Journal of Food and Nutrition Research, 2020, Vol. 8, No. 8, 399-409
Available online at http://pubs.sciepub.com/jfnr/8/8/3
Published by Science and Education Publishing
DOI:10.12691/jfnr-8-8-3

Differential Effects of Agraz (*Vaccinium meridionale* Swartz) Consumption in Overweight and Obese Women with Metabolic Syndrome

Catalina Marín-Echeverri[1], Manuela Piedrahita-Blandón[1], Yeisson Galvis-Pérez[1], Vitelbina Nuñez-Rangel[1], Christopher N. Blesso[2], María-Luz Fernández[2], Jacqueline Barona-Acevedo[1,*]

[1]Research Group of Toxinology, Therapeutic and Food Alternatives; School of Microbiology; Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia
[2]Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA
*Corresponding author: maria.barona@udea.edu.co

Received July 03, 2020; Revised August 04, 2020; Accepted August 13, 2020

Abstract Obesity implies higher cardiovascular risk (CVR) than overweight. Polyphenol-rich fruits have shown to ameliorate CVR factors (CVRF). It is not clear whether differential effects could be observed between obese and overweight people consuming these fruits. Objective: To evaluate the effects of agraz on CVRF in overweight and obese women with metabolic syndrome (MetS). Methods: Overweight (n=22) and obese (n=18) women (25-60 years) with MetS, were included in this crossover, double-blind and placebo-controlled study. They consumed agraz or placebo over 4-weeks separated by a 4-wk washout period. At the end of each period, the following parameters were measured: anthropometrics, blood pressure, serum lipid profile, glucose, insulin, adipokines, apolipoprotein (apo)-A1, high sensitivity C-reactive protein (hs-CRP), serum total antioxidant capacity (TAC), endogenous antioxidant enzymes and oxidative stress (OxS) markers. Results: Compared to placebo, agraz consumption significantly ($p<0.05$) reduced hs-CRP and urinary 8-hydroxy 2 deoxyguanosine (8-OHdG) levels in overweight and obese women, respectively. In both groups, changes in antioxidant markers were significant ($p<0.05$) and negatively correlated with changes in CVR factors and OxS markers, respectively. Positive correlations were observed with cardioprotective markers. Conclusions: Agraz consumption had differential effects in overweight and obese women, with better effects on inflammation and OxS markers, respectively. Further studies should consider these differential responses when analyzing the results of an intervention, and eventually adjust to get better outcomes.

Keywords: *Andean berry, obesity, oxidative stress, total antioxidant capacity, antioxidant enzymes, inflammation, cardiovascular risk factors*

Cite This Article: Catalina Marín-Echeverri, Manuela Piedrahita-Blandón, Yeisson Galvis-Pérez, Vitelbina Nuñez-Rangel, Christopher N. Blesso, María-Luz Fernández, and Jacqueline Barona-Acevedo, "Differential Effects of Agraz (*Vaccinium meridionale* Swartz) Consumption in Overweight and Obese Women with Metabolic Syndrome." *Journal of Food and Nutrition Research*, vol. 8, no. 8 (2020): 399-409. doi: 10.12691/jfnr-8-8-3.

1. Introduction

Obesity is a global epidemic and represents an important public health problem [1].

Obese people have higher risk of coronary heart disease than overweight people (61% and 22% respectively) [2]. Compared with overweight subjects, obese people have higher prevalence of metabolic syndrome (MetS) [3], a complex of cardiometabolic factors that doubles the risk for developing cardiovascular diseases (CVD) [4], which represent the first cause of mortality worldwide [5].

Besides the cardiometabolic alterations in obese people, endogenous antioxidant enzymes are not enough to neutralize the overproduction of reactive oxidative species [6], resulting in oxidative stress (OxS) and high levels of oxidation markers [7,8]. These alterations lead to dysregulated adipocytokine production, chronic inflammation, insulin resistance [9,10], and atherosclerotic lesion development [11].

Lifestyle changes have shown to contribute importantly to the treatment and prevention of cardiovascular risk factors (CVRF) in overweight and obese people. For example, studies in people with high BMI (≥25 kg/m^2) have shown that chronic consumption of polyphenol-rich foods results in reductions of lipid peroxidation [12,13] and protein oxidation markers [14]; and increases in antioxidant markers like blood total antioxidant capacity [TAC] [15] and antioxidant enzymes activity [16,17]. Likewise, polyphenol consumption has shown to improve insulin resistance [18], adiponectin levels [14], BMI, waist circumference (WC), LDL-cholesterol (LDL-c) [19], systolic and diastolic blood pressure [13] and inflammation

(e.g., by reducing high sensitivity C-reactive protein [hs-CRP] levels) [20]. In contrast, some studies did not find beneficial effects of polyphenol consumption in people with high BMI [21,22]. One reason for these contradictory results might be a differential response to polyphenol consumption between obese and overweight people. Recently, Herranz-López *et al.*, found that consumption of polyphenol-rich extracts (Metabolaid®, 500 mg/day) had a better effect in reducing BMI, WC, body fat percentage and blood pressure in overweight, compared to obese participants [23], suggesting that it is important to evaluate differences in the response between overweight and obese participants in intervention studies.

Lately, there has been a great interest in studying the effects of *Vaccinium meridionale* Swartz (also called agraz or Andean berry) in human health, due to its high antioxidant capacity and phenol concentration (mainly anthocyanins) [24]. In 2018, Torres *et al.*, showed that 21 days of osmodehydrated Andean berry consumption in overweight subjects caused reductions of some CVRF like blood pressure, BMI and WC [25]. Previously, we reported beneficial effects after 4-weeks of agraz consumption in this same group of women with MetS, with increases in TAC [26] and reduction in DNA oxidative damage [27]. However, there are limited studies evaluating the effect of consumption of a polyphenol-rich beverage of agraz on OxS markers and antioxidant capacity comparing people with different BMI classification. Therefore, we aimed to evaluate the effects of agraz, compared to placebo, in cardiometabolic variables, OxS markers, endogenous antioxidant enzyme activity and serum TAC in overweight and obese women with MetS.

2. Materials and Methods

2.1. Study Design

Forty women (25-60 years) with MetS from Medellín- Colombia, were included in this double-blind, crossover design and placebo-controlled study. MetS was defined according to the NCEP ATP-III guidelines [4], as previously described [27]. Women were analyzed according to their BMI as overweight (n=22) and obese (n=18) [28]. Exclusion criteria included kidney disease, heart disease, diabetes; having TG ≥ 500 mg/dL, fasting plasma glucose ≥ 126 mg/dL, LDL-c ≥ 190 mg/dL, blood pressure >140/90 mmHg. In addition, consumption of anti-inflammatory, lipid-lowering, hypoglycemic, and anti-hypertensive medications; smoking, consuming more than 20 g alcohol per day, being pregnant or planning to become pregnant, being a professional athlete, and consumption of supplements or nutraceuticals.

Women were assigned through an alternating quasi-randomization allocation method, to consume over 4 weeks either a freeze-dried agraz powder dissolved in 200 mL of water (containing 1027.97 ± 41.99 mg gallic acid equivalents /L of total phenols) or 200 mL of placebo (with sensorial and physicochemical characteristics of the agraz beverage, but devoid of any polyphenols). Both beverages were prepared and characterized by food engineers of the University of Antioquia as previously described [26]. There was a 4-week washout between both periods, in which neither agraz nor placebo were consumed.

During the whole study, women were asked to continue with their habitual diet and exercise, as well as to avoid the consumption of polyphenol-rich foods. To verify compliance, a 7-day physical activity record and a food frequency questionnaire were used at the beginning and end of each period. This last questionnaire was designed and adjusted specifically for the academic community of the University of Antioquia [29], where the participants were recruited. The frequency of consumption of some foods and the energy and macronutrients consumed during each period were obtained. In addition, the adherence to the study was evaluated on a weekly basis using a questionnaire to verify the daily beverage consumption and abstinence from consuming polyphenol-rich foods. An adherence below 80% was a criterion for withdrawing study participants.

