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Extracts Enriched in Different Polyphenolic Families Normalize Increased Cardiac NADPH Oxidase Expression while Having Differential Effects on Insulin Resistance, Hypertension, and Cardiac Hypertrophy in High-Fructose-Fed Rats

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Insulin resistance and oxidative stress act synergistically in the development of cardiovascular complications. The present study compared the efficacy of three polyphenolic extracts in their capacity to prevent hypertension, cardiac hypertrophy, increased production of reactive oxygen species (ROS) by the aorta or the heart, and increased expression of cardiac NAD(P)H oxidase in a model of insulin resistance. Rats were fed a 60%-enriched fructose food and were treated once a day (gavage) for 6 weeks with 10 mL/kg of water only (F group) or the same amount of solution containing a red grape skin polyphenolic extract enriched in anthocyanins (ANT), a grape seed extract enriched in procyanidins and rich in galloylated procyanidins (PRO), or the commercial preparation Vitaflavan (VIT), rich in catechin oligomers. All treatments were administered at the same dose of 21 mg/kg of polyphenols. Our data indicate that (a) the ANT treatment prevented hypertension, cardiac hypertrophy, and production of ROS, (b) the PRO treatment prevented insulin resistance, hypertriglyceridemia, and overproduction of ROS but had only minor effects on hypertension or hypertrophy, while (c) Vitaflavan prevented hypertension, cardiac hypertrophy, and overproduction of ROS. All polyphenolic treatments prevented the increased expression of the p91phox NADPH oxidase subunit. In summary, our study suggest that (a) the pathogeny of cardiac hypertrophy in the fructose-fed rat disease involves both hypertension and hyperproduction of ROS, (b) polyphenolic extracts enriched in different types of polyphenols possess differential effects on insulin resistance, hypertension, and cardiac hypertrophy, and (c) polyphenols modulate the expression of NAD(P)H oxidase.

KEYWORDS: Polyphenols; tannins; anthocyanins; galloylated procyanidins; hypertension; cardiac hypertrophy; oxidative stress; NADPH oxidase; insulin resistance

INTRODUCTION

Feeding rats with fructose has been reported to generate many metabolic features of syndrome X or metabolic syndrome (*1*), which associates glucose intolerance, visceral obesity, hypertension, insulin resistance, and dyslipidemia, and predisposes to type II diabetes development and atherosclerotic cardiovascular disease (*2*−*4*). The involvement of oxidative stress in the murine model was suggested by an increase in lipid peroxides and decrease in free radical defense mechanisms (*5*, *6*). We previously showed that high fructose feeding was associated with a precocious increase in ROS production by aorta, heart, and polynuclear cells as well as moderate hypertension and cardiac hypertrophy after 1 week of diet only. Cardiac hypertrophy was strongly correlated with cardiac superoxide anion production, suggesting that reactive oxygen species (ROS) could be a key event in cardiovascular complications of insulin resistance. Giving some antioxidants molecules could therefore decrease the insulin resistance-related oxidative stress and the subsequent hypertension, as antioxidants such as vitamin E have been shown to improve the free radical defense system and subsequently the insulin sensitivity in fructose fed rats (*5*).

Polyphenols are natural compounds found in most vegetables and fruits and are responsible for their taste and pigmentation.

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They represent a wide family of molecules and are mainly known for their antioxidants properties. The main classes of polyphenols are phenolic acids (i.e., caffeic acid) and flavonoids with the flavanols (catechins, procyanidins) and the anthocyanins.

Catechins and procyanidins are found in grape seeds: (+)-catechin and (−)-epicatechin are the basic units of this group. The procyanidins are formed from the association of several of these monomeric units: 2−5 units for catechin oligomers, over 5 units for catechin polymers. Procyanidins differ in the position and configuration of their monomeric linkages. The structures of procyanidin dimers B1, B2, B3, and B4 are the best known. Galloylated procyanidins (catechins as esters of gallic acid, e.g., epigallocatechin, epicatechin gallate, epigallocatechingallate) give gallic acid after hydrolysis. Anthocyanins are responsible for the color of black grapes and red wines and are predominantly the 3-*O*-glucosides of delphinidin, cyanidin, petunidin, peonidin, and the major compound, malvidin, although coumaric, caffeic, and acetic acid esters have also been detected. They are also known to contribute to the antioxidant and vasodilatation activity of red wines (*7*).

