Effect of low molecular grape seed proanthocyanidins on blood pressure and lipid homeostasis in cafeteria diet-fed rats.

Pons Z[†], Guerrero L[†#], Margalef M[†], Arola L[†§], Arola-Arnal A[†], Muguerza B[†§]

[†] Department of Biochemistry and Biotechnology, Rovira i Virgili University, Tarragona, Spain.

[#] Department of Research, Nutrition and Innovation, ALPINA S.A, Bogotá, Colombia.

[§] Centre Tecnològic de Nutrició i Salut (CTNS), TECNIO, CEICS, Catalonia, Spain

*Dra. Anna Arola Arnal

Biochemistry and Biotechnology Department, Rovira i Virgili University, C/ Marcel·lí Domingo s/n 43007 Tarragona, Spain.

Telephone: 34 977 55 8630

Fax: 34 977 55 8232

E-mail: anna.arola@urv.cat

Abstract

Cardiovascular disease (CVD) and related pathologies are the leading cause of death worldwide. Fruits and vegetables are known to improve CVD, an effect that has been associated with flavonoid intake. The aim of this study was to simultaneously evaluate the acute effect of a low molecular grape-seed proanthocyanidin extract (LM-GSPE) on two of the main risk factors of CVD, high blood pressure (BP) and dyslipidaemia, using high fat diet-fed rats. Therefore, male Wistar rats that were cafeteria diet fed for 10 weeks were administered 375 mg/kg of body weight of LM-GSPE, and the BP as well as plasmatic and hepatic parameters were determined at 6 h post-administration. The BP and plasmatic and hepatic lipid were decreased 6 h after the LM-GSPE administration. Moreover, the liver lipid peroxidation products decreased after the LM-GSPE treatment, indicating a reduction in oxidative stress. However, hepatic reduced glutathione or plasma angiotensin converting enzyme activity was not altered by the LM-GSPE. In conclusion, grape proanthocyanidins is able to simultaneously reduce more than one risk factor for CVD by decreasing the BP and improving hypertriglyceridaemia at least in part due to an improvement in oxidative stress. These results open up the possibility of using grape proanthocyanidins in functional foods for CVD improvement.

Keywords: Cardiovascular disease; flavonoids; grape proanthocyanidins; hypertension; dyslipidaemia; Oxidative stress

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. A total of 17.3 million people died from CVD in 2008, which represents 30% of the global deaths; and in 2030, approximately 23.3 million people are estimated to die annually from this disease [68]. Two of the major risk factors for CVD, which often occur together, are hypertension (HTN) and dyslipidaemia, which is the elevation of total cholesterol (TC) and/or triglyceride (TG) levels. In fact, 20% of adults in the USA have been diagnosed with HTN and dyslipidaemia, with the frequency increasing to 50% in elderly adults [69]. The molecular mechanisms that explain the relationship of these two risk factors are mainly related to the damage of the endothelial wall. Dyslipidaemia produces endothelial damage [41-43], which in addition to diminishing the anti-atherogenic role of the vascular endothelium [25] and increasing the oxidative stress [10], has a causal role in the molecular mechanisms leading to HTN.

Animals fed a high-fat diet are a good model for studying different risk factors associated with CV events, conditions that cannot be regarded as a single disease and that together comprise what is known as metabolic syndrome (MS), which is defined as a group of interconnected factors that directly increase the risk of CVD [31]. The cafeteria diet is a robust experimental model of a western high sugar and high fat diet extensively used to produce obesity and MS in rats because its palatability induces the animals to increase their energy intake [59; 62]. In fact, the cafeteria diet-fed rats present increased body weight and more abdominal fat and develop hyperinsulinaemia, hyperglycaemia and hepatic steatosis (see review of animal models of MS in [46]). In addition, dyslipidaemia [51] and elevated BP have been reported in these animals [13; 14; 19; 29; 47].