This study was performed according to the Helsinki declaration and was approved by the Human Bioethics Committee of the *Sede de Investigación Universitaria, University of Antioquia* (Act No. 15-35-558-02). Before the intervention, all participants signed an informed consent.

2.2. Blood Collection

Blood samples were collected after 12 hours of overnight fasting at baseline and at the end of each intervention period, using serum separator tube (Vacutainer ®, Franklin Lakes, NJ, USA). Samples were centrifuged at 2000 x g for 10 minutes and kept frozen at -70°C until analysis.

2.3. Anthropometric, Metabolic and Inflammatory markers

Body weight was measured using a calibrated digital scale (Seca 813, Seca, Chino, CA, USA) and height was determined with a portable stadiometer (Seca 213, Seca, Chino, CA, USA). BMI was calculated by dividing body weight (kg) by height squared (m^2). WC was measured using a non-stretching body measuring tape (Lufkin W606PM, Sparks, MD, USA) according to the Anthropometry Procedures Manual of the CDC (2017) [30]. Systolic and diastolic blood pressure were measured using an automated monitor (Omron, Healthcare, Hoffman Estates, IL, USA) and following the protocol described in the Health Tech/ Blood Pressure Procedures Manual of the CDC (2007) [31]. Serum glucose and lipid profile concentrations were measured with colorimetric and enzymatic kits (Siemens, Washington, DC, USA). The levels of hs-CRP were measured with a turbidimetric immunoassay (Siemens, Washington, DC, USA); values above 10mg/L were excluded for the analysis. All measurements were made using an automatic analyzer (Dimension RxL®, Siemens, Washington, DC, USA). Insulin levels were determined with direct chemiluminescence technology following manufacturer's instructions (Siemens, Washington, DC, USA) and using ADVIA Centaur® CP immunoassay System (Siemens, Washington, DC, USA). Adiponectin and resistin levels were determined with the human adipocyte magnetic panel kit following manufacturer's instructions and using Luminex® technology (Millipore Sigma, Burlington, MA, USA). Apo-A1 was determined

with the apo-A1 (human) ELISA kit based on a competitive assay (Cayman Chemical, MI, USA) in a microplate reader (Multiskan Go®, Thermo Scientific, MA, USA). The Friedewald formula was used to calculate LDL-c [32]. HOMA-IR index was calculated with the formula described by Matthews *et al* [33], and insulin sensitivity was calculated using Quantitative Insulin Sensitivity Check Index (QUICKI index) developed by Katz, *et al* [34].

2.4. Serum Total Antioxidant Capacity (TAC)

Serum TAC was measured through several methods: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was determined via a modified method described by Re, *et al* (1999) [35]; ferric reducing ability of plasma (FRAP) was measured using the modified method of Benzie and Strain (1996) [36]; oxygen radical absorbance capacity (ORAC) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) were measured following the methodology reported by Quintero *et al* [26].

2.5. Endogenous Antioxidant Enzymes

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzyme activities were determined in serum using the following kits, respectively: OxiSelect™ kit Superoxide Dismutase Activity Assay (Cell Biolabs, Inc, CA, San Diego, USA), OxiSelect™ Catalase Activity Assay Kit (Cell Biolabs, Inc, CA, San Diego, USA), and Glutathione Peroxidase Assay Kit (Cayman Chemical, MI, USA), following manufacturer's instructions. The results were expressed in U/mL for SOD and CAT enzyme activities and in nmol/min/mL for GPx activity.

Paraoxonase 1 (PON1) lactonase activity was measured according to the methodology described by Millar *et al* [37]. The concentration was determined using a standard curve generated with 0.1M HCl (0-115 µM) and results were expressed in kU/L.

2.6. Oxidative Stress (OxS) Markers

Advanced oxidation protein products (AOPP) levels were measured in apo-B depleted serum as previously described [38]. The results were expressed in µM. Myeloperoxidase was determined using the Myeloperoxidase human ELISA Kit, (Cayman Chemical, MI, USA) following manufacturer's instructions. The results were expressed in ng/mL.

8-isoprostane levels and 8-hydroxy 2 deoxyguanosine (8-OHdG) were measured in 24-hours' urine using the following kits, respectively: OxiSelect™ 8-iso-prostaglandin F2 ELISA kit (Cell Biolabs, Inc., San Diego, CA, USA) and 8-OHdG ELISA Kit (Abcam, Cambridge, MA, USA), following manufacturer's instructions. The concentrations were calculated with standard curves. Finally, results were normalized to urinary creatinine levels and expressed in ng/mg creatinine.

2.7. Statistical Analysis

Shapiro Wilk test was used to evaluate data distribution. Accordingly, data are presented as mean ± SD or median and 25 (p25) and 75 (p75) percentiles. Repeated measures ANOVA was used to determine the effect of time, intervention and the interaction between time*intervention in variables measured at baseline and end of intervention (anthropometric measures, blood pressure, lipid profile and fasting glucose). For variables measured only at the end of intervention, paired samples t-test or Wilcoxon test were used to compare the results between agraz and placebo periods, according to data distribution. Mann- Whitney U test or Student's t-test were used to evaluate the differences between obese and overweight women. Finally, changes after agraz consumption (agraz minus placebo period) were calculated and the correlations between changes were determined with Pearson´s or Spearman´s correlation coefficients. All analyses were done using SPSS version 21 for Windows (SPSS, IBM Corporation, 2012). Differences with a p value <0.05 were considered significant.

3. Results

3.1. Study Population Characteristics

Forty women, 22 overweight (49±9 years old) and 18 obese (45±9 years old) with MetS finished the study (Table 1). Adherence was 94.8%, indicating a suitable consumption of both beverages. No changes in diet and physical activity were found during the whole intervention (agraz versus placebo) as previously reported [27]. Importantly, there were not differences in age, energy, fat, carbohydrate, and protein intakes, nor physical activity between obese and overweight women (Table 1 and Table 2).

Table 1. Baseline characteristics of overweight and obese women with metabolic syndrome included in the study[1]

Variables	Overweight (n= 22)			Obesity (n=18)			p value
	Mean ±SD or median (p25-p75)			Mean ±SD or median (p25-p75)			
Age (years)[a]	49.0	±	9.0	45.0	±	9.0	0.210
Weight (Kg)[a]	69.8	±	7.4	84.5	±	10.4	**0.000***
BMI (kg/cm^2)[b]	28.1 (27.3 - 28.9)			32 (30.9 - 33.7)			**0.000***
Waist Circumference (cm)[a]	97.7	±	3.5	107.2	±	11.2	**0.001***
Systolic blood pressure (mm Hg)[a]	112.8	±	11.2	124.6	±	11.0	**0.002***
Diastolic blood pressure (mm Hg)[a]	72.5	±	9.9	80.5	±	6.6	**0.006***
Fasting glucose (mg/dL)[a]	94.7	±	7.4	93.5	±	7.2	0.609
TC (mg/dL)[b]	229.8 (209.8 - 255.9)			225 (192.6 - 239.9)			0.447
HDL-c (mg/dL)[a]	43.5	±	7.1	40.6	±	5.2	0.164
Triglycerides (mg/dL)[b]	204.2 (175.8 - 281)			194.8 (149.4 - 274.9)			0.254
TG/HDL ratio[a]	4.6 (3.8 - 6.8)			4.5 (3.7 - 6.9)			0.775
LDL-c (mg/dL)[a]	136.2	±	37.5	131.8	±	31.7	0.692
Non HDL-c (mg/dL)[a]	182.8	±	36.6	172.9	±	39.9	0.418

[a] t student test; [b] Mann-Whitney U test; * significance p<0.05; [1]Women were classified according to BMI [28] and metabolic syndrome definition by ATP-III [4]. Abbreviations: HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; p25, percentile 25; p75, percentile 75; TC, total cholesterol.

