF remains unclear, and further study is necessary to elucidate the specific mechanisms.

Both RC and RCMG consumption led to lower LCAT expression in the liver as compared to their respective controls. LCAT is known to be involved in extracellular cholesterol esterification and cholesterol transport.[39] Lowering of LCAT is consistent with small but significant decreases in HDL levels in HFRC group and the decrease in LDL levels in the HFMG group compared to those of the HF group. However, a change in LCAT also occurred in LFRC and LFMG groups relative to the LF diet group, suggesting dietary component(s) from RCMG and RC may play a role in the regulation of hepatic LCAT activity. The consequence of the changes in expression remained less clear and warrant further study. Additionally, recent studies suggested that lower LCAT may be associated with an increase in obesity-related insulin sensitivity.[40,41] The effect of RC or RCMG on insulin sensitivity was not examined in this study and warrants further validation.

Diets high in fat content are known to induce a variety of physiological changes, including chronic, low-grade inflammation in the liver.[30] Our observation of inhibitory effects of RC and RCMG on HF diet induced increases in CRP and TNF-α expression, inflammatory markers in the liver,[42] suggested that supplementation of RC or RCMG in HF diets may help alleviate hepatic inflammation. Therefore, the inclusion of RC or RCMG in the Western style diet may contribute to the prevention of diet-related chronic conditions in the U.S. population.

Given that food intake was similar in all groups, the effect on lowering weight gain elicited by RCMG or RC in animals fed the HF diet was unexpected. The mechanism of this novel finding is unclear. Because the movement, activity, and behavior of animals in this study were not specifically monitored, it is possible that altered baseline energy expenditure may be a contributing factor and warrant additional study. One possible regulatory mechanism may involve UCP1,[43] a protein involved in energy balance, which is known to be affected by phytochemicals in the diet.[44−46] Analysis of adipose tissue from different depots and UCP1 expression would be an important next step. The increase in body weight in LFRC and LFMG groups compared to those of the LF group was also unexpected. The animals were transitioning from juvenile to adulthood from the start of the experiment (6 weeks old) toward the end (14 weeks old), so it is possible that both RC and RCMG may provide additional components that maximize their growth. Future studies are needed to identify the active components.

Polyphenols and glucosinolates are known bioactive compounds in fruits and vegetables and major phytochemical components in RC.[20−26] In this study, RCMG was determined to contain more polyphenols (71.01 μmol/g) and glucosinolates (17.15 μmol/g) than RC (50.58 and 8.30 μmol/g, respectively). Polyphenols and glucosinolates have been shown in previous studies to possess cholesterol-lowering,[47−49] antioxidant,[50] and anti-inflammation[51−53] properties. Therefore, it is possible that these bioactive components in RCMG and RC may contribute to the observed differential cholesterol-lowering and anti-inflammation effects in this study. Anthocyanins give a red hue, and consistent with the color differences between RC and RCMG (Figure 1), RCMG contained about one-third of the anthocyanins in RC. Hence, the compositions of RCMG and its mature counterpart are quite different. However, the precise components from RC or RCMG that provide health beneficial effects remain to be elucidated in future studies.

In summary, supplementing diets with 1.09% RCMG or 1.66% RC, the equivalent of 200 g vegetables/day/person, modulated body weight, plasma lipoprotein profiles, and inflammatory status. In mice fed high-fat diets, supplementation with RCMG or RC had a beneficial effect against lipid risk factors for the development of CVD. This study also provided the first evidence that RCMG and its mature counterpart may regulate the lipid and cholesterol metabolisms differently. One could speculate that other microgreens may have similar effects, although additional studies are needed. Further delineation of the mechanisms of action is also needed, especially as related to energy expenditure and lipid metabolism.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b03805.

Figure 1, HPLC chromatogram of microgreen and red cabbage at 330 nm; Figure 2, HPLC chromatogram of microgreen and red cabbage at 520 nm; Figure 3, HPLC chromatogram of desulfo glucosinolates in MG and RC; Figure 4, initial and final animal body weights (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*(T.T.Y.W.) Mail: 10300 Baltimore Ave, USDA-ARS, BHNRC, Bldg 307C, Rm 132, Beltsville, MD 20705, USA. Phone: (301) 504-8459. E-mail: Tom.Wang@ars.usda.gov.

ORCID

Haiqiu Huang: 0000-0003-1543-3901

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Notes
The authors declare no competing financial interest.