We have previously shown that a red wine total polyphenolic extract was able to prevent hypertension, cardiac hypertrophy, and production of cardiac and aorta ROS, without a significant change in the degree of insulin resistance (*8*). The present study was initiated to compare the efficacy of three polyphenolic extracts, one enriched in anthocyanins and two enriched in procyanidins (one rich in galloylated procyanidins and one rich in catechin oligomers), in preventing the cardiovascular changes associated with insulin resistance. In addition, we explored the possible modulating effect of polyphenols on the ROS-producing enzyme NADPH oxidase.

MATERIALS AND METHODS

Characterization of Polyphenolic Extracts. Three dry phenolic powders were used:

(i) Vitaflavan (VIT), a grape seeds extract reference (# OC-DRT-02−03885) from DRT (Dérivés Résine Terpénique, Dax, France) (called VIT) containing 660 mg/g of total phenolic compounds expressed as gallic acid.

(ii) Anthocyanines (ANT), a grape skin extract reference (# LO2 063 PPR) from La Gardonnenque SCEA, Cuviers-Lascours, France, containing 455 mg/g of total phenolic compounds expressed as gallic acid.

(iii) Procyanidins (PRO), a grape seeds extract reference (#LO2 050 OPC_{40}) from La Gardonnenque SCEA, Cuviers-Lascours, France, containing 720 mg/g of total phenolic compounds expressed as gallic acid.

Phenolic levels of individual compounds in the three extracts were obtained according to the HPLC analysis procedure as follows: gallic acid, (+)-catechin, and (−)-epicatechin were obtained from Aldrich (St. Quentin Fallavier, France). Epigallocatechin, epigallocatechingallate, epicatechingallate, delphinidin, cyanidin-3-glucoside, malvidin-3-glucoside, cyanidin, and peonidin were obtained from Extrasynthése (Genay, France). Procyanidin dimers B1, B2, B3, and B4 were obtained from grape seeds as previously reported (*9*). For stilbenes compounds monomers or oligomers and astilbin, *trans*-piceid was obtained from cell suspension cultures as previously described (*10*). Viniferin and astilbin were obtained from merlot stalks (*11*). *cis*-Resveratrol was isolated from V. vinifiera cell cultures (*12*, *13*).

HPLC analysis of phenolic acids, tannins, and anthocyanines was obtained with UV detection. A Hewlett-Packard model 1090 with three low-pressure pumps and a diode array detector coupled to a Hewlett-Packard Chem Station was used for solvent delivery and detection. A Hewlett-Packard column packed with a Nucleosil 100 C18 (250 × 4 mm, 5 μm particle size) was used for stationary phase with a flow of 0.7 mL/min. The solvents used for separation (*14*) were as follows: solvent A, 50 mmol/L dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% acetonitrile; solvent C, 200 mmol/L, orthophosphoric acid adjusted with ammoniac to pH 1.5. Elution was performed with a gradient previously described, and detection was carried out at 280, 313, 365, and 520 nm (*15*). For stilbenes compounds monomers or oligomers and astilbin, separation and quatification of stilbenes were carried out by HPLC. A Hewlett-Packard model 1090 with three low-pressure pumps and a diode array detector coupled to a Hewlett-Packard Chem Station was used for solvent delivery and detection. A Hewlett-Packard column packed with Nucleosil 100 C18 (250 × 4 mm, 5 μm particle size) thermostated at 30 °C was used as stationary phase with a flow rate of 0.5 mL/min. The solvents used for the separation were as follows: solvent A acetic acid in H_2O, pH 2.4; solvent B 20% phase A with 80% acetonitrile; performed with a gradient previously described (*16*). Detection was carried out at 280, 286, 306, and 321 nm.