There is evidence that a diet rich in vegetables and fruits decreases the risk of CVD [27; 33; 35], which has been attributed to the phenolic compounds present in plants. Specifically, grapes and wine are known to exert beneficial health effects. The major bioactive compounds in these products are known to be the flavonoids [3], which are the most abundant polyphenols in human diet [60]. Grape seeds, which are a by-product of the grape/wine industry, are one of the richest sources of flavanols and proanthocyanidins (PAs), a class of flavonoids [39], and their beneficial effects have been extensively investigated. Grape seed PAs have been reported by many studies as being bio-active, exhibiting different

beneficial properties on some MS-related parameters and CVD, acting as antioxidants [50], limiting adipogenesis [49], presenting anti-inflammatory properties [37] and acting as insulin-mimetic agents [11]. Moreover, a reduction of the *de novo* synthesis of hepatic lipids, mainly TG, by grape seed PAs has been established [17]. Furthermore, the antihypertensive effect of a low molecular grape-seed proanthocyanidin extract (LM-GSPE) in spontaneously hypertensive rats (SHR) was recently reported by our group [52].

Therefore, the aim of this study was to assess the simultaneous effects of LM-GSPE on two of the major risk factors for CVD, elevated BP and dyslipidaemia, in cafeteria diet-fed rats.

Material and methods

Low-molecular proanthocyanidin-rich grape seed extract

LM-GSPE was obtained from white grape seeds and was kindly provided by *Les Dérives Résiniques et Terpéniques* (Dax, France). Table 1 shows the flavanol and phenolic acid contents of the grape seed extract used in this study (taken from [52]).

Animal experimental procedure

Male Wistar rats Crl:WI (Charles River Laboratories, Barcelona, Spain) that were 8 weeks old and weighed 150-175 g were singly housed in animal quarters at 22ºC with a light/dark period of 12 h. The rats (n=16) were fed standard chow Panlab A04 (Panlab, Barcelona, Spain) and tap water *ad libidum*. After a quarantine period (1 week), the animals were fed standard chow diet or the cafeteria diet consisting of bacon, biscuits with paté, cheese, muffins, carrots, milk with 20% sucrose (w/v) daily and tap water in addition to the standard chow diet. The cafeteria diet was composed of 13.6% fat, 21% carbohydrates, 9% protein, 51.3% water and 5.1% other nutrients [5]. The different diets were maintained until sacrifice.

Ten weeks after the beginning of the experiment, a group of cafeteria diet-fed rats (n=8) were treated with 375 mg/kg LM-GSPE 6 h before being sacrificed, and the remaining animals (n=8) were given water 6 h before being sacrificed. The sacrifice was conducted by

live decapitation without anaesthesia after overnight fasting. The LM-GSPE and water were orally administered by gastric intubation between 9 and 10 am. The volume orally administered to the rats was consistently 1 mL. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded in the rats by the tail-cuff method [8], before and 6 h post-administration. Before the measurement, the rats were kept at 38°C for 10 min to allow the dilatation of blood vessels to increase the level of the heartbeat signal and also to calm the animal, as recommended by the manufacturer. The equipment used in this study, the LE 5001 Non Invasive Blood Pressure Meter (Panlab - Harvard apparatus, Barcelona, Spain), can detect the SBP and DBP. Both the SBP and DBP values were determined by calculating the average of at least seven measurements. The animals were maintained during the measurements in a heater (LE 5610, Panlab - Harvard apparatus, Barcelona, Spain) to maintain the animal in a state of relaxation as well as to retain an external view and minimise environmental noises. To minimise stress-induced variations in BP, all measurements were taken by the same person in the same peaceful environment. After the quarantine period, the BP method was performed during a training period to acclimatise the animals to the procedure. To ensure the accuracy of the results, the animals were subjected to the experimental procedure for 2 weeks prior to testing, enabling them to adapt to the measurement protocol and reducing the rat stress-induced errors during the test.

The animal protocol followed in this study was approved by the Bioethical Committee of Rovira i Virgili University (European Commission Directive 86/609 and Spanish Royal Decree 223/1988).

Plasma and liver lipid determinations

Blood samples from the sacrificed animals were collected in tubes containing the anticoagulant lithium heparin. These samples were centrifuged at 2000 x g for 15 minutes at 4°C to obtain the plasma. In addition, the liver was removed and a lipid total extraction was performed using the method described by Caimari et al. [9] . Both plasma and the tissue were stored at -80°C until their use. Plasmatic and hepatic TC and TG concentrations were determined by a colorimetric enzymatic kit (QCA, Barcelona, Spain).