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■ REFERENCES

(1) Milani, R. V.; Lavie, C. J. Health care 2020: reengineering health care delivery to combat chronic disease. *Am. J. Med.* **2015**, *128*, 337−343.
(2) Ward, B. W.; Schiller, J. S.; Goodman, R. A. Multiple chronic conditions among US adults: a 2012 update. *Preventing Chronic Dis.* **2014**, *11*, E62.
(3) *CDC Chronic Disease Notes & Report*; Cultivating Healthy Communities; Centers for Disease Control and Prevention: 2009; p 49.
(4) Hung, H.-C.; Joshipura, K. J.; Jiang, R.; Hu, F. B.; Hunter, D.; Smith-Warner, S. A.; Colditz, G. A.; Rosner, B.; Spiegelman, D.; Willett, W. C. Fruit and vegetable intake and risk of major chronic disease. *J. Natl. Cancer Inst.* **2004**, *96*, 1577−1584.
(5) Slavin, J. L.; Lloyd, B. Health benefits of fruits and vegetables. *Adv. Nutr.* **2012**, *3*, 506−516.
(6) Minich, D. M.; Bland, J. S. A review of the clinical efficacy and safety of cruciferous vegetable phytochemicals. *Nutr. Rev.* **2007**, *65*, 259−267.
(7) Sarkar, F. H.; Li, Y. W. Indole-3-carbinol and prostate cancer. *J. Nutr.* **2004**, *134*, 3493S−3498S.
(8) Chan, R.; Lok, K.; Woo, J. Prostate cancer and vegetable consumption. *Mol. Nutr. Food Res.* **2009**, *53*, 201−216.
(9) Lee, C.-S.; Pill, W. G. Advancing greenhouse establishment of radish, kale and amaranth microgreens through seed treatments. *Hortic., Environ., Biotechnol.* **2005**, *46*, 363−368.
(10) Xiao, Z.; Lester, G. E.; Luo, Y.; Wang, Q. Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens. *J. Agric. Food Chem.* **2012**, *60*, 7644−7651.
(11) Singh, J.; Upadhyay, A.; Bahadur, A.; Singh, B.; Singh, K.; Rai, M. Antioxidant phytochemicals in cabbage (*Brassica oleracea* L. var. *capitata*). *Sci. Hortic.* **2006**, *108*, 233−237.
(12) Mizgier, P.; Kucharska, A. Z.; Sokół-Łętowska, A.; Kolniak-Ostek, J.; Kidoń, M.; Fecka, I. Characterization of phenolic compounds and antioxidant and anti-inflammatory properties of red cabbage and purple carrot extracts. *J. Funct. Foods* **2016**, *21*, 133−146.
(13) Demirdöven, A.; Karabıyıklı, Ş.; Tokatlı, K.; Öncül, N. Inhibitory effects of red cabbage and sour cherry pomace anthocyanin extracts on food borne pathogens and their antioxidant properties. *LWT−Food Sci. Technol.* **2015**, *63*, 8−13.
(14) Al-Dosari, M. S. Red cabbage (*Brassica oleracea* L.) mediates redox-sensitive amelioration of dyslipidemia and hepatic injury induced by exogenous cholesterol administration. *Am. J. Chin. Med.* **2014**, *42*, 189−206.
(15) Nordestgaard, B. G.; Chapman, M. J.; Ray, K.; Borén, J.; Andreotti, F.; Watts, G. F.; Ginsberg, H.; Amarenco, P.; Catapano, A.; Descamps, O. S. Lipoprotein (a) as a cardiovascular risk factor: current status. *Eur. Heart J.* **2010**, *31*, 2844−2853.
(16) Sowers, J. R. Obesity as a cardiovascular risk factor. *Am. J. Med.* **2003**, *115*, 37−41.
(17) Hokanson, J. E.; Austin, M. A. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a metaanalysis of population-based prospective studies. *J. Cardiovasc. Risk* **1996**, *3*, 213−219.
(18) Rapeseed I, Determination of glucosinolates content—part 1: Method using high performance liquid chromatography; 9167-1:1992 (E); International Standard Organization, Geneva, Switzerland, 1992; pp 1−9
(19) Lin, L. Z.; Harnly, J.; Zhang, R. W.; Fan, X. E.; Chen, H. J. Quantitation of the hydroxycinnamic acid derivatives and the glycosides of flavonols and flavones by UV absorbance after identification by LC-MS. *J. Agric. Food Chem.* **2012**, *60*, 544−553.
(20) Sun, J.; Xiao, Z.; Lin, L.-z.; Lester, G. E.; Wang, Q.; Harnly, J. M.; Chen, P. Profiling polyphenols in five *Brassica* species microgreens by UHPLC-PDA-ESI/HRMS*n*. *J. Agric. Food Chem.* **2013**, *61*, 10960−10970.
