Food & Function

PAPER

Cite this: *Food Funct.*, 2016, **7**, 1876

Modifications in nitric oxide and superoxide anion metabolism induced by fructose overload in rat heart are prevented by (−)-epicatechin

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Received 12th January 2016,
Accepted 25th February 2016

DOI: 10.1039/c6fo00048g

www.rsc.org/foodfunction

Fructose overload promotes functional and metabolic derangements in humans and in animal experimental models. Evidence suggests that dietary flavonoids have the ability to prevent/attenuate the development of metabolic diseases. In this work we investigated the effects of (−)-epicatechin on the modifications induced by fructose overload in the rat heart in terms of nitric oxide and superoxide metabolism. Male Sprague Dawley rats received 10% (w/v) fructose in the drinking water for 8 weeks, with or without (−)-epicatechin (20 mg per kg body weight per day) in the rat chow diet. These conditions of fructose overload did not lead to overt manifestations of heart hypertrophy or tissue remodeling. However, biochemical and molecular changes were observed and could represent the onset of functional alterations. (−)-Epicatechin prevented a compromised NO bioavailability and the development of oxidative stress produced by fructose overload essentially acting on superoxide anion metabolism. In this line, the increase in superoxide anion production, the overexpression of NOX2 subunit p47phox and of NOX4, the decrease in superoxide dismutase activity, and the higher oxidized/reduced glutathione ratio installed by fructose overload were absent in the rats receiving (−)-epicatechin. These results support the hypothesis that diets rich in (−)-epicatechin could prevent the onset and progression of heart dysfunctions associated with metabolic alterations.

Introduction

In humans, a high consumption of fructose from sugar-sweetened beverages and foods has been associated with several undesirable health conditions, including increased blood pressure (BP) and a higher risk of coronary heart disease.[1] In experimental animals, fructose overload has been used to promote functional and metabolic derangements, *e.g.* higher triglyceride (TG) levels, adiposity, hypertension, and insulin resistance.[2]

Compromised nitric oxide (NO) bioavailability and oxidative stress are conditions that could be related to the deleterious effects of fructose overconsumption on the cardiovascular system.[3–6] NO is generated by nitric oxide synthase (NOS) isoenzymes, and modulates various aspects of cardiac and vascular physiology such as heart contractility and rate, hypertrophy and apoptosis, cardiac vagal and vessel tone, coronary perfusion and angiogenesis, among others.[7,8] NO bioavailability depends on both: its synthesis from NOS, and its degradation. What is important for NO degradation is its reaction with a superoxide anion. Then, an increased steady state level of superoxide anions will be associated with diminished NO bioavailability.[9–11] A relevant source of superoxide anions is the NADPH oxidase (NOX), with NOX2 and NOX4 being the main isoforms present in heart cells, *i.e.* cardiomyocytes, fibroblasts and endothelial cells.[12,13]

Fruits and vegetables, and food and beverages derived from them, provide the human diet with large amounts of flavonoids,[14] flavanol (−)-epicatechin being one of the most abundant. (−)-Epicatechin is present in cacao, grape, tea, and berries, among other plants.[15] Beyond the association between flavanol consumption and better cardiovascular health,[16–18] in experimental models (−)-epicatechin administration prevented cardiac hypertrophy in NO-deficiency,[19] ventricular remodeling in permanent coronary occlusion,[20] and ischemia-reperfusion injury.[21]

To better understand the mechanisms underlying the cardiovascular benefits of (−)-epicatechin, we studied its protective effects on the heart modifications triggered by fructose in rats in terms of NO and superoxide anion metabolism.