Thiobarbituric acid reactive substances (TBARS) analysis

The lipid peroxidation products in the liver were measured by a thiobarbituric acid assay [58], modified as previously described [36]. The liver homogenate was mixed with 20% trichloroacetic acid in 0.6 M HCl (1:1, v/v), and the sample tubes were kept on ice for 20 min to precipitate the plasma components and thus avoid any interference. The samples were centrifuged at 1500 x g for 15 min before adding thiobarbituric acid (120 mM in Tris 260 mM, pH 7) to the supernatant in a ratio of 1:5 (v/v). The mixture was subsequently boiled at 97°C for 30 min. Spectrophotometric measurements at 540 nm were conducted at 20°C. The liver TBARS were expressed as nmol TBARS /g tissue protein.

Reduced glutathione (GSH) assay

The GSH levels in the liver were measured by the monochlorobimane fluorometric method [34]. For this analysis, 90 μL of homogenised supernatant from the liver was mixed with monochlorobimane (100 mM; Sigma, Barcelona, Spain) and 10 μL of the catalyst (glutathione S-transferase) solution (1 U/mL), which was obtained from horse liver (Sigma) The levels of GSH were quantified using a fluorometer, the FLx800 Fluorescence Microplate Reader (Bio-Tek-IZASA, Barcelona, Spain) and expressed as μmol/g tissue protein.

Determination of angiotensin converting enzyme (ACE) activity in plasma

The ACE activity in plasma was measured using a fluorometric method, as previously reported [38]. Briefly, plasma aliquots were incubated in triplicate for 15 min at 37ºC with 40 μL of assay buffer containing the ACE substrate (5 mM Hip-His-Leu, 0,1 M sodium tetraborate decahydrate, 300 mM NaCl, pH 8.3; Sigma, Barcelona, Spain). The reaction was quenched by the addition of 0.35 M NaOH. The concentration of the product, His-Leu, was measured fluorimetrically after 30 min of incubation with 2% O-phthaldialdehyde in methanol. The fluorescence measurements were performed at 37°C in an FLx800 Fluorescence Microplate Reader (Bio-Tek-IZASA) with 350 nm excitation and 520 nm emission filters and Gen5TM Data Analysis Software. Black 96-well polystyrene microplates (Thermo Scientific, MERCK, Barcelona, Spain) were used. A calibration curve was constructed by adding

different concentrations of rabbit lung ACE (Sigma, Barcelona, Spain) to each plate. The ACE activity was expressed as mU ACE/mL of plasma.

Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM) and were analysed by the unpaired *Student t*-test for independent samples using IBM SPSS Statistics software (Version 20.0.0). Differences between the averages were considered significant when $p < 0.05$.

Results

Effects of cafeteria diet on BP and plasma lipid levels

The ingestion of a cafeteria diet for 10 weeks resulted in a significant increase in BP compared with the values recorded before the diet administration. The measurements showed increases of 24.75 ± 3.06 and 25.39 ± 3.88 mmHg for the SBP and DBP, respectively, reaching values of 140.1 ± 2.86 mmHg for the SBP and 119.0 ± 2.08 mmHg for the DPB.

At the end of the cafeteria-feeding period, the plasma TC and TG levels were 67.5 ± 4.9 mg/dL and 132.1 ± 9.3 mg/dL, respectively.

Effect of LM-GSPE on BP and lipid levels in cafeteria diet-fed rats

Previous studies have demonstrated that the greatest antihypertensive effect of GSPE in SHR rats was obtained 6 h after the administration of 375 mg/kg LM-GSPE [52]. Therefore, in this study using cafeteria diet-fed rats, a dose of 375 mg/kg LM-GSPE was selected to be administered for 6 h. The administration of LM-GSPE (375 mg/kg) resulted in a decrease in both the SBP (Figure 1A) and DBP (Figure 1B) within 6 h, being significant for the SBP. In contrast, the water administration did not lead to changes in either the SBP or DBP (Figures 1A and 1B). The LM-GSPE administration produced a decrement in both the plasmatic and hepatic TC and TG levels (Figure 2) compared with the cafeteria diet-fed animals receiving water.

Effect of LM-GSPE on the lipid peroxidation products and GSH levels and ACE activity in the cafeteria diet-fed rats

The TBARS levels as the lipid peroxidation products levels and GSH levels and the plasma ACE activity were measured in cafeteria diet-fed rats that were untreated and treated with 375 mg/kg LM-GSPE 6 h after the administration. The livers of the LM-GSPE-treated and untreated animals had very similar GSH levels (Figure 3A). The levels of TBARS were decreased in the livers of the treated cafeteria diet-fed rats (Figure 3B), whereas the plasma ACE activity was similar in the untreated and LM-GSPE-treated rats 6 h after the administration of 375 mg/kg LM-GSPE (Figure 4).