Table 1. Phenolics Levels in Grape Extracts (in mg/g)[a]

phenolic compounds (mg/g of powder)	procyanidins PRO	anthocyanines ANT	vitaflavan VIT
catechin	172.66	NF	47.62
epicatechin	182.58	45.32	68.94
dimer B1	90.42	59.11	58.77
dimer B2	252.57	NF	NF
dimer B3	60.57	NF	22.49
dimer B4	NF	NF	250.77
epigallocatechin	86.77	NF	NF
epigallocatechingallate	30.97	NF	NF
epicatechingallate	NF	NF	18.36
gallic acid	NF	8.31	38.97
delphinidin	NF	113.2	NF
cyanidin-3-glucoside	NF	7.92	NF
malvidin-3-glucoside	NF	37.69	NF
cyanidin	NF	3.87	NF
peonidin	NF	8.53	NF
trans-piceid	NF	0.4	NF
astilbin	0.51	NF	0.53
cis-resveratrol	NF	0.51	1.2
viniferin	0.57	2.08	0.34

[a] NF, not found.

The composition of the three extracts is given in **Table 1**. Differences in the phenolic composition appear for skins and seeds grape extracts. The procyanidins extract (PRO) is the richest in catechins monomers, procyanidin dimers (B1 and B2), and galloylated catechins (epigallocatechin, epigallocatechingallate). The anthocyan extract (ANT) is rich in delphinidin and malvidin-3glucoside. The vitaflavan extract (VIT) contains tannins (monomeric catechins and procyanidins dimers) and is richest in dimer B4 and gallic acid.

Animals and Treatments. Sprague−Dawley rats (Iffa-Credo, Labresle, France) weighing 185−220 g, maintained in a 12 h light/dark, temperature- and humidity-controlled environment, were used in this study. After an adaptation period of 1 week, they were subdivided into five groups of homogeneous weight of nine animals each (three rats per cage): a control group (C), a fructose-enriched diet group (F), an anthocyan-treated fructose-fed group (FANT), a procyanidin and galloylated procyanidins-treated fructose-fed group (FPRO), and a vitaflavan-treated fructose-fed (FVITA) group. Rats were allowed free access to water and food. Control rats were fed with standard chow containing 60% vegetable starch, 11% fat, and 29% protein (UAR company, France), whereas fructose-fed rats received a diet containing 66% fructose, 22% proteins, and 12% fat (Harlan Teklad Co., USA). Mineral contents of the two diets were similar. Rats had access to tap water ad libitum. They were daily treated by gavage for 6 weeks. C and F groups received water only. Doses were calculated on the basis of a daily human (70 kg weight) consumption of 0.5 L wine (containing 3000 mg/L of total polyphenols), that is, 21.42 mg/kg of polyphenols. Therefore, the daily dose of each polyphenolic extract was calculated as 21.42 mg/kg of total polyphenols, according to the polyphenolic content of each extract. Therefore, the FANT group received 10 mL/kg of an anthocyan-enriched solution (3.852 g/L, 55.6% polyphenols).

The FPRO group received 10 mL/kg of an extract procyanidin and galloylated procyanidins enriched solution, (3.326 g/L, 64.3% polyphenols), and the FVIT group received 10 mL/kg of a Vitaflavan solution (2.142 g/L, 100% polyphenols).

Metabolic Parameters. Food and water intake were recorded every day, and the weight of animals was recorded twice a week. Tail-cuff pressure blood pressures were assessed twice a week with a Letica Scientific Instrument electrosphygmomanometer (Lc 5002 Storage Pressure Meter) composed in a thermoregulated room containing six restrainers and a microprocessor, after the rats were warmed at 35 °C for 10 min in a Le 5650/6 Heater and Scanner. Blood pressure was measured under conscious conditions. In this method, the reappearance of pulsation on a digital display of the blood pressure cuff is detected by a pressure transducer and is amplified and recorded digitally as the SBP. The average of three pressure readings was recorded for each measurement.

At the end of the treatment period, blood was collected on heparin-coated tubes, and the thoracic aorta was immediately removed, cleaned of adherent fat, washed in an ice-cold bicarbonate buffer, and kept at 4 °C until measurement of superoxide anion production. The heart was removed and weighed for the calculation of heart to body weight ratio, and the left ventricular was used for the detection of superoxide anion production.

All procedures were designed in accordance with French law and institutional guidelines for the care and use of laboratory animals.