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Materials and methods

(−)-Epicatechin, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH), *N*,*N*′-dimethyl-9,9′-biacridinium dinitrate (lucigenin), bovine serum albumin (BSA), epinephrine, superoxide dismutase (S-7571), glutathione reductase (G3664), reduced glutathione (GSH) (G-4251), oxidized glutathione (GSSG) (G-4376), diphenyleneiodonium, tempol, N^{ω}-nitro-L-arginine, nicotinamide adenine dinucleotide (NADH) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (St Louis, MO, USA). Primary antibodies for $p47^{phox}$ (sc-7660), eNOS (sc-654), p-ERK (sc-7383), ERK1 (sc-93), NOX4 (sc-21860) and β-actin (sc-47778) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The primary antibody for p-eNOS (Ser1177) (#9570) was from Cell Signaling Technology (Boston, MA, USA). [^{14}C] L-arginine was from Perkin Elmer Life and Analytical Sciences (Boston, MA, USA). Fructose was obtained from Droguería Saporiti (Buenos Aires, Argentina). Commercial rat chow was from Gepsa-Feeds (Buenos Aires, Argentina). All other reagents were of the highest commercially available purity.

Animal groups and animal care

All procedures were in agreement with the standards for the care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals, and performed according to the institutional guidelines for animal experimentation approved by Comité Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL protocol approval no. 36/2014), Technical and Science Secretary at the National University of Cuyo School of Medicine, Argentina, where the rats were maintained. Male Sprague-Dawley rats, weighing approximately 100 g at the beginning of the study were housed under conditions of controlled temperature (21–25 °C) and humidity with a 12 h light/dark cycle. The animals were randomly divided into three groups (10 rats per group) receiving: (i) standard rat chow and water *ad libitum* (control group, C); (ii) standard rat chow and 10% (w/v) fructose in the drinking water (fructose group, F); or (iii) standard rat chow added with the amount of (−)-epicatechin necessary to provide 20 mg of (−)-epicatechin per kg body weight per d, and 10% (w/v) fructose in the drinking water (fructose-(−)-epicatechin group, FEC). The food and beverage intakes were recorded twice per week. Systolic BP was measured *via* tail plethysmography in conscious, pre-warmed, slightly restrained rats and recorded on a Grass Model 7 polygraph (Grass Instruments Co., Quincy, MA, USA) at the beginning and one day before the end of the 8-week experimental period. After dietary treatment, and following overnight fasting, the rats were weighted, anesthetized with a mixture of ketamine (50 mg per kg of body weight) and acepromazine (1 mg per kg of body weight), and subjected to abdominal surgery to expose the organs. Blood was collected from the abdominal aorta into heparinized tubes, and immediately centrifuged at 1000*g* for 15 min at 4 °C to obtain plasma. The heart was excised for further determination.

Heart tissue preparation

Heart tissues were minced and homogenized in phosphate buffer, pH 7.4 at 4 °C. After centrifugation of the homogenates at 600*g* for 10 min, the pellet was discarded. A fraction of the supernatant ("total homogenate") was separated and the remaining portion was further centrifuged at 10 000*g*, for 10 min at 4 °C, to obtain a fraction free of mitochondria and peroxisomes ("homogenate"). The protein content was assayed using bovine serum albumin as the standard.[22]

Metabolic parameters

Plasma glucose, total and HDL cholesterol and TGs concentrations were determined using commercial kits (Wiener Lab, Rosario, Argentina and Laboratorios G.T., S.R.L., Rosario, Argentina) following manufacture's guidelines.

Matrix metalloprotease activity

Matrix metalloprotease (MMP) activity was evaluated *via* zymography.[23] Proteins were stained with Coomassie Brilliant Blue and the gelatinolytic activity was visualized as clear areas of lysis in the gel. To confirm that the gelatinolytic activity was due to MMP, the enzyme activity was inhibited by incubating the samples with 10 mM EDTA (final concentration) for 1 h. The values are expressed in arbitrary units.