Discussion

Among the modifiable risk factors for CVD, HTN and dyslipidaemia, together with smoking and *diabetes mellitus*, have particular relevance [63]. In addition, patients with one risk factor tend to present other factors, and the probability of occurrence increases as the number of risk factors increases, with the risk being more pronounced as the MS itself is presented [30]. Thus, interventions for all risk factors present and assessing the "global CV risk of the patients" are recommended [21; 23]. However, currently, there is not a pharmacological treatment completely successful for the clustering of CVD risk factors, and the overall approach used is the treatment of each individual component [57]. The experimental model of rats fed a cafeteria diet proposed in this study can be especially useful because, in addition to being induced by the diet, the increased BP is associated with other risk factors of CVD, such as elevated lipid concentrations. Although the measured SBP after 10 weeks of feeding a cafeteria diet was not as high as that reached in SHR [52], the obtained values can be considered to be borderline or a state that could be designated as pre-hypertension, which thus already implies a risk factor.

Regarding the grape PAs effect on the lipid levels in this animal model, the LM-GSPE treatment decreases the plasmatic and hepatic TC levels in animals fed with a cafeteria diet after overnight fasting (22% and 21% decrement, respectively).Other studies have also found a decrease in plasmatic TC after the administration of PAs from grape seeds [15-17; 45; 51]. However, a lack of an effect by grape seed PAs on plasma lipids has been previously

224 reported for these animals [7]. These discrepancies could be due to the different doses of
225 extract used, the time of the administration of the extracts, the different types of cafeteria diet
226 used, the fasting period of the animals and even the use of different animal models [7; 9]. In
227 addition, to the findings for TC, a decrease in the plasmatic and hepatic TG levels after the
228 administration of LM-GSPE was recorded only 6 h after the administration of grape PAs (20
229 and 16% decrement, respectively). In agreement, a liver lipid decrement was previously
230 described in a lipid tolerance test after an acute treatment with a grape seed extract only 3 h
231 after the administration [6]. A repression of genes related to TG synthesis was highlighted as
232 the cause of this quick modulation of the concentration of hepatic TG by the grape seed
233 extract [15; 17; 51] (see the review about the hypolipidaemic effect of PAs and its biochemical
234 mechanisms in [7]).

235 The results of this study also showed that the administration of LM-GSPE exhibited
236 an antihypertensive effect with respect to the animals administered water in this model of pre-
237 hypertension. This antihypertensive effect of PAs has been previously reported in both short-
238 term [12; 40; 53] and long-term studies conducted with SHR [55; 65], once the hypertension
239 has been fully established.

240 One of the mechanisms involved in endothelial damage and the control of BP is
241 oxidative stress. The elevated production of free radicals in the endothelium, as has been
242 described for high lipid circulation, augments the contractibility of the vascular smooth muscle
243 and promotes its proliferation [64], which has been recognised as the central pathology for
244 HTN and atherosclerosis [61]. Moreover, free radicals in the endothelium can directly
245 scavenge NO and avoid NO-dependent vasodilatation. In addition, the oxidative stress also
246 stimulates the production of endothelium-derived vasoconstrictor factors and pro-
247 inflammatory agents [10]. Nevertheless, it is controversial whether the antioxidant properties
248 of polyphenols could explain their health benefits. In fact, an important feature of flavonoids is
249 the changes that occur to these molecules during first-pass metabolism. Moreover,
250 considerable quantities of the ingested PAs reach the large intestine where they are
251 degraded by colonic microbiota, yielding other smaller molecules that are also absorbed into
252 the body [18]. Consequently, the molecular forms that reach the peripheral circulation and
253 tissues are different from those that are present in foods. Therefore, their physiological

properties differ from the original compounds ingested, including their antioxidant activities. Two of the known biomarkers for the global oxidative stress status in the body are the liver concentrations of GSH and TBARS. The latter are the final products of lipidic peroxidation [32; 66]. In contrast, the GSH concentration can be directly related to the concentration of free radicals in the tissue [67]. Although we did not find changes in this antioxidant system in the liver, we describe in the present study a decrement in the hepatic lipid peroxidation products of the rats treated with LM-GSPE, which indicates an improvement in oxidative stress. These results are in concordance with other rat [54] and human [22] studies that revealed a decrease in oxidative stress biomarkers after the administration of PAs.