Table 2. Baseline kilocalories, macronutrients intake and physical activity in obese and overweight women with metabolic syndrome[1]

Variables	Overweight		Obese		p value
	n	Mean ±SD or median (p25-p75)	n	Mean ±SD or median (p25-p75)	
Kilocalories (Kcal)[a]	22	1800.7 (1519.6 - 2419.1)	18	1643 (1227.3 - 2486.8)	0.610
Protein (g)[a]	22	65.9 (54.1 - 81.2)	18	63 (47.7 - 81.5)	0.770
Total fat (g)[a]	22	58.8 (52.1 - 84.5)	18	63.1 (49.5 - 88)	0.830
Saturated fatty acids (g)[a]	22	21.4 (19 - 31.2)	18	23.6 (17.5 - 33.1)	0.550
Monounsaturated fatty acids (g)[a]	22	23.1 (19.8 - 33.9)	18	22.7 (17.5 - 34.3)	0.980
Polyunsaturated fatty acids (g)[a]	22	11.8 (8.3 - 15.9)	18	12 (8 - 17)	0.790
Cholesterol (mg)[b]	22	313.4 ± 151.3	18	310.5 ± 131.7	0.950
Total carbohydrates (g)[a]	22	255.8 (203.1 - 358.8)	18	206.2 (174.1 - 329.5)	0.230
Dietary Fiber (mg)[a]	22	20.3 (13.6 - 25.1)	18	14.9 (9.8 - 18.5)	0.100
Physical activity (min) [a]	22	210(110-330)	18	220 (70-300)	0.730
Total phenols (mg)[a]	17	1105.9 (848-1319)	14	940.8 (706-1616)	0.812

[a] Mann-Whitney U test; [b] t student test; * significance p<0.05; [1]Women were classified according to BMI [28] and ATP-III (4).

3.2. Anthropometric, Metabolic and Inflammatory Markers

As expected, women with obesity had significantly higher body weight, BMI, WC, systolic and diastolic blood pressure (p<0.05), compared with overweight women. However, there were no significant differences in fasting glucose and lipid profile (Table 1).

Changes in hs-CRP levels were significantly different between obese and overweight women (p=0.028) (Table 3), with a significant reduction of hs-CRP levels in overweight women (p=0.011) after agraz consumption, compared to placebo (Figure 1).

Differences between obese and overweight women, in other cardiovascular variables after agraz intake were not observed (Table 3, Table S1, Online Supporting Information).

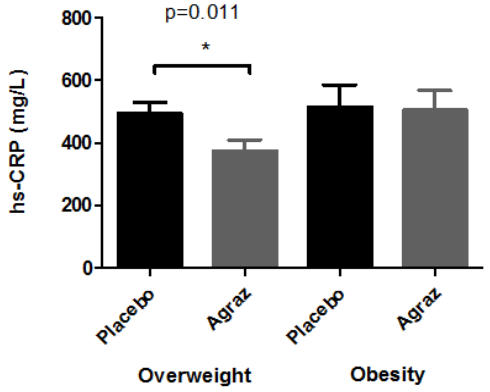


Figure 1. Differential effects after 4 weeks of agraz consumption, compared to placebo on inflammation (Abbreviations: hs-CRP, high sensitivity C-reactive protein)

Table 3. Changes in anthropometric, metabolic and inflammatory markers between agraz and placebo periods for participants classified with overweight *versus* those with obesity[1]

Change between agraz and Placebo	Overwerweigh			Obesity			p
	Mean ±SD or median (p25-p75)			Mean ±SD or median (p25-p75)			
Weight (Kg)[a]	-0.1 (-0.5, 0.5)			-0.4 (-1.4, 0.4)			0.360
BMI (kg/cm^2)[a]	0.0 (-0.2, 0.2)			-0.2 (-0.5, 0.1)			0.310
Waist Circumference (cm)[a]	-0.1 (-1.4, 0.8)			0.4 (-2.3, 3.5)			0.438
SBP (mm Hg) [b]	-2.6	±	11.3	4.8	±	13.0	0.061
DBP (mm Hg) [b]	-1.4	±	8.2	2.6	±	7.4	0.116
Fasting glucose (mg/dL) [b]	-1.4	±	5.9	-2.8	±	5.4	0.450
TC (mg/dL)[b]	2.9	±	26.9	-7.5	±	28.6	0.258
HDL-c (mg/dL) [b]	1.6	±	7.8	-0.1	±	4.5	0.411
Non HDL-c (mg/dL) [b]	2.0	±	27.6	-7.4	±	28.0	0.307
TG (mg/dL) [b]	0.5	±	142.8	-3.6	±	81.5	0.914
LDL-c (mg/dL) [b]	1.3	±	25.6	-6.5	±	20.7	0.351
TG/HDL ratio [b]	-0.3	±	4.3	0.0	±	2.3	0.846
Apo A1 (mg/dL) [b]	-4.8	±	37.0	13.5	±	44.5	0.229
Insulin (µUI/mL) [a]	0.7 (-2.6, 2.3)			0.1 (-2.2, 1.9)			0.887
HOMA-IR Index [a]	0.2 (-0.6, 0.4)			0.1 (-0.7, 0.7)			0.910
QUICKI Index [b]	0.0	±	0.0	0.0	±	0.0	1.000
Adiponectin (µg/mL) [a]	0.9 (-1.5, 3.0)			0.9 (-2.3, 2.4)			0.665
hs-CRP (mg/L) [a]	-1.0 (-2.5, -0.5)			0.4 (-0.5, 1.2)			0.028*

[1]Women were classified according to BMI [28]; [a] Mann-Whitney U test; [b] Student t-test; * significance p<0.05
Abbreviations: p25, percentile 25; p75, percentile 75; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; TC, total cholesterol; TG, triglycerides; Apo, apolipoprotein; HOMA, Homeostatic Model Assessment for Insulin Resistance; QUICKI Index, Quantitative Insulin Sensitivity Check Index; hs-CRP, high sensitivity C-reactive protein.

In obese women, there were significant correlations between changes in different cardiometabolic factors after agraz consumption compared to placebo (Figure S1, Online Supporting Information). Changes in HDL-cholesterol (HDL-c) levels had a positive correlation with changes in QUICKI index (r= 0.57) (Figure S1A) and a negative correlation with changes in fasting glucose levels (r= -0.59) (Figure S1B). Likewise, changes in apo-A1 concentration had negative significant correlations with changes in LDL-c, Non-HDL-c and TC levels (r= -0.608; r= -0.585; r= -0.615, respectively) (Figure S1C-E).

3.3. Antioxidant and Oxidative Stress (OxS) Markers

There was a significant reduction in urinary 8-OHdG levels in obese women after agraz consumption compared to placebo (p=0.031) (Figure 2). No other significant changes were observed (Table 4).

The correlation analyses showed significant correlations among antioxidant, metabolic and inflammatory markers in obese women (Figure S2, Online Supporting Information). Changes in PON1 lactonase activity were negatively correlated with changes in WC and hs-CRP levels (r= -0.52; r= -0.57, respectively) (Figure S2A and B). In addition, changes in TAC measured by DPPH had a positive correlation with changes in HDL-c levels (r= 0.51) (Figure S2C). Changes in CAT activity correlated negatively with changes in HOMA-IR (r= -0.60) (Figure S2D) and 8-isoprostanes (r= -0.55) (Figure S2F).

Important correlations were also found in overweight women (Figure S3, Online Supporting Information). Changes in ORAC were negatively correlated with BMI (Figure S3A), and changes in SOD activity were also negatively correlated with changes in 8-OHdG levels (Figure S3B). On the contrary, positive correlations were found between changes in DPPH and changes in adiponectin concentration (Figure S3C). Changes in FRAP were also positively correlated with changes in PON1 lactonase activity (Figure S3D).