Nitric oxide synthase (NOS) activity

NOS activity was evaluated in heart slices *via* the formation of [^{14}C]-L-citrulline using [^{14}C]-L-arginine as the substrate.[24] The specific NOS activity was assessed by substracting the citrulline formation measured in the presence of 10^{-4} M N^{ω}-nitro-L-arginine methyl ester (L-NAME). The values are expressed as pmol of citrulline per g tissue per min.

Superoxide anion production

Superoxide anion production was determined in homogenates free of mitochondria and peroxisomes following the chemiluminescent emission of the samples added with 25 μM lucigenin and 40 μM NADH (final concentrations).[25] Chemiluminescence was monitored for 7 min. The samples were added with SOD or tempol to confirm the superoxide anion identity; and with diphenyleneiodonium, oxypurinol, indomethacin, or N^{ω}-nitro-L-arginine to confirm the enzymatic sources of the superoxide anion. Superoxide anion production was calculated as the SOD-inhibitable chemiluminescence. The amount of superoxide production was estimated using the xanthine/xanthine oxidase production of the superoxide anion under the same assaying conditions.[25] The results are expressed as μmol of superoxide anion per min per mg protein.

Superoxide dismutase, glutathione peroxidase, and catalase activities

The superoxide dismutase (SOD) activity was determined in total homogenates following the inhibition of epinephrine oxidation by the superoxide anion at 480 nm.[26] One unit of SOD

was defined as the amount of enzyme necessary to cause a 50% inhibition of the oxidation of epinephrine. The results are expressed as units of SOD per mg of protein. The glutathione peroxidase (GPx) activity was determined in total homogenates in the presence of the glutathione reductase enzyme by following the oxidation of NADPH at 340 nm.[27] One unit of GPx was defined as the amount of enzyme that catalyzes the oxidation of one micromol of NADPH per min; the values are expressed as units of GPx per mg protein. The catalase activity was evaluated in total homogenates following the consumption of added hydrogen peroxide at 240 nm and calculating the pseudo-first order constant of the reaction.[28] The catalase activity was expressed as pmol of catalase per mg protein.

Thiobarbituric reactive substances

Oxidative stress in the heart was evaluated through the presence of thiobarbituric acid reactive substances (TBARS) using a fluorescence method.[29] Aliquots of the total homogenates (50 μl) were added with 100 μl of butylated hydroxytoluene (4% w/v in ethanol) before measurement to prevent non-physiological TBARS formation during sample processing. The results are expressed as μmol of malondialdehyde per mg protein.

Reduced and oxidized glutathione

A portion of approximately 100 mg of heart was homogenized in 1.0 M $HClO_4$ containing 2 mM EDTA. The samples were centrifuged for 20 min at 12 000*g*. The supernatants were filtered through 0.22 μm cellulose acetate membranes and subjected to HPLC separation using a LC-18-DB column (25 cm × 4.6 mm, 5 μm particle size). Reduced (GSH) and oxidized (GSSG) glutathione were eluted with 20 mM sodium phosphate (pH 2.7) and detected electrochemically at 0.800 V.[30]

Western blot analysis

A portion of heart was homogenized in radio-immunoprecipitation assay (RIPA) buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% w/v sodium dodecylsulfate (SDS), 1% w/v Triton X-100, 1% sodium deoxycholate, 5 mM EDTA, 1 mM NaF, 1 mM sodium orthovanadate, and protease inhibitors). Homogenates were centrifuged at 10 000*g* for 10 min at 4 °C, the supernatant was the fraction used for western blot analysis. Aliquots containing 40–50 μg of protein were denatured with the sample buffer, separated on SDS polyacrylamide gels and then transferred to poly-vinylidene fluoride membranes using liquid electroblotting (MiniProtean 3, Bio-Rad) in a transfer solution containing 25 mM Tris, pH 8.3, 190 mM glycine, and 20% (v/v) methanol, at 110 V for 90 min. The membranes were blocked with phosphate buffered saline (PBS) containing 5% (w/v) dry low-fat milk (except for p-eNOS in which the membranes were blocked with 3% BSA (w/v) in PBS), for 2 h at room temperature, and subsequently incubated in the presence of the corresponding primary antibody (1 : 1000 dilution for all of the antibodies except 1 : 500 for p-eNOS^{S1177}), overnight at 4 °C. After incubation for 90 min with the corresponding peroxidase-conjugated secondary antibody (1 : 10 000 dilution) (or for 60 min with biotinylated secondary antibody and another 60 min with biotin streptavidin-HRP-conjugated tertiary antibody (1 : 10 000 dilution) in the case of p-eNOS), complexes were detected using the ECL system (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The films were scanned and densitometric analysis was performed using Image J (National Institute of Health, Bethesda, MD, USA). The sample protein band densities were normalized to β-actin content.