Finally, as the renin–angiotensin–aldosterone system (RAAS) is a key factor in the maintenance of arterial BP and one of its main components is the ACE, we studied whether the administration of LM-GSPE modified the activity of this enzyme. ACE catalyses the conversion of angiotensin I into the potent vasoconstrictor angiotensin II [62]. In addition, angiotensin II stimulates the cell intake of lipids and their free radical production by promoting the action of the enzyme NADPH oxidase [20]. The ACE inhibitory activity of PAs has been demonstrated both *in vitro* [1; 2; 24; 44] and *in vivo* [48]. However, in this study, we did not find any change in the plasmatic ACE activity 6 h after the administration of LM-GSPE, although these results did not eliminate the participation of this enzyme before this moment or the participation of the RAAS system in the improvement of the BP.

As is well known, lifestyle habits, such as dietary habits or physical activity, have been associated with improvements in CVD, and the regular consumption of vegetables and fruits has been associated with the reduced mortality and risk of CVD [4; 26; 28; 56]. In this context, the study of the beneficial effects of plant-derived compounds, such as PAs, on different risk factors associated with CVD can be very useful because these compounds can be used as functional ingredients, especially when the limits set to begin pharmacological treatment have not been reached yet for the pre-hypertensive state. Nevertheless, the quantity of LM-GSPE necessary to decrease arterial BP in humans should be definitively established when clinical trials are conducted.

Therefore, the results obtained in this study have demonstrated a simultaneous beneficial effect of grape seed PAs on HTN and hepatic TG. In addition, the beneficial effect

of LM-GSPE on hepatic lipids and HTN is mediated, at least in part, by a reduction in oxidative stress. In conclusion, we have described some mechanisms implicated in the cardiovascular protective effect of LM-GSPE, a grape seed extract that could be used as a functional food ingredient for simultaneously controlling different risk factors associated with CVD.

Acknowledgment

This work was supported by grant number AGL 2008–00387/ALI from the Spanish Government and by the Universitat Rovira i Virgili-Banco Santander (2011 LINE-13 and 2011 LINE-12).

References

1. Actis-Goretta L, Ottaviani JI Fraga CG (2006) Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J Agric Food Chem 54:229-34
2. Actis-Goretta L, Ottaviani JI, Keen CL Fraga CG (2003) Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. FEBS Lett 555:597-600
3. Aherne SA & O'Brien NM (2002) Dietary flavonols: Chemistry, food content, and metabolism. Nutrition 18:75-81
4. Ariga T (2004) The antioxidative function, preventive action on disease and utilization of proanthocyanidins. Biofactors 21:197-201
5. Baiges I, Palmfeldt J, Blade C, Gregersen N Arola L (2010) Lipogenesis is decreased by grape seed proanthocyanidins according to liver proteomics of rats fed a high fat diet. Mol Cel Proteomics 9:1499-513
6. Baselga‐Escudero L, Bladé C, Ribas‐Latre A, Casanova E, Salvadó MJ, Arola L Arola‐Arnal A (2012) Grape seed proanthocyanidins repress the hepatic lipid regulators miR‐33 and miR‐122 in rats. Mol Nutr Food Res 56:1636-46
7. Bladé C, Arola L Salvadó MJ (2010) Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms. Mol Nutr Food Res 54:37-59
8. Buñag RD (1973) Validation in awake rats of a tail-cuff method for measuring systolic pressure. J Appl Physiol 34:279-82

9. Caimari A, Del Bas J, Crescenti A Arola L (2013) Low doses of grape seed procyanidins reduce adiposity and improve the plasma lipid profile in hamsters. Int J Obes 37:576-83

10. Campia U, Tesauro M Cardillo C (2012) Human obesity and endothelium‐dependent responsiveness. Br J Pharmacol 165:561-73

11. Castell-Auví A, Cedó L, Pallarès V, Blay MT, Pinent M, Motilva MJ, Garcia-Vallvé S, Pujadas G, Maechler P Ardévol A (2012) Procyanidins modify insulinemia by affecting insulin production and degradation. J Nutr Biochem 23:1565-72

12. Cienfuegos-Jovellanos E, Quiñones MM, Muguerza B, Moulay L, Miguel M Aleixandre A (2009) Antihypertensive effect of a polyphenol-rich cocoa powder industrially processed to preserve the original flavonoids of the cocoa beans. J Agric Food Chem 57:6156-62