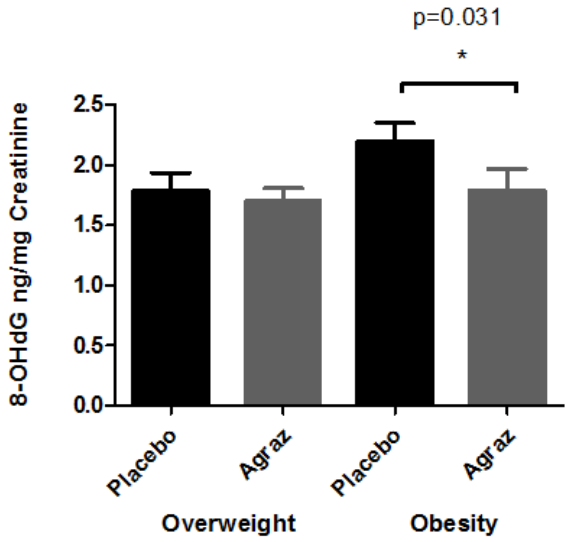


Figure 2. Differential effects after 4 weeks of agraz consumption, compared to placebo on inflammation (Abbreviations: 8-OHdG, 8-hydroxy 2 deoxyguanosine)

Table 4. Changes in antioxidant and oxidative stress markers between agraz and placebo periods for participants classified with overweight *versus* those with obesity[1]

Change between agraz and Placebo	Overweight				Obesity				p
	n	Mean ±SD or median (p25-p75)			n	Mean ±SD or median (p25-p75)			
Antioxidants markers									
Total phenols (mgGA/L)	22	7.9	±	65.0	18	16.2	±	73.7	0.159
DPPH (% Scavenging effect)	21	1.2	±	5.2	18	1.9	±	3.9	0.642
ORAC (µM Trolox Eq/mL)	22	-0.4	±	3.4	18	0.7	±	3.3	0.337
ABTS (µM Trolox Eq/mL)	22	0.0	±	0.1	18	0.0	±	0.1	0.500
FRAP (µM Trolox Eq/mL)	22	-20.1	±	115.5	18	14.7	±	106.0	0.331
SOD activity (U/mL)	20	32.5	±	80.0	17	-6.1	±	45.6	0.088
CAT activity (U/mL)	21	15.9	±	61.7	18	-3.2	±	94.7	0.454
GPx activity (nmol/min/mL)	22	4.0	±	58.7	17	-8.5	±	48.2	0.482
PON1 Lactonase Activity (kU/L)	22	-1.1	±	8.3	16	-0.1	±	9.7	0.736
Oxidative stress markers									
AOPP (µM)	16	-3.2	±	22.4	13	-0.5	±	16.8	0.727
8-Isoprostane (pg/mL)	19	0.5	±	2.2	15	-0.3	±	2.2	0.321
Urinary 8-OHdG (mg/g creatinine)	19	-0.1	±	0.7	16	-0.4	±	0.7	0.186
MPO (ng/mL)	19	-3.5	±	82.4	17	-19.7	±	59.5	0.197

[1]Women were classified according to BMI [28]. Student t test; * significance p<0.05
Abbreviations: p25, percentile 25; p75, percentile 75; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; ORAC, Oxygen radical absorbance capacity; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); FRAP, ferric reducing ability of plasma; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; PON1, paraoxonase1; AOPP, advanced oxidation protein products; 8-OHdG, 8-hydroxy 2 deoxyguanosine; MPO, myeloperoxidase.

4. Discussion

In this study, we evaluated the effects of agraz consumption on anthropometric, metabolic, inflammatory, antioxidant and OxS markers in women with MetS according to their BMI classification (overweight and obesity). We found that after agraz intake, the effects on inflammatory and oxidative markers were different in obese and overweight women.

It is well known that BMI is highly associated with CVRF [39]. As expected, obese women included in this study had a greater risk profile with higher WC and blood pressure than those with overweight, as others have reported [40].

A meta-analysis including 1251 subjects demonstrated that berry consumption has improved CVRF like obesity, hypertension, hyperglycemia, and dyslipidemia, not only in normal weight subjects, but also in overweight and obese people [41]. Interestingly, these beneficial effects were observed only after interventions longer than 8 weeks [41]. The time of intervention of our study was 4 weeks, which seemed to be insufficient to modify traditional cardiovascular markers in both groups of women. In addition, the beneficial effects reported in the meta-analysis, were observed only in studies with a parallel design, but not in those with a crossover design [41]. Although our study did not show effects in these CVRF, a crossover design study, as the one we used, has some advantages over studies with a parallel design, such as higher statistical power, as it requires a smaller sample size and it reduces the inter-individual variation because each person is her/his own control [42]. Interestingly, Torres *et al.* demonstrated that *V. meridionale* consumption over 21 days, significantly improved blood pressure, WC and BMI in overweight adults [25]. However, they used a pretest/post-test design with a single group.

We observed that positive changes in atheroprotective markers (HDL-c and apo-A1) were negatively and significantly associated with CVRF. Currently, identifying potential therapies targeting improvements in HDL functionality is of great interest, given the relationship of HDL with CVD reduction [43,44,45]. A study in adults with prediabetes consuming anthocyanins during 12 weeks showed increases in apo-A1 levels [46] - the major component of HDL with atheroprotective roles [47]. Although we did not measure the expression of the *ApoA1* gene in this women to explain the improvement in apo-A1 levels, a study in mice demonstrated that anthocyanin consumption significantly increased *ApoA1* gene expression and reduced atherosclerosis progression [48]. The authors concluded these effects could be attributed to the anthocyanin content in the black elderberry extract consumed by the mice.

In obesity, adipose tissue expansion is an important source of cytokines triggering a systemic inflammation [49], which is associated with high levels of CRP, an independent predictor of cardiac risk [50]. It has been demonstrated that the levels of pro-inflammatory markers are significantly different between overweight and obese individuals, being higher in the latter [51]. As expected, in our study, hs-CRP levels were higher in obese than overweight women, however, in both groups the levels of this pro-inflammatory marker were above 3 mg/L, indicating a high risk of CVD [50]. Interestingly, the levels of hs-CRP significantly decreased by more than 1 mg/L after consuming agraz during 4 weeks, only in overweight women; indicating a better effect in this group of women. This result is similar to the reported by Karlsen *et al.* who observed a reduction in hs-CRP, among other cytokines, in overweight subjects consuming bilberry juice (*V. myrtillus*) over 4 weeks [52]. These anti-inflammatory effects of polyphenols could be associated to their capacity of reducing pro-inflammatory protein expression through the inhibition of NF-kB translocation [52] and activation of liver X receptor alpha (LXRα) pathway (which induces a trans-repression of NF-kB) [53]. The lack of *Vaccinium* anti-inflammatory effects in obese women have also been reported by others, even after 6 [18] and 8 weeks of intervention [13]. It is possible that obese subjects, with a more inflammatory environment, need to make more drastic changes in their lifestyle or receive longer treatments to achieve more consistent results.

Interestingly, we found that after agraz consumption, obese women with lower levels of hs-CRP correlated with higher activity of the antioxidant enzyme PON1. Other mechanistic studies with polyphenols have reported increases in PON1 expression [54] and the reduction of inflammation mediated by NF-kB [52]. However, molecular studies with *V. meridionale* will be necessary to corroborate this mechanism.

In addition to inflammation, people with MetS have low levels of TAC which is inversely associated with some CVRF [55]. In this study, agraz consumption showed positive effects on serum TAC in the participants. In obese women, there was a tendency to increase serum TAC measured by DPPH scavenging capacity by 18.2% after agraz consumption, compared to placebo. Recently, we reported an increase in serum antioxidant status by 19% with this method after 30 days of agraz consumption, in the whole group of women with MetS [26]. This could indicate there was a greater neutralization of DPPH radicals in serum through donation of an electron and/or a hydrogen by antioxidant molecules (i.e. in agraz). This could be hypothesized as a protective effect of agraz intake given that it has been demonstrated that TAC is negatively associated with OxS [56].

Likewise, changes in serum TAC correlated positively with changes in HDL-c levels in obese women, and with changes in PON1 activity in overweight women, after agraz consumption, compared to placebo. OxS affects the antioxidant activity on the HDL particle [57], which also alters its ability to accept cholesterol, thereby decreasing cholesterol transported by HDL [58]. Therefore, when antioxidant capacity is improved, there is also an increase in the cholesterol efflux capacity by the HDL particle, increasing HDL-c levels. This was demonstrated in a study where anthocyanin consumption improved HDL-associated PON1 activity and cholesterol efflux capacity [59]. Thus, anthocyanins present in the agraz could have the same effect in these women.