Biochemical Analysis and Markers of Oxidative Stress. Plasma insulin was evaluated by radioimmunoassay using the Kit Rat Insulin RIA (ref #RI-13K, Linco). In each subject, the degree of insulin resistance was estimated at the baseline by HOMA according to the method described by Matthews et al. (*17*). In particular, an insulin resistance score HOMA-IR was computed with the formula: plasma glucose (mmol/L) times serum insulin (mU/L) divided by 22.5. Beta cell function was assessed by the HOMA-BCF score: 20 times serum insulin (mU/L) divided by (plasma glucose (mmol/L) − 3.5). Insulin values were expressed in International Units (1 UI = 0.04167 mg).

Determination of plasma glucose, ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase), and urea was performed on final blood sampling with a HITACHI 704 apparatus. Glucose and urea analysis was performed with kits n° 1448668, 1489364, 851124, and 851132 from Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, respectively. HDL (high-density lipoprotein) cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), and phospholipids (PL) were determined using a Konelab automatic plasma analyzer. Non-HDL-cholesterol (non-HDL-C) was calculated as total cholesterol (CT) minus HDL-cholesterol.

Determination of Superoxide Anion Production. Superoxide anion production was evaluated in tissues as previously described (*18*) by chemiluminescence using lucigenin bis *N*-methyl acridinium. Briefly, thoracic aorta or left ventricle (150 mg) was placed in Krebs buffer containing 250 μM of lucigenin, the intensity of luminescence was measured on a luminometer (Wallac LKB 1251, Finland), and the chemoluminescence was measured for 10 s for aorta and 60 s for left ventricle. This concentration of lucigenin was found not to affect $O_2^{\bullet -}$ production when compared to lower concentrations (5−30 μM) (*19*). Results were expressed as mv/g of tissue.

Immunoblotting. Proteins were extracted from frozen left ventricular of control and high fructose rats. Samples were homogenized with the use of an ultra turrax T25 basic (Irka-Werke) in an ice-cold lysis buffer containing 120 mM NaCl, 25 mM KCl, 2 mM $CaCl_2$, 15 mM Tris-HCl pH 7.5, 0.5% Triton, 1 mM PMSF, 0.1 mM DTT, 10 μM leupeptin, and 1 μM pepstatin. Protein concentrations in each samples were determined by a Bio-Rad DC protein microassay using BSA as protein standard. In the experiments for quantitation, proteins (30 μg/lane) from each sample were loaded onto 12% SDS-polyacrylamide gels. Colored molecular weight markers (Amersham Biosciences) were run on each gel. Proteins were separated for 1 h at 120 V at room temperature. The gels were then electroblotted to nitrocellulose membranes (30 min 90 V, 60 min 100 V at 4 °C). The proteins bound to the nitrocellulose membranes were shown via Ponceau red staining (0.1%) of the nitrocellulose blots. Membranes were then saturated in blocking buffer (10% nonfat dried milk in TBS-Tween, 0.1% Tween 20) for 1 h at room temperature to reduce nonspecific binding sites. Membranes were incubated overnight at 4 °C with primary polyclonal antibody against gp91 protein (1/100, Santa Cruz Biotechnology, Santa Cruz, CA) in 5% nonfat dried milk in TBS-Tween. After six washes in TBS-Tween/sodium deoxycholate (2% sodium deoxycholate) (10 min each), blots were then probed with 4000-fold-diluted horseradish peroxidase-conjugated secondary rabbit anti-goat antibody, 1 h at room temperature in the dark. After six washes as detailed above, the immunoreactive bands were detected using enhanced chemiluminescence detection reagents (ECL Plus System, Amersham) according to the manufacturer's instructions and quantified by scanning densitometry (software BIO-profil 1D, Fisher Bioblock).

Table 2. Metabolic Features of the Various Treatment Groups[a]

treatment group	glucose (mM)	insulin (ng/mL)	HOMA: ir
C	7.83 ± 0.32ab	2.42 ± 0.28a	12.56 ± 4.59a
F	8.93 ± 0.49a	4.00 ± 0.61a	38.69 ± 5.60b
FANT	8.04 ± 0.56ab	3.21 ± 0.66a	26.26 ± 5.95ab
FPRO	7.94 ± 0.58ab	2.27 ± 0.55a	19.48 ± 5.11a
FVIT	7.56 ± 0.36b	3.27 ± 0.55a	26.16 ± 4.41ab

[a] Values are means (±SEM) of plasma glucose, insulin, and index of insulin-resistance (HOMA: ir) determined in control (C), fructose (F), and fructose-anthocyan (FANT), -procyanidins, and galloylated procyanidins (FPRO), and -vitaflavan (FVIT) treated groups ($n = 9$). For each treatment group, means in a column with different letters differ ($p < 0.05$).