13. Coatmellec-Taglioni G, Dausse JP, Giudicelli Y Ribière C (2003) Sexual dimorphism in cafeteria diet-induced hypertension is associated with gender-related difference in renal leptin receptor down-regulation. J Pharmacol Exp Ther 305:362-7

14. Coatmellec-Taglioni G, Dausse JP, Ribière C Giudicelli Y (2000) Hypertension in cafeteria-fed rats: Alterations in renal α2-adrenoceptor subtypes. Am J Hypertens13:529-34

15. Del Bas JM, Ricketts M, Vaqué M Sala Eea, (2009) Dietary procyanidins enhance transcriptional activity of bile acid-activated FXR in vitro and reduce triglyceridemia in vivo in a FXR-dependent manner. Mol Nutr Food Res 53:805–814

16. Del Bas JM, Fernández-Larrea J, Blay M, Ardèvol A, Salvadó MJ, Arola L Bladé C (2005) Grape seed procyanidins improve atherosclerotic risk index and induce liver CYP7A1 and SHP expression in healthy rats. FASEB J 19:479-81

17. Del Bas JM, Ricketts ML, Baiges I, Quesada H, Ardevol A, Salvadó MJ, Pujadas G, Blay M, Arola L Bladé C (2008) Dietary procyanidins lower triglyceride levels signaling through the nuclear receptor small heterodimer partner. Mol Nutr Food Res 52:1172-81

18. Del Rio D, Mateos AMR, Spencer JPE, Massimiliano T, Borges G Crozier A (2012) Dietary (poly) phenolics in human health: Structures, bioavailability and evidence of protective effects against chronic diseases. Antioxid Redox Signaling 18:1818-92

19. du Toit EF, Nabben M Lochner A (2005) A potential role for angiotensin II in obesity induced cardiac hypertrophy and ischaemic/reperfusion injury. Basic Res Cardiol 100:346-54

20. Epstein FH & Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340:115-26

21. Expert Panel on Detection, Evaluation,and Treatment of High Blood Cholesterol in Adults (2001) EXecutive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). Jama 285:2486-97

22. Fraga CG, Actis-Goretta L, Ottaviani JI, Carrasquedo F, Lotito SB, Lazarus S, Schmitz HH Keen CL (2005) Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. Clin Dev Immunol 12:11-7

23. Grundy SM, Bilheimer D, Chait A, Clark LT, Denke M, Havel RJ, Hazzard WR, Hulley SB, Hunninghake DB Kreisberg RA (1993) Summary of the second report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel II). JAMA 269:3015-23

24. Guerrero L, Castillo J, Quiñones M, Garcia-Vallvé S, Arola L, Pujadas G Muguerza B (2012) Inhibition of angiotensin-converting enzyme activity by flavonoids: Structure-activity relationship studies. PloS One 7:e49493

25. Hadi HA & Al Suwaidi J (2007) Endothelial dysfunction in diabetes mellitus 3(6): 853–876. Vasc Health Risk Manag 3:853-76

26. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A Nedeljkovic S (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 155:381-6

27. Hollman P, Hertog M Katan M (1996) Role of dietary flavonoids in protection against cancer and coronary heart disease. Biochem Soc Trans 24:785-9

28. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL Cassidy A (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. Am J Clin Nutr 88:38-50

29. Howitt L, Sandow SL, Grayson TH, Ellis ZE, Morris MJ Murphy TV (2011) Differential effects of diet-induced obesity on BKCa β1-subunit expression and function in rat skeletal muscle arterioles and small cerebral arteries. Am J Physiol-Heart C 301:H29-40

30. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K Pyorala K (2004) Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic european men and women. Arch Intern Med 164:1066-76

31. Huang PL (2009) A comprehensive definition for metabolic syndrome. Dis Model Mech 2:231-7

32. Janero DR (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 9:515-40

33. Joshipura KJ, Hu FB, Manson JAE, Stampfer MJ, Rimm EB, Speizer FE, Colditz G, Ascherio A, Rosner B Spiegelman D (2001) The effect of fruit and vegetable intake on risk for coronary heart disease. Ann Intern Med 134:1106-14

34. Kamencic H, Lyon A, Paterson PG Juurlink BHJ (2000) Monochlorobimane fluorometric method to measure tissue glutathione. Anal Biochem 286:35-7