(21) Reagan-Shaw, S.; Nihal, M.; Ahmad, N. Dose translation from animal to human studies revisited. *Faseb Journal* **2008**, *22*, 659−661.
(22) German, J. B.; Xu, R.; Walzem, R.; Kinsella, J. E.; Knuckles, B.; Nakamura, M.; Yokoyama, W. H. Effect of dietary fats and barley fiber on total cholesterol and lipoprotein cholesterol distribution in plasma of hamsters. *Nutr. Res.* **1996**, *16*, 1239−1249.
(23) Ametaj, B. N.; Bobe, G.; Lu, Y.; Young, J. W.; Beitz, D. C. Effect of sample preparation, length of time, and sample size on quantification of total lipids from bovine liver. *J. Agric. Food Chem.* **2003**, *51*, 2105−2110.
(24) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Bio. Chem.* **1957**, *226*, 497−509.
(25) Finley, J. W. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds. *Ann. Bot.* **2005**, *95*, 1075−1096.
(26) Gliszczyńska-Świgło, A.; Ciska, E.; Pawlak-Lemańska, K.; Chmielewski, J.; Borkowski, T.; Tyrakowska, B. Changes in the content of health-promoting compounds and antioxidant activity of broccoli after domestic processing. *Food Addit. Contam.* **2006**, *23*, 1088−1098.
(27) Cohen, J. C. Contribution of cholesterol 7α-hydroxylase to the regulation of lipoprotein metabolism. *Curr. Opin. Lipidol.* **1999**, *10*, 303−307.
(28) Jelinek, D. F.; Andersson, S.; Slaughter, C. A.; Russell, D. W. Cloning and regulation of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* **1990**, *265*, 8190−8197.
(29) Giugliano, D.; Ceriello, A.; Esposito, K. The effects of diet on inflammation − emphasis on the metabolic syndrome. *J. Am. Coll. Cardiol.* **2006**, *48*, 677−685.
(30) Wouters, K.; van Gorp, P. J.; Bieghs, V.; Gijbels, M. J.; Duimel, H.; Lutjohann, D.; Kerksiek, A.; van Kruchten, R.; Maeda, N.; Staels, B.; van Bilsen, M.; Shiri-Sverdlov, R.; Hofker, M. H. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. *Hepatology* **2008**, *48*, 474−486.
(31) Lecerf, J. M.; de Lorgeril, M. Dietary cholesterol: from physiology to cardiovascular risk. *Br. J. Nutr.* **2011**, *106*, 6−14.
(32) Aggarwal, B. B.; Shishodia, S.; Sandur, S. K.; Pandey, M. K.; Sethi, G. Inflammation and cancer: how hot is the link? *Biochem. Pharmacol.* **2006**, *72*, 1605−1621.
(33) Bosma-den Boer, M. M.; van Wetten, M. L.; Pruimboom, L. Chronic inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering. *Nutr. Metab.* **2012**, *9*, 32.
(34) Reuter, S.; Gupta, S. C.; Chaturvedi, M. M.; Aggarwal, B. B. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biol. Med.* **2010**, *49*, 1603−1616.
(35) Coleman, R. A.; Lee, D. P. Enzymes of triacylglycerol synthesis and their regulation. *Prog. Lipid Res.* **2004**, *43*, 134−176.
(36) Harris, C. A.; Haas, J. T.; Streeper, R. S.; Stone, S. J.; Kumari, M.; Yang, K.; Han, X.; Brownell, N.; Gross, R. W.; Zechner, R. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. *J. Lipid Res.* **2011**, *52*, 657−667.
(37) Chang, T. Y.; Chang, C. C. Y.; Ohgami, N.; Yamauchi, Y. Cholesterol sensing, trafficking, and esterification. *Annu. Rev. Cell Dev. Biol.* **2006**, *22*, 129−157.
(38) Thompson, S. L.; Krisans, S. Rat liver peroxisomes catalyze the initial step in cholesterol synthesis. The condensation of acetyl-CoA units into acetoacetyl-CoA. *J. Biol. Chem.* **1990**, *265*, 5731−5735.
(39) Glomset, J.; Janssen, E.; Kennedy, R.; Dobbins, J. Role of plasma lecithin: cholesterol acyltransferase in the metabolism of high density lipoproteins. *J. Lipid Res.* **1966**, *7*, 639−648.
(40) Li, L.; Hossain, M. A.; Sadat, S.; Hager, L.; Liu, L.; Tam, L.; Schroer, S.; Huogen, L.; Fantus, I. G.; Connelly, P. W. Lecithin