Expression of Results and Statistics. Data are shown as the mean ± SEM. Statistical comparisons were performed using the Statgraphics software (Uniware, Paris, France). Groups were compared using the one-way ANOVA. When a significant difference was obtained ($p \leq 0.05$), the Newman−Keuls test was used to compare each pair of means.

RESULTS

General Feature of the Animals. Body weight at sacrifice was not significantly different within the groups, averaging 434 ± 5 g. No difference was observed between groups in plasma ALAT, ASAT, and urea, averaging 20.93 ± 3.38 IU/mL, 96.80 ± 14.34 IU/mL, and 3.55 ± 0.82 g/L, respectively.

Table 2 indicates mean plasma glucose, insulin, and the HOMA index of insulin-resistance (HOMA: ir) in control and experimental animals. Blood glucose was not significantly different between C and F groups, while it was lower in FVIT than in the F group. Plasma insulin was not different within the groups. Determination of HOMA: ir index showed a significant elevation in fructose-fed (F) rats as compared to control (C) rats. Procyanidin and galloylated procyanidins treatment (FPRO group) restored this parameter to a level not significantly different from that of C rats and significantly different from F rat, while values obtained from ANT or VIT-treated rats were intermediate between and not significantly different from C or F animals.

Table 3 indicates plasma lipids from the various treatment groups. As expected, fructose feeding induced an increase in triglycerides, phospholipids, and non-HDL-cholesterol and a decrease of LDL-cholesterol. Triglycerides were normalized by the procyanidin treatment. Phospholipids were not changed. HDL-cholesterol was increased by anthocyanins and procyanidins, but not Vitaflavan, while LDL-cholesterol was unchanged.

Cardiovascular Alterations. Figure 1 represents the evolution of the mean systolic blood pressure during the 6-week study. As previously described, the high-fructose diet (F group) is associated with the development of moderate hypertension, as compared to the C group. Fructose-fed rats had a progressive increase in their blood pressure, which became significantly

Table 3. Plasma Lipids of the Various Treatment Groups[a]

treatment group	TG (g/L)	PL (g/L)	TC (g/L)	HDL-C (g/L)	non-HDL-C (g/L)
C	1.06 ± 0.11a	1.94 ± 0.07a	1.98 ± 0.11a	1.49 ± 0.11a	0.49 ± 0.06a
F	3.38 ± 0.62b	2.47 ± 0.18b	2.05 ± 0.09a	0.84 ± 0.14b	1.21 ± 0.18b
FANT	2.62 ± 0.44ab	2.57 ± 0.15b	2.37 ± 0.13b	1.22 ± 0.16a	1.14 ± 0.11b
FPRO	1.68 ± 0.29a	2.14 ± 0.22b	2.27 ± 0.22b	1.26 ± 0.10a	1.00 ± 0.19b
FVIT	3.82 ± 0.78b	2.81 ± 0.13b	2.25 ± 0.12ab	0.81 ± 0.15b	1.44 ± 0.18b

[a] Plasma triglycerides (TG), phospholipids (PL), total cholesterol (TC), HDL-cholesterol (HDL-C), and non HDL-cholesterol (non-HDL-C) of rats in control (C), fructose (F), fructose+anthocyanins (FANT), fructose+procyanidins, and galloylated procyanidins (FPRO), and fructose+Vitaflavan (FVIT) treated groups ($n = 9$). For each treatment group, means in a column with different letters differ ($p \leq 0.05$).