35. Liu S, Lee IM, Ajani U, Cole SR, Buring JE Manson JAE (2001) Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The physicians' health study. Int J Epidemiol 30:130-5

36. Manso MA, Miguel M, Even J, Hernández R, Aleixandre A López-Fandiño R (2008) Effect of the long-term intake of an egg white hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats. Food Chem 109:361-7

37. Martinez‐Micaelo N, González‐Abuín N, Ardèvol A, Pinent M Blay MT (2012) Procyanidins and inflammation: Molecular targets and health implications. Biofactors 38:257-65

38. Miguel M, Alonso MJ, Salaices M, Aleixandre A López-Fandiño R (2007) Antihypertensive, ACE-inhibitory and vasodilator properties of an egg white hydrolysate: Effect of a simulated intestinal digestion. Food Chem 104:163-8

39. Nakamura Y, Tsuji S Tonogai Y (2003) Analysis of proanthocyanidins in grape seed extracts, health foods and grape seed oils. J Health Sci 49:45-54

402 40. Negishi H, Xu J, Ikeda K, Njelekela M, Nara Y Yamori Y (2004) Black and green tea
403 polyphenols attenuate blood pressure increases in stroke-prone spontaneously
404 hypertensive rats. J Nutr 134:38-42
405 41. Nickenig G (2002) Central role of the AT (1)-receptor in atherosclerosis. J Hum Hypertens
406 16:S26-33
407 42. Nickenig G & Harrison DG (2002) The AT1-type angiotensin receptor in oxidative stress
408 and atherogenesis part I: Oxidative stress and atherogenesis. Circulation 105:393-6
409 43. Nickenig G & Harrison DG (2002) The At1-type angiotensin receptor in oxidative stress
410 and atherogenesis part II: At1 receptor regulation. Circulation 105:530-6
411 44. Ottaviani JI, Actis-Goretta L, Villordo JJ Fraga CG (2006) Procyanidin structure defines
412 the extent and specificity of angiotensin I converting enzyme inhibition. Biochimie
413 88:359-65
414 45. Pajuelo D, Quesada H, Díaz S, Fernández-Iglesias A, Arola-Arnal A, Bladé C, Salvadó J
415 Arola L (2011) Chronic dietary supplementation of proanthocyanidins corrects the
416 mitochondrial dysfunction of brown adipose tissue caused by diet-induced obesity in
417 wistar rats. Br J Nutr 1:1-9
418 46. Panchal SK & Brown L (2010) Rodent models for metabolic syndrome research. J
419 Biomed Biotechnol 2011:351982
420 47. Pasarín M, La Mura V, Gracia-SanTCJ, García-Calderó H, Rodríguez-Vilarrupla A,
421 García-Pagán JC, Bosch J Abraldes JG (2012) Sinusoidal endothelial dysfunction
422 precedes inflammation and fibrosis in a model of NAFLD. PloS One 7:e32785
423 48. Persson IAL, Persson K, Hägg S Andersson RGG (2011) Effects of cocoa extract and
424 dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial
425 cells and healthy volunteers–a nutrigenomics perspective. J Cardiovasc Pharmacol
426 57:44-50
427 49. Pinent M, Blade M, Salvado M, Arola L, Hackl H, Quackenbush J, Trajanoski Z Ardevol A
428 (2005) Grape-seed derived procyanidins interfere with adipogenesis of 3T3-L1 cells at
429 the onset of differentiation. Int J Obes 29:934-41

50. Puiggròs F, Llópiz N, Ardévol A, Bladé C, Arola L Salvadó MJ (2005) Grape seed procyanidins prevent oxidative injury by modulating the expression of antioxidant enzyme systems. J Agric Food Chem 53:6080-6

51. Quesada H, Del Bas J, Pajuelo D, Diaz S, Fernandez-Larrea J, Pinent M, Arola L, Salvado M Blade C (2009) Grape seed proanthocyanidins correct dyslipidemia associated with a high-fat diet in rats and repress genes controlling lipogenesis and VLDL assembling in liver. Int J Obes 33:1007-12

52. Quiñones M, Guerrero L, Suarez M, Pons Z, Aleixandre A, Arola L Muguerza B (2013) Low-molecular procyanidin rich grape seed extract exerts antihypertensive effect in males spontaneously hypertensive rats. Food Res Int 51:587-95

53. Quiñones M, Miguel M, Muguerza B Aleixandre A (2011) Effect of a cocoa polyphenol extract in spontaneously hypertensive rats. Food Funct. 2:649-53