DOI: 10.1021/acs.jafc.6b03805
J. Agric. Food Chem. 2016, 64, 9161−9171

cholesterol acyltransferase null mice are protected from diet-induced obesity and insulin resistance in a gender-specific manner through multiple pathways. *J. Biol. Chem.* **2011**, *286*, 17809−17820.
(41) Li, L.; Naples, M.; Song, H.; Yuan, R.; Ye, F.; Shafi, S.; Adeli, K.; Ng, D. S. LCAT-null mice develop improved hepatic insulin sensitivity through altered regulation of transcription factors and suppressors of cytokine signaling. *Am. J. Physiol.−Endocrinol. Metab.* **2007**, *293*, E587−E594.
(42) Lind, L. Circulating markers of inflammation and atherosclerosis. *Atherosclerosis* **2003**, *169*, 203−214.
(43) Wanders, D.; Burk, D. H.; Cortez, C. C.; Van, N. T.; Stone, K. P.; Baker, M.; Mendoza, T.; Mynatt, R. L.; Gettys, T. W. UCP1 is an essential mediator of the effects of methionine restriction on energy balance but not insulin sensitivity. *FASEB J.* **2015**, *29*, 2603−2615.
(44) Alvarez-Crespo, M.; Csikasz, R. I.; Martínez-Sánchez, N.; Diéguez, C.; Cannon, B.; Nedergaard, J.; López, M. Essential role of UCP1 modulating the central effects of thyroid hormones on energy balance. *Mol. Metab.* **2016**, *5*, 271−282.
(45) Goldwasser, J.; Cohen, P. Y.; Yang, E.; Balaguer, P.; Yarmush, M. L.; Nahmias, Y. Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPARα, PPARγ and LXRα. *PLoS One* **2010**, *5*, e12399.
(46) Kamiya, T.; Nagamine, R.; Sameshima-Kamiya, M.; Tsubata, M.; Ikeguchi, M.; Takagaki, K. The isoflavone-rich fraction of the crude extract of the Puerariae flower increases oxygen consumption and BAT UCP1 expression in high-fat diet-fed mice. *Global J. Health Sci.* **2012**, *4*, 147.
(47) Ngamukote, S.; Mäkynen, K.; Thilawech, T.; Adisakwattana, S. Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules* **2011**, *16*, 5054−5061.
(48) Manach, C.; Mazur, A.; Scalbert, A. Polyphenols and prevention of cardiovascular diseases. *Curr. Opin. Lipidol.* **2005**, *16*, 77−84.
(49) Saeed, S. A.; Manzoor, I.; Quadri, J.; Tasneem, S.; Simjee, S. U. Demystifying phytochemicals: an insight. *Int. J. Pharmacol.* **2005**, *1*, 234−238.
(50) Wiczkowski, W.; Szawara-Nowak, D.; Topolska, J. Red cabbage anthocyanins: profile, isolation, identification, and antioxidant activity. *Food Res. Int.* **2013**, *51*, 303−309.
(51) Villarreal-García, D.; Jacobo-Velázquez, D. A. Glucosinolates from broccoli: nutraceutical properties and their purification. *Curr. Trends Nutraceuticals* **2016**, *1*, in press.
(52) Traka, M. Health benefits of glucosinolates. *Adv. Bot. Res.* **2016**, *80*, 247.
(53) Gonzalez, R.; Ballester, I.; Lopez-Posadas, R.; Suarez, M. D.; Zarzuelo, A.; Martinez-Augustin, O.; De Medina, F. Effects of flavonoids and other polyphenols on inflammation. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 331−362.

DOI: 10.1021/acs.jafc.6b03805
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