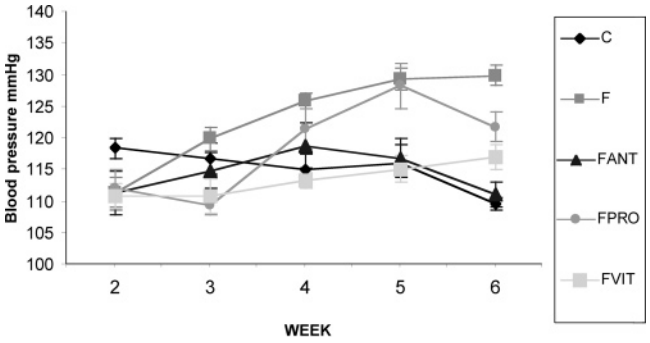

Figure 1. Evolution of systolic blood pressure in rats from control (C), fructose (F), and fructose-anthocyanin (FANT), -procyanidins, and galloylated procyanidins (FPRO), and -vitaflavan (FVIT) treated groups ($n = 9$) during the 6-week study. As compared to C animals, blood pressure (BP) was significantly higher ($p \leq 0.05$) in the untreated fructose-fed (F group) from week 3 to week 6, not significantly different in FANT and FVIT from week 4 to week 6, and higher in FPRO animals at weeks 4, 5, and 6.

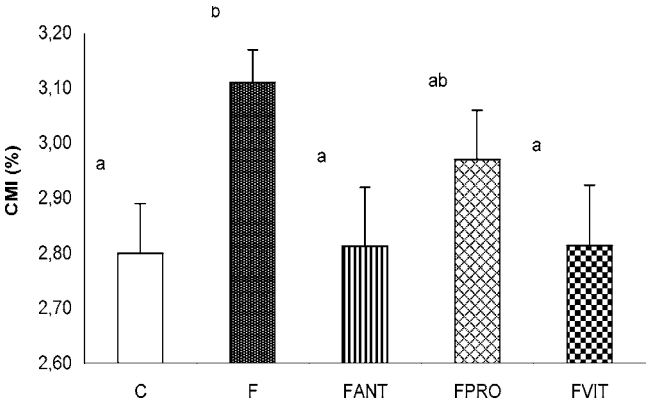

Figure 2. Cardiac mass index (means ± SEM) of rats in control (C), fructose (F), and fructose-anthocyanin (FANT), -procyanidins, and galloylated procyanidins (FPRO), and -vitaflavan (FVIT) treated groups ($n = 9$) during the 6-week study. For each treatment group, means in a column with different superscripts differ ($p \leq 0.05$).

different between the third and the fourth weeks of the study. While anthocyans and vitaflavan prevented the development of hypertension, the FPRO group had a significantly lower blood pressure than the F group on the sixth week only. As depicted in **Figure 2**, rats fed with fructose developed a significant cardiac hypertrophy, as demonstrated by the increase in heart weight index when compared to C rats. Treatments with anthocyanins (FANT) or vitafavan (FVIT) prevented the development of cardiac hypertrophy, while the FPRO group heart weight index was intermediate between and not significantly different from C or F groups.

NADPH Activity and Expression in Left Ventricule. The production of superoxide anion in the heart or the aorta was higher in the F than in the C groups. The various polyphenolic treatments normalized the production of superoxide anion in the heart and significantly reduced it in the aorta. The heart weight index was positively ($r2 = 0.532$, $p < 0.0008$) correlated with the cardiac superoxide anion production (**Figure 3**).

To further examine the effects of the polyphenolic extract on the synthesis of the gp91 NAD(P)H subunit, we examined its protein expression in the heart. **Figure 4a** shows the effects of the various treatments on the relative gp91phox amounts determined from Western blotting analysis after densitometric quantification of the respective bands recorded in the fluorogram, while **Figure 4b** illustrates a representative experiment. As shown, the expression of gp91 phox was markedly increased (about 4.5-fold) in left ventricles from high fructose-fed rats and normalized by treatments with the three polyphenolic extracts.

DISCUSSION

The aim of the present study was to explore the effect of purified polyphenolic extracts enriched in anthocyanins or procyanidins and galloylated procyanidins, in the latter case with different compositions in the polymers content, on insulin resistance and the cardiovascular changes associated with it. Indeed, we also determined the effects of enriched extracts on hypertension, cardiac hypertrophy, increased production of ROS by the aorta and the heart, and increased expression of cardiac NAD(P)H oxidase in the heart. In the current study, we showed (a) that a red grape skin polyphenolic extract enriched in anthocyanins (ANT) prevented hypertension, cardiac hypertrophy, and production of ROS, (b) that a procyanidin-enriched extract rich in galloylated procyanidins (PRO) prevented insulin resistance and overproduction of ROS but had only minor effects on hypertension or hypertrophy, while (c) another tannin-enriched extract rich in catechin oligomers (VIT) prevented hypertension, cardiac hypertrophy, and overproduction of ROS.