Increased levels of adiponectin- secreted by adipose tissue with insulin sensitizing effects [60]- have been reported in people with high TAC and vice versa [61]. Although, we did not observe significant changes in this

hormone, we found a positive correlation between serum TAC and adiponectin levels in overweight women after consuming agraz, compared to placebo. This could be associated to the antioxidants present in agraz which showed to increase serum TAC [26].

We also evaluated the effects of agraz consumption in endogenous antioxidant enzymes (CAT, SOD and GPx) which have an important role in the prevention of oxidative damage in the cells. A study reported that obese women had significant lower levels of these endogenous enzymes than overweight women [6]; these low enzyme levels were strongly associated with abdominal obesity [6]. In our study, there were not significant changes in antioxidant enzyme activities in overweight and obese women after agraz intake, compared to placebo. Nevertheless, changes in CAT activity had negative and significant correlations with a lipid peroxidation marker in obese women; and changes in SOD activity were negatively correlated with a DNA oxidative marker in overweight women. Our results are similar to the reported by Lee *et al.* in subjects consuming a chokeberry supplement [56]. They found a negative association between SOD activity and 8-isoprostane levels, and concluded these results indicate the prevention of lipid peroxidation mediated by the endogenous antioxidant system [56]. In addition, a recent study in rats evaluating the effects of *Vaccinium meridionale*, demonstrated the treatment with this extract significantly increased the activity of CAT and SOD associated with an increase of AKT expression [62], which seems to be modulated by polyphenols [63], as a defense mechanism against free radical damage.

Catalase has an important role as an antioxidant enzyme through the decomposition of the reactive species of hydrogen peroxide (H_2O_2) in O_2 and water, which indirectly could diminish insulin resistance [64]. We found that changes in CAT activity were negatively correlated with changes in HOMA-IR- an insulin resistance marker- after agraz consumption, compared to placebo, in obese women. This could be associated with reductions in H_2O_2 levels mediated by this endogenous enzyme.

Similarly, agraz consumption had positive effects over OxS markers in both groups of women. The effects were more evident in obese women, in which there was a significant reduction in urinary 8-OHdG levels. This biomarker of DNA oxidative damage is associated with CVD [65]. The reduction of this marker was also reported in a study with a pretest-post-test design (without a control group) evaluating healthy people consuming a strawberry beverage for one month [66]. Different from our study, they also found decreases in other OxS markers such as malondialdehyde and isoprostane levels [66]. Other studies with parallel designs in obese people with MetS have reported significant reductions in OxS markers, including oxidized LDL, malondialdehyde, hydroxynonenal and AOPP, after 8 weeks of consuming berry beverages [13,14,15]. It is important to note, these studies had different designs (pretest-post-test without a control group, parallel arm) than the one used in our study (crossover placebo-controlled design). In addition, most of them had double the time of supplementation than our study (8 versus 4 weeks). It seems that a longer intervention is required to observe significant changes in OxS, especially in people with high OxS, as the women included in this study.

5. Conclusions

In conclusion, in this study we evaluated anthropometric, metabolic, inflammatory, antioxidant and OxS markers, to determine the differential effects of agraz consumption between overweight and obese women with MetS. Agraz consumption had a better anti-inflammatory effect in overweight women. Interestingly, the effect on OxS markers was better in obese women. Regarding the anti-inflammatory effects of Vaccinium supplementation, it seems to be difficult to observe these effects in obese cohorts, possibly associated with a more inflammatory state that requires a more drastic dietary intervention in this specific population. Although there were positive effects on OxS markers in obese individuals in this study, evaluation of agraz intervention using different dosages and a longer duration of supplementation should be explored to obtain better responses in this population. Finally, the associations observed after agraz intake on antioxidant markers in both groups of women, suggest a potential antioxidant role of the bioactive compounds present in this fruit.

Acknowledgements

We thank all the volunteers for the time and commitment dedicated to this study. We thank members of the research group - Dr. Gelmy Ciro-Gómez, Julián Quintero and Santiago Galeano, for the design and characterization of the agraz and placebo beverages used in this study.

Statement of Competing Interests

The authors have no competing interests

List of Abbreviations

apo-A1, Apolipoprotein-A1; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); AOPP, advanced oxidation protein products; BMI, body mass index; CAT, catalase; CVR, cardiovascular risk; CVRF, CVR factors; CVD; cardiovascular diseases; CVRF, cardiovascular risk factors; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; FRAP, ferric reducing ability of plasma; GPx, glutathione peroxidase; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance index; hs-CRP, high sensitivity C reactive protein; LDL-c, low-density lipoprotein-cholesterol; LXRα, liver X receptor alpha; MetS, metabolic syndrome; NF-kB, nuclear factor kB; Nrf2, nuclear factor erythroid 2-related factor 2; OxS, oxidative stress; ORAC, oxygen radical absorbance capacity; p, percentile; PON1, Paraoxonase 1; QUICKI; Quantitative Insulin Sensitivity Check Index; SOD, superoxide dismutase; SD, standard

deviation; TAC, total antioxidant capacity; TG, triglycerides; WC, waist circumference; 8-OHdG, 8-hydroxy 2 deoxyguanosine.

References

[1] Collaborators G 2015 O, Afshin A, Forouzanfar M, Reitsma M, Sur P, Estep K, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; 377: 13-27.

[2] Mongraw-Chaffin ML, Peters SAE, Huxley RR, Woodward M. The sex-specific association between BMI and coronary heart disease: a systematic review and meta-analysis of 95 cohorts with 1·2 million participants. *Lancet Diabetes Endocrinol* 2015; 3: 437-49.

[3] Damiri B, Abualsoud MS, Samara AM, Salameh SK. Metabolic syndrome among overweight and obese adults in Palestinian refugee camps. *Diabetol Metab Syndr* 2018; 10: 34.

[4] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; 112: 2735-52.

[5] WHO (World Health Organization). Global status report on noncommunicable diseases 2014 2014: 79-93. http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf (accessed February 8, 2018).

[6] Amirkhizi F, Siassi F, Djalali M, Shahraki SH. Impaired enzymatic antioxidant defense in erythrocytes of women with general and abdominal obesity. *Obes Res Clin Pract* 2014; 8: e26-34.

[7] Morelli NR, Scavuzzi BM, Miglioranza LH da S, Lozovoy MAB, Simão ANC, Dichi I, et al. Metabolic syndrome components are associated with oxidative stress in overweight and obese patients. *Arch Endocrinol Metab* 2018; 62: 309-18.

[8] Venojärvi M, Korkmaz A, Wasenius N, Manderoos S, Heinonen OJ, Lindholm H, et al. 12 Weeks' aerobic and resistance training without dietary intervention did not influence oxidative stress but aerobic training decreased atherogenic index in middle-aged men with impaired glucose regulation. *Food Chem Toxicol* 2013; 61: 127-35.

[9] Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, et al. Oxidative stress is associated with adiposity and insulin resistance in men. *J Clin Endocrinol Metab* 2003; 88: 4673-6.

[10] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest.* 2004; 114: 1752-61.

[11] Abu-Saleh N, Aviram M, Hayek T. Aqueous or lipid components of atherosclerotic lesion increase macrophage oxidation and lipid accumulation. *Life Sci.* 2016; 154: 1-14.

[12] Basu A, Wilkinson M, Penugonda K, Simmons B, Betts NM, Lyons TJ. Freeze-dried strawberry powder improves lipid profile and lipid peroxidation in women with metabolic syndrome: baseline and post intervention effects. *Nutr J* 2009; 8: 43.

[13] Basu A, Du M, Leyva MJ, Sanchez K, Betts NM, Wu M, et al. Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *J Nutr* 2010; 140: 1582-7.

[14] Name T, Simão C, Alysson M, Lozovoy B, Name A, Oliveira SR, et al. Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome. *Br J Nutr* 2013; 110: 1885-94.

[15] Basu A, Betts NM, Ortiz J, Simmons B, Wu M, Lyons TJ. Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. *Nutr Res* 2011; 31: 190-6.

[16] Broncel M, Kozirog M, Duchnowicz P, Koter-Michalak M, Sikora J, Chojnowska-Jezierska J. Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. *Med Sci Monit* 2010; 16: CR28-34.