54. Quiñones M, Sánchez D, Muguerza B, Miguel M Aleixandre A (2011) Mechanisms for antihypertensive effect of CocoanOX, a polyphenol-rich cocoa powder, in spontaneously hypertensive rats. Food Res Int 44:1203-8

55. Quiñones M, Sánchez D, Muguerza B, Moulay L, Laghi S, Miguel M Aleixandre A (2010) Long-term intake of CocoanOX attenuates the development of hypertension in spontaneously hypertensive rats. Food Chem 122:1013-9

56. Rasmussen SE, Frederiksen H, Struntze Krogholm K Poulsen L (2005) Dietary proanthocyanidins: Occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. Mol Nutr Food Res 49:159-74

57. Redon J, Cifkova R, Laurent S, Nilsson P, Narkiewicz K, Erdine S Mancia G (2008) The metabolic syndrome in hypertension: European society of hypertension position statement. J Hypertens 26:1891-900

58. Rodriguez-Martinez M & Ruiz-Torres A (1992) Homeostasis between lipid peroxidation and antioxidant enzyme activities in healthy human aging. Mech Ageing Dev 66:213-22

59. Sampey BP, Vanhoose AM, Winfield HM, Freemerman AJ, Muehlbauer MJ, Fueger PT, Newgard CB Makowski L (2011) Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: Comparison to high-fat diet. Obesity 19:1109-17

60. Scalbert A & Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S-85S

61. Schwartz SM, Campbell GR Campbell JH (1986) Replication of smooth muscle cells in vascular disease. Circ Res 58:427-44

62. Sclafani A & Berner CN (1976) Influence of diet palatability on the meal taking behavior of hypothalamic hyperphagic and normal rats. Physiol Behav 16:355-63

63. Smith SC,Jr (2007) Multiple risk factors for cardiovascular disease and diabetes mellitus. Am J Med 120:S3-S11

64. Taniyama Y & Griendling KK (2003) Reactive oxygen species in the vasculature. Hypertension 42:1075-81

65. Thandapilly SJ, LeMaistre JL, Louis XL, Anderson CM, Netticadan T Anderson HD (2012) Vascular and cardiac effects of grape powder in the spontaneously hypertensive rat. Am J Hypertens 25:1070-6

66. Valenzuela A (1991) The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. Life Sci 48:301-9

67. Wang W & Ballatori N (1998) Endogenous glutathione conjugates: Occurrence and biological functions. Pharmacol Rev 50:335-56

68. World Health Organization (WHO) (2013) A global brief on hypertension. Silent killer, global public health crisis. World Health Organization. WHO Press. Available in http://www.who.int/cardiovascular_diseases/publications/global_brief_hypertension/en/index.html. Accessed 1 September 2013.

69. Wong ND, Lopez V, Tang S Williams GR (2006) Prevalence, treatment, and control of combined hypertension and hypercholesterolemia in the united states. Am J Cardiol 98:204-8

485 **Figure Legends**

486 **Figure 1.** Decrease in systolic blood pressure (SBP) (A) and diastolic blood pressure (DBP)
487 (B) in cafeteria diet-fed rats 6 h after the administration of 375 mg/kg LM-GSPE (●) or water
488 (○). The results are expressed as the mean ± standard error (SEM). ** indicates significant
489 differences at $p<0.01$.

490 **Figure 2.** Plasma cholesterol (mg/dL) (A), plasma triglyceride (mg/dL) (B), hepatic cholesterol
491 (mg/g tissue) (C) and hepatic triglyceride (mg/g tissue) (D) levels in cafeteria diet-fed rats 6 h
492 after the administration of 375 mg/kg LM-GSPE (●) or water (○). The results are expressed
493 as the mean ± standard error (SEM).

494 **Figure 3.** Hepatic TBARS (A) and reduced glutathione (GSH) (B) in cafeteria diet-fed rats 6 h
495 after the administration of 375 mg/kg LM-GSPE (●) or water (○). The results are expressed
496 as the mean ± standard error (SEM). ** indicates significant differences at $p<0.01$.

497 **Figure 4.** Plasma angiotensin-converting enzyme activity (ACE) in cafeteria diet-fed rats 6 h
498 after the administration of 375 mg/kg LM-GSPE (●) or water (○). The results are expressed
499 as the mean ± standard error (SEM).