Statistical analyses

The values in the text, tables, and figures are expressed as mean ± SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) using Gaphpad Prism 4 (GraphPad Software Inc., San Diego, CA, USA) followed by Bonferroni's Multiple Test to examine the differences between the group means. *P* values <0.05 were considered to be significant.

Results

Metabolic parameters

At the end of the 8-week treatment, body weight and food and water intake were not significantly different in the 3 groups of rats (Table 1). Systolic BP was similar in the 3 groups at the beginning, but at the end of the study was higher in F with respect to C and FEC (Table 1). To investigate the potential effects of (−)-epicatechin in the prevention and/or amelioration of the metabolic parameters altered by fructose overload, plasma TG, cholesterol, HDL, LDL, and glucose were determined after the 8-week treatment (Table 2). Dyslipidemia developed in F, evidenced by a higher plasma TG and LDL content (p < 0.05 *vs.* C), were absent in FEC. Regarding the cholesterol and HDL content, there were no differences between C and F. However, in FEC, cholesterol was lower and HDL was higher compared to the two other groups (Table 2). The plasma glucose concentration was not affected by the treatments.

Hypertrophy and remodeling

The heart weight was not different among the 3 groups at the end of the study (Table 1). A histological analysis of the

Table 1 Systolic blood pressure, body and heart weight, and food and water consumption in fructose fed rats

Variable	C	F	FEC
Initial body weight (g)	97 ± 8	104 ± 12	99 ± 8
Final body weight (g)	334 ± 12	313 ± 11	325 ± 13
Final heart weight (g)	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Food intake (g d^{-1})	24.9 ± 1.5	19.9 ± 0.8	18.1 ± 1.3
Water intake (ml d^{-1})	45 ± 2	53 ± 4	57 ± 4
Final SBP (mm Hg)	130 ± 4	142 ± 3*	133 ± 3

C, control group; F, fructose group; and FEC, fructose + (−)-epicatechin group. The final values were taken after 8 weeks of treatment. Values are expressed as mean ± SEM (n = 10 per group). *p < 0.05 *vs.* C and FEC.

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Table 2 Metabolic parameters in fructose-fed rats

Variable	C	F	FEC
Triglycerides (mg g^{-1})	46 ± 3	97 ± 7*	75 ± 7
Cholesterol (mg dl^{-1})	46 ± 2	51 ± 3	42 ± 3**
HDL (mg dl^{-1})	20 ± 4	16 ± 5	26 ± 6$^{\#}$
LDL (mg dl^{-1})	26 ± 2	35 ± 3*	18 ± 2‡
Glucose (mg dl^{-1})	88 ± 6	85 ± 11	86 ± 6

C, control group; F, fructose group; and FEC, fructose + (−)-epicatechin group. The values were taken after 8 weeks of treatment, and are expressed as mean ± SEM (n = 10 per group). *$p < 0.05$ *vs.* C and FEC; **$p < 0.05$ *vs.* F; $^{\#}p < 0.05$ *vs.* C and F; $^{‡}p < 0.05$ *vs.* C.