The development of cardiac hypertrophy is a complex phenomenon involving various factors such as high blood pressure, hyperinsulinemia (*20*), and production of reactive oxygen species (ROS) by NAD(P)H oxidase (*21*). Our data suggest that hypertension and ROS production act synergistically to induce cardiac hypertrophy. Indeed, left ventricular hypertrophy was prevented by treatments able to prevent both hypertension and overproduction of ROS (ANT and VIT extracts) but not by the PRO extract (which prevent insulinoresistance but have only a minor effect on hypertension). Our results may also suggest that insulin resistance itself was not a determining factor of cardiac hypertrophy in this model, as the PRO extract was not able to prevent hypertrophy.

It has been previously suggested in different murine models including suprarenal abdominal aortic banding (*22*) or angio-

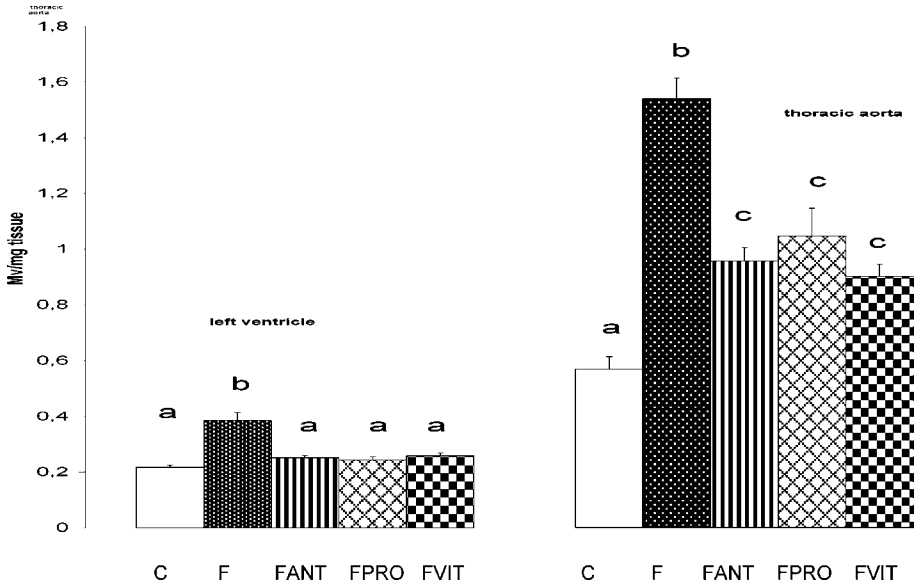

Figure 3. Superoxide anion production Mv/mg tissue from left ventricle and thoracic aorta (mean ± SEM) of rats in control (C), fructose (F), and fructose-anthocyanin (FANT), -procyanidins, and galloylated procyanidins (FPRO), and -vitaflavan (FVIT) treated groups ($n = 9$) during the 6-week study. For each treatment group, means in a column with different superscripts differ ($p \leq 0.05$).

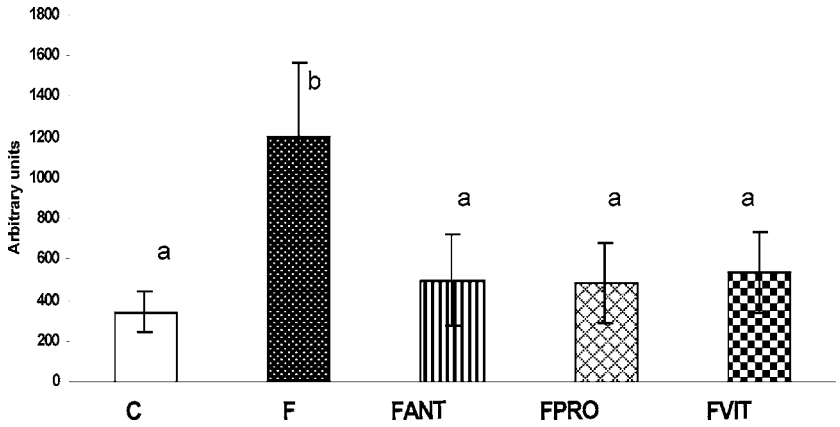

Figure 4. Expression of left ventricle NAD(P)H oxidase (gp91 subunit) (mean ± SEM) from control (C), fructose (F), and fructose-anthocyanin (FANT), -procyanidins, and galloylated procyanidins (FPRO), and -vitaflavan (FVIT) treated groups ($n = 9$). For each treatment group, means in a column with different superscripts differ ($p \leq 0.05$).