[17] Chu S, Fu H, Yang J, Liu G, Dou P, Zhang L, et al. A randomized double-blind placebo-controlled study of Pu'er tea extract on the regulation of metabolic syndrome. *Chin J Integr Med* 2011; 17: 492-8.

[18] Stull AJ, Cash KC, Johnson WD, Champagne CM, Cefalu WT. Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *J Nutr* 2010; 140: 1764-8.

[19] Sulibursk J, Bogdanski P, Szulinska M, Stepien M, Pupek-Musialik D, Jablecka A. Effects of green tea supplementation on elements, total antioxidants, lipids, and glucose values in the serum of obese patients. *Biol Trace Elem Res* 2012; 149: 315-22.

[20] Moazen S, Amani R, Homayouni Rad A, Shahbazian H, Ahmadi K, Taha Jalali M. Effects of freeze-dried strawberry supplementation on metabolic biomarkers of atherosclerosis in subjects with type 2 diabetes: a randomized double-blind controlled trial. *Ann Nutr Metab* 2013; 63: 256-64.

[21] van der Made SM, Plat J, Mensink RP. Resveratrol does not influence metabolic risk markers related to cardiovascular health in overweight and slightly obese subjects: a randomized, placebo-controlled crossover trial. *PLoS One* 2015; 10: e0118393.

[22] Murkovic M, Abuja PM, Bergmann AR, Zirngast A, Adam U, Winklhofer-Roob BM, et al. Effects of elderberry juice on fasting and postprandial serum lipids and low-density lipoprotein oxidation in healthy volunteers: a randomized, double-blind, placebo-controlled study. *Eur J Clin Nutr* 2004; 58: 244-9.

[23] Herranz-López M, Olivares-Vicente M, Boix-Castejón M, Caturla N, Roche E, Micol V. Differential effects of a combination of Hibiscus sabdariffa and Lippia citriodora polyphenols in overweight/obese subjects: A randomized controlled trial. *Sci Rep* 2019; 9: 2999.

[24] Garzón GA, Narváez CE, Riedl KM, Schwartz SJ. Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (Vaccinium meridionale Swartz) from Colombia. *Food Chem* 2010; 122: 980-6.

[25] Torres D, Reyes-Deeck C, Gallego E, Gómez-García AC, Posada G, Maldonado-Celis ME. Impact of osmodehydrated Andean berry (Vaccinium meridionale Swartz) on overweight adults. *Vitae* 2018; 25: 141-7.

[26] Quintero-Quiroz J, Galvis-Pérez Y, Galeano-Vásquez S, Marín-Echeverri C, Franco-Escobar C, Ciro-Gómez G, et al. Physico-chemical characterization and antioxidant capacity of the Colombian berry (Vaccinium meridionale Swartz) with a high-polyphenol content: potential effects in people with metabolic syndrome. *Food Sci Technol* 2019.

[27] Espinosa-Moncada J, Marín-Echeverri C, Galvis-Pérez Y, Ciro-Gómez G, Aristizábal JC, Blesso CN, et al. Evaluation of Agraz Consumption on Adipocytokines, Inflammation, and Oxidative Stress Markers in Women with Metabolic Syndrome. *Nutrients* 2018; 10: 1639.

[28] WHO (World Health Organization). Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000; 894: i-xii, 1-253.

[29] Álvarez Monsalve JM, González Zapata LI. Diseño de un cuestionario de frecuencia para evaluar ingesta alimentaria en la Universidad de Antioquia, Colombia. *Nutr Hosp* 2011; 26: 1333-44.

[30] CDC. Anthropometry Procedures Manual. Centers for Disease Control and Prevention. 2017.

[31] CDC. Health Tech/Blood Pressure Procedures Manual. Centers for Disease Control and Prevention. 2009.

[32] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.

[33] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412-9.

[34] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85: 2402-10.

[35] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; 26: 1231-7.

[36] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 1996; 239: 70-6.

[37] Millar CL, Duclos Q, Garcia C, Norris GH, Lemos BS, DiMarco DM, et al. Effects of Freeze-Dried Grape Powder on High-Density Lipoprotein Function in Adults with Metabolic Syndrome: A Randomized Controlled Pilot Study. *Metab Syndr Relat Disord* 2018; 16: 464-9.

[38] Marín-Echeverri C, Blesso CN, Fernández ML, Galvis-Pérez Y, Ciro-Gómez G, Núñez-Rangel V, et al. Effect of Agraz (Vaccinium meridionale Swartz) on High-Density Lipoprotein Function and Inflammation in Women with Metabolic Syndrome. *Antioxidants (Basel, Switzerland)* 2018; 7: E185.

[39] Zhang R, Dong S-Y, Wang F, Ma C, Zhao X-L, Zeng Q, et al. Associations between Body Composition Indices and Metabolic Disorders in Chinese Adults: A Cross-Sectional Observational Study. *Chin Med J (Engl)* 2018; 131: 379-88.

[40] Félix-Redondo FJ, Grau M, Baena-Díez JM, Dégano IR, León AC de, Guembe MJ, et al. Prevalence of obesity and associated cardiovascular risk: the DARIOS study. *BMC Public Health* 2013; 13: 542.

[41] Huang H, Chen G, Liao D, Zhu Y, Xue X. Effects of Berries Consumption on Cardiovascular Risk Factors: A Meta-analysis with Trial Sequential Analysis of Randomized Controlled Trials. *Sci Rep* 2016; 6: 23625.

[42] Lucey A, Heneghan C, Kiely ME. Guidance for the design and implementation of human dietary intervention studies for health claim submissions. *Nutr Bull* 2016; 41: 378-94.

[43] Michael Gibson C, Korjian S, Tricoci P, Daaboul Y, Yee M, Jain P, et al. Safety and Tolerability of CSL112, a Reconstituted, Infusible, Plasma-Derived Apolipoprotein A-I, After Acute Myocardial Infarction: The AEGIS-I Trial (ApoA-I Event Reducing in Ischemic Syndromes I). *Circulation* 2016; 134: 1918-30.

[44] Waksman R, Torguson R, Kent KM, Pichard AD, Suddath WO, Satler LF, et al. A first-in-man, randomized, placebo-controlled study to evaluate the safety and feasibility of autologous delipidated high-density lipoprotein plasma infusions in patients with acute coronary syndrome. *J Am Coll Cardiol* 2010; 55: 2727-35.

[45] Khera A V., Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, et al. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. *N Engl J Med* 2011; 364: 127-35.

[46] Yang L, Ling W, Yang Y, Chen Y, Tian Z, Du Z, et al. Role of Purified Anthocyanins in Improving Cardiometabolic Risk Factors in Chinese Men and Women with Prediabetes or Early Untreated Diabetes-A Randomized Controlled Trial. *Nutrients* 2017; 9: 1104.

[47] Aasvee K, Rasmussen M, Kelly C, Kurvinen E, Giacchi MV, Ahluwalia N. Validity of self-reported height and weight for estimating prevalence of overweight among Estonian adolescents: the Health Behaviour in School-aged Children study. *BMC Res Notes* 2015; 8: 606.

[48] Farrell N, Norris G, Lee SG, Chun OK, Blesso CN. Anthocyanin-rich black elderberry extract improves markers of HDL function and reduces aortic cholesterol in hyperlipidemic mice. *Food Funct* 2015; 6: 1278-87.

[49] Castro AM, Macedo-de la Concha LE, Pantoja-Meléndez CA. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. *Rev Médica Del Hosp Gen México* 2017; 80: 101-5.

[50] Ridker PM, Buring JE, Cook NR, Rifai N. C-Reactive Protein, the Metabolic Syndrome, and Risk of Incident Cardiovascular Events. *Circulation* 2003; 107: 391-7.

[51] Azizian M, Mahdipour E, Mirhafez SR, Shoeibi S, Nematy M, Esmaily H, et al. Cytokine profiles in overweight and obese subjects and normal weight individuals matched for age and gender. *Ann Clin Biochem* 2016; 53: 663-8.

[52] Karlsen A, Paur I, Bøhn SK, Sakhi AK, Borge GI, Serafini M, et al. Bilberry juice modulates plasma concentration of NF-kappaB related inflammatory markers in subjects at increased risk of CVD. *Eur J Nutr* 2010; 49: 345-55.