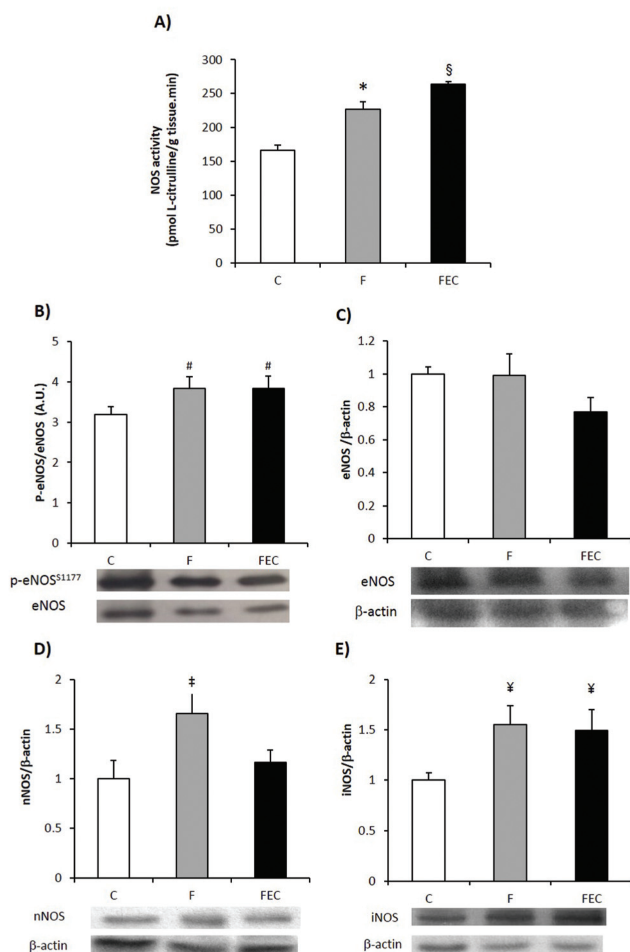

Fig. 1 Effect of dietary (−)-epicatechin on NOS activity, eNOS phosphorylation and expression of eNOS, nNOS and iNOS. (A) NOS activity in heart tissue slices from C, F and FEC with respect to C), (B) eNOS phosphorylation expressed as p-eNOS/eNOS, (C) total eNOS expression, (D) nNOS expression and (E) iNOS expression, in heart homogenates from the C, F and FEC groups, determined using western blot. The determination was done after 8 weeks of the corresponding treatment as described in Materials and methods. Values are expressed as mean ± SEM (n = 9 per group). *$p < 0.05$ with respect to C and FEC groups; $^{§}p < 0.01$; $^{\#}p < 0.05$ *vs.* C; $^{‡}p < 0.05$ F *vs.* C, $^{¥}p < 0.05$ F and FEC *vs.* C.

cardiac tissue showed no differences after the 8-week treatment (data not shown). Markers of cardiac hypertrophy and/or remodeling, as MMP-9 activity and the relative phosphorylation levels of ERK1/2 at Thr188 were not affected by the treatment. MMP-9 activities were 2.5 ± 0.3, 3.4 ± 0.5, and 2.2 ± 0.2 A. U. for the C, F, and FEC groups, respectively. The p-ERK^{thr188}/ERK levels were 1.2 ± 0.1, 1.0 ± 0.1, and 1.0 ± 0.1 for the C, F, and FEC groups, respectively.

Nitric oxide and superoxide anion

Both NO and superoxide anion metabolism in cardiac tissue were evaluated to define NO bioavailability. Regarding NO production, NOS activity plus eNOS, nNOS and iNOS protein expression were measured.

The NOS activity was higher in F with respect to C (37%, $p < 0.01$). FEC showed the highest NOS activity (59% with respect to C, $p < 0.001$; 16% with respect to F, $p < 0.05$) (Fig. 1A). These results were associated with increased levels of eNOS phosphorylation at ser1177 in F and FEC with respect to C (19%, $p < 0.05