tensin II-induced hypertension (*18*, *23*) that ROS production could be involved in left ventricular hypertrophy. The significant correlation observed between cardiac superoxide production and left ventricular mass strongly supports this hypothesis. Moreover, we also showed that wine polyphenols were able to prevent the overproduction of ROS and normalize the expression of NADPH oxidase in vivo. Interestingly, one major stimulant of ROS production by cardiovascular tissues is angiotensin II (*24*). Noticeably, fructose feeding was shown to enhance the expression of AT1a receptor mRNA in fructose-fed mice, whereas expression of either AT1b or AT2 was unaltered. In addition, protein expression of each subunit of NADPH oxidase was increased in fructose-fed mice, whereas the expression was significantly decreased in fructose-fed AT1a KO mice (*25*). Those data suggest that angiotensin may play a major role in the overexpression of NADPH oxidase in fructose-fed rats. Very interestingly, green tea polyphenols were recently shown to lower the overexpression of NADPH oxidase induced by angiotensin II in endothelial cells in vitro (*26*). Various data indicate that activation of NADPH oxidase and production of ROS will induce an autoamplification loop, leading to an increased expression of the enzyme (*27*), while antioxidant molecule inhibitors of the activity of the enzyme will reduce its expression. For example, Park et al. (*21*) showed that apocynin, an inhibitor of NADPH oxidase activity, was able to reduce the increased NADPH mRNA aortic content associated with infusion of aldosterone. It is therefore possible that polyphenols modulate the expression of NADPH oxidase by scavenging ROS. Our current data suggest that polyphenols may downregulate the overproduction of ROS induced by angiotensin II in the fructose-fed rat.

The fact that the three polyphenolic extracts had differential effects on blood pressure, cardiac hypertrophy, and insulin resistance, although all three extracts lowered the production of ROS and the expression of NAD(P)H oxidase, suggests that the different types of polyphenols do not have the same biological activity in vivo. In particular, ANT (rich in delphinidin and malvidin-3-glucoside) and VIT (rich in catechin oligomers) extracts were able to correct high blood pressure and cardiac hypertrophy, while PRO (richer in procyanidins and galloylated procyanidins) normalized insulin sensitivity. Interestingly, PRO also prevented hypertriglyceridemia, a major feature of the insulin resistance syndrome. Recently, green tea supplementation was found to correct insulin resistance and

increased the expression of GLUT IV in fructose-fed rats (*28*). The fact that green tea is particularly rich in galloylated catechins is in accordance with our own data and suggests that galloylated procyanidins have a particular potential to modulate insulin resistance.

In agreement with the improvement of insulin sensitivity induced by procyanidin and, to a lower extent, by anthocyanin extracts, we observed a decrease in triglycerides and an increase in HDL-cholesterol levels. This improvement of the lipid profile is in agreement with data obtained by Yokozawa et al. (*29*) regarding the effect of green tea polyphenols on HDL levels in cholesterol-fed rats or those obtained by De Gaetano et al. (*30*) on the effects of wine (as compared to alcohol) in healthy volunteers. The mechanism of the effect of polyphenols on HDL-cholesterol levels deserves to be further studied. However, insulin-resistance is associated in murine models (*31*) or in clinical studies (*32*, *33*) with impairment of lipoprotein lipase activity resulting in the occurrence of lipid triade characterized by an increase in TG, a decrease in HDL, and an increase in small dense LDL prevalence. It could be postulated that improvement of insulin sensitivity could restore lipoprotein lipase activity and prevent dyslipidemia.

In summary, our study indicates that polyphenolic extracts enriched in different types of polyphenols possess differential effects on insulin resistance, hypertension, cardiac hypertrophy, and hyperlipidemia and that polyphenols modulate the expression of NAD(P)H oxidase.

Future studies will determine the respective role and mechanism of the various polyphenolic families in prevent the cardiovascular complications associated with insulin resistance.

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