[53] Du C, Shi Y, Ren Y, Wu H, Yao F, Wei J, et al. Anthocyanins inhibit high-glucose-induced cholesterol accumulation and inflammation by activating LXRα pathway in HK-2 cells. *Drug Des Devel Ther* 2015; 9: 5099-113.

[54] Gouédard C, Barouki R, Morel Y. Dietary polyphenols increase paraoxonase 1 gene expression by an aryl hydrocarbon receptor-dependent mechanism. *Mol Cell Biol* 2004; 24: 5209-22.

[55] Bakhtiari A, Hajian-Tilaki K, Omidvar S, Nasiri Amiri F. Association of lipid peroxidation and antioxidant status with metabolic syndrome in Iranian healthy elderly women. *Biomed Reports* 2017; 7: 331-6.

[56] Lee SG, Wang T, Vance TM, Hubert P, Kim D-O, Koo SI, et al. Validation of Analytical Methods for Plasma Total Antioxidant Capacity by Comparing with Urinary 8-Isoprostane Level. *J Microbiol Biotechnol* 2017; 27: 388-94.

[57] Kontush A, de Faria EC, Chantepie S, Chapman MJ. A normotriglyceridemic, low HDL-cholesterol phenotype is characterised by elevated oxidative stress and HDL particles with attenuated antioxidative activity. *Atherosclerosis* 2005; 182: 277-85.

[58] Thérond P, Bonnefont-Rousselot D, Laureaux C, Vasson M-P, Motta C, Legrand A, et al. Copper Oxidation of in Vitro Dioleolylphosphatidylcholine-Enriched High-Density Lipoproteins: Physicochemical Features and Cholesterol Effluxing Capacity. *Arch Biochem Biophys* 1999; 362: 139-47.

[59] Zhu Y, Huang X, Zhang Y, Wang Y, Liu Y, Sun R, et al. Anthocyanin supplementation improves HDL-associated paraoxonase 1 activity and enhances cholesterol efflux capacity in subjects with hypercholesterolemia. *J Clin Endocrinol Metab* 2014; 99: 561-9.

[60] Furler SM, Gan SK, Poynten AM, Chisholm DJ, Campbell L V., Kriketos AD. Relationship of Adiponectin with Insulin Sensitivity in Humans, Independent of Lipid Availability. *Obesity* 2006; 14: 228-34.

[61] Kim JH, Baik HW, Yoon YS, Joung HJ, Park JS, Park SJ, et al. Measurement of antioxidant capacity using the biological antioxidant potential test and its role as a predictive marker of metabolic syndrome. *Korean J Intern Med* 2014; 29: 31-9.

[62] Shen M, Li K, Jing H, Zheng L. In Vivo Therapeutic Effect of Vaccinium Meridionale Swartz in Ischemia-Reperfusion Induced Male Albino Rats. *J Food Sci* 2018; 83: 221-8.

[63] Wu J, Li Q, Wang X, Yu S, Li L, Wu X, et al. Neuroprotection by curcumin in ischemic brain injury involves the Akt/Nrf2 pathway. *PLoS One* 2013; 8: e59843.

[64] Iwakami S, Misu H, Takeda T, Sugimori M, Matsugo S, Kaneko S, et al. Concentration-dependent dual effects of hydrogen peroxide on insulin signal transduction in H4IIEC hepatocytes. *PLoS One* 2011; 6: e27401.

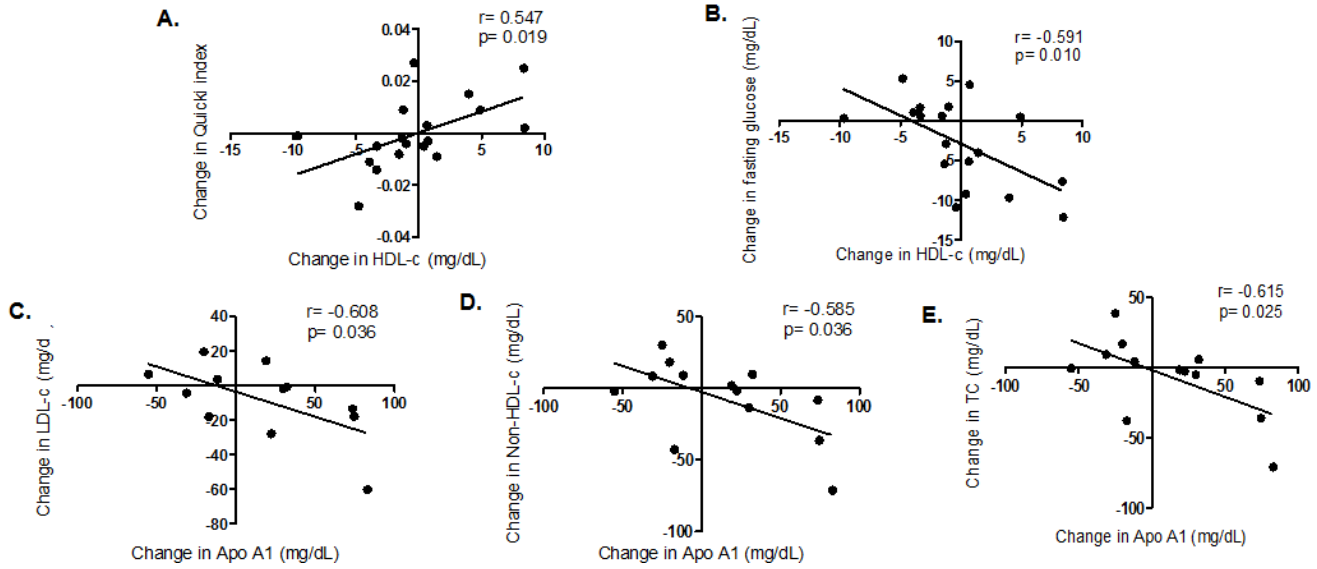
[65] Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, et al. 8-Hydroxy-2-Deoxyguanosine Levels and Cardiovascular Disease: A Systematic Review and Meta-Analysis of the Literature. *Antioxid Redox Signal* 2016; 24: 548-55.

[66] Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Di Stefano G, González-Paramás AM, et al. One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. *J Nutr Biochem* 2014; 25: 289-94.

Table S1. Effects of 4 weeks intervention with agraz or placebo on anthropometric and biochemical characteristics in obese and overweight women with metabolic syndrome[1]

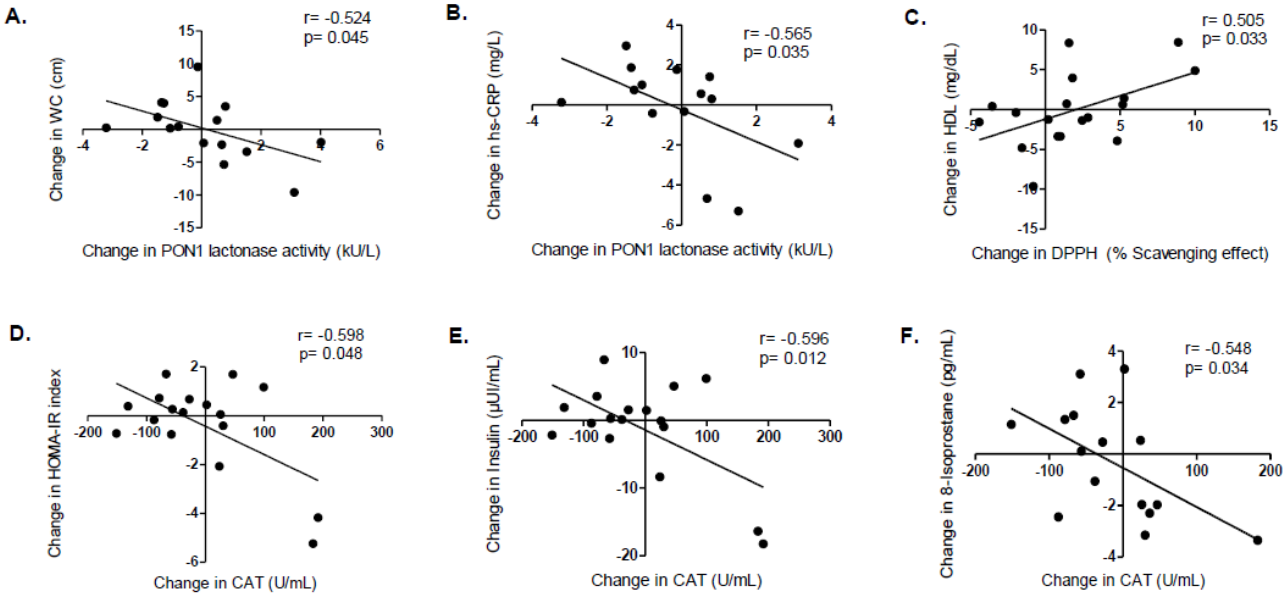
	Placebo						Agraz						Repeated Measures ANOVA p-value		
	Before			After			Before			After			Time	Intervention	Time * Intervention
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD			
Overweight															
Weight (Kg)	69.6	±	7.2	70.3	±	7.5	69.8	±	7.3	69.9	±	7.6	0.346	0.054	0.231
BMI (kg/cm2)	27.9	±	1.2	28.2	±	1.5	27.9	±	1.2	28.0	±	1.3	0.276	0.062	0.272
Waist Circumference (cm)	96.8	±	3.5	97.3	±	4.1	96.5	±	3.9	96.7	±	4.2	0.307	0.414	0.69
SBP (mm Hg)	113	±	13	114	±	12	114	±	13	112	±	12	0.541	0.979	0.284
DBP (mm Hg)	73	±	10	74	±	9	73	±	8	72	±	10	0.275	0.952	0.422
Fasting glucose (mg/dL)	95.0	±	6.1	95.0	±	7.8	96.6	±	7.9	95.2	±	7.6	0.364	0.543	0.268
TC (mg/dL)	232.9	±	38.1	226.9	±	41.9	226.8	±	44.6	223.8	±	46.8	0.182	0.374	0.83
HDL-c (mg/dL)	44.2	±	7.9	43.0	±	6.6	43.1	±	6.3	43.5	±	6.9	0.659	0.613	0.339
Triglycerides (mg/dL)	224.7	±	79.3	227.9	±	92.7	198.1	±	73.3	201.7	±	74.0	0.009	0.752	0.987
TG/HDL ratio ‡	5.2	±	2.0	5.5	±	2.7	4.7	±	2.2	4.8	±	2.0	0.050	0.846	0.983
LDL-c (mg/dL)	143.7	±	32.0	138.4	±	39.1	144.4	±	41.2	139.9	±	44.0	0.781	0.391	0.991
Non HDL-c (mg/dL)	188.7	±	34.5	184.0	±	39.2	183.8	±	43.6	180.3	±	45.2	0.186	0.386	0.927
Obese															
Weight (Kg)	84.5	±	10.3	84.8	±	10.6	85.0	±	10.3	84.7	±	10.4	0.269	0.975	0.104
BMI (kg/cm2) ‡	33.0	±	2.9	33.1	±	3.0	33.1	±	2.9	33.0	±	3.0		0.772 †	
Waist Circumference (cm)	106.8	±	10.5	105.5	±	10.9	106.3	±	10.7	105.3	±	10.7	0.479	0.077	0.87
SBP (mm Hg)	124	±	12	122	±	12	118	±	11	120	±	10	0.012	0.778	0.138
DBP (mm Hg)	80	±	8	79	±	9	77	±	8	78	±	9	0.034	0.763	0.157
Fasting glucose (mg/dL) ‡	93.8	±	6.9	96.3	±	8.3	97.1	±	8.6	96.8	±	8.6	0.120	0.309	0.040
TC (mg/dL)	210.1	±	37.8	213.6	±	40.7	210.8	±	41.2	206.8	±	42.2	0.230	0.952	0.283
HDL-c (mg/dL)	40.4	±	5.3	40.2	±	4.9	39.9	±	6.1	39.6	±	6.2	0.334	0.678	0.926
Triglycerides (mg/dL)	204.2	±	80.8	222.3	±	115.2	200.2	±	80.3	208.7	±	97.6	0.706	0.232	0.676
TG/HDL ratio ‡	5.2	±	2.3	5.8	±	3.3	5.2	±	2.6	5.6	±	3.0	0.515	0.739	0.999
LDL-c (mg/dL)	128.9	±	33.8	129.4	±	29.8	129.9	±	35.0	121.2	±	35.8	0.109	0.302	0.241
Non HDL-c (mg/dL)	169.7	±	36.9	173.5	±	40.9	170.9	±	40.1	167.3	±	42.1	0.311	0.981	0.280

[1]Women were classified according to BMI [28] and ATP-III (4). ‡ Log-transformed variable; † p-value from Friedman test; significance p<0.05 Abbreviations: p25, percentile 25; p75, percentile 75; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; TC, total cholesterol; TG, triglycerides.



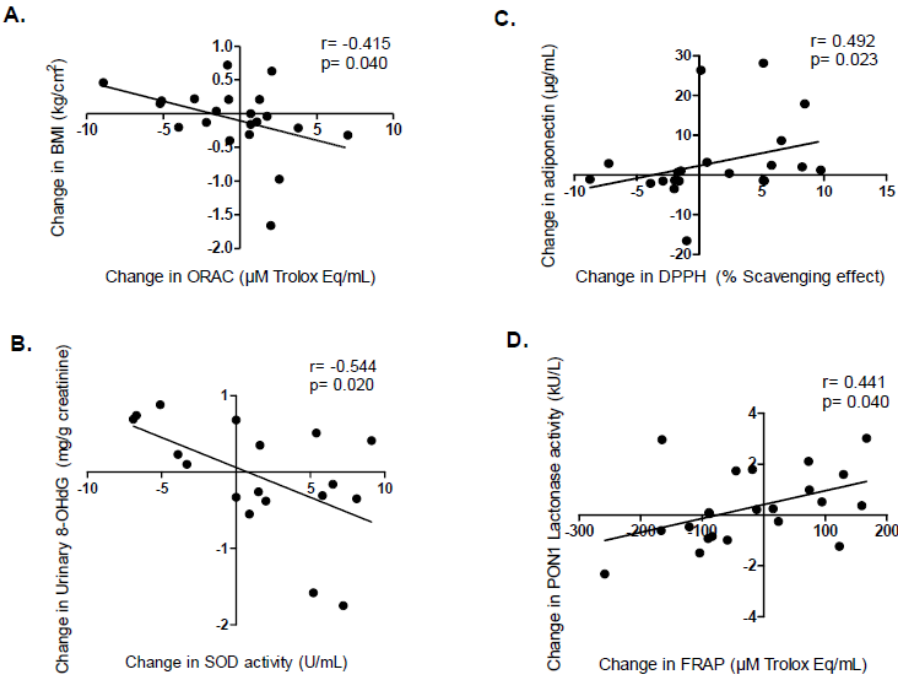
Significance p<0.05. Abbreviations. HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-colesterol; Apo, apolipoprotein; TC, total cholesterol.

Figure S1. Pearson correlations between changes in cardiometabolic factors after agraz consumption, in obese women with MetS



Abbreviations. WC, waist cercumference; PON1, paraoxonase 1; hs-CRP, high sensitivity- C reactive protein; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance index; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; CAT, catalase.

Figure S2. Correlations between changes in antioxidant markers and changes in cardiometabolic factors after agraz consumption, in obese women with MetS. Significance <0.05. A, C and F, Pearson correlations; B, D and E, Spearman correlations.



Abbreviations. BMI, body mass index; ORAC, Oxygen radical absorbance capacity; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; PON1, paraoxonase 1; SOD, superoxide dismutase; 8-OHdG, 8-hydroxy 2 deoxyguanosine; FRAP, ferric reducing ability of plasma.

Figure S3. Correlations between changes in antioxidant markers, cardiometabolic factors and oxidative stress markers, after agraz consumption, in overweight women with MetS. Significance p<0.05; A and B Spearman correlations; C and D Pearson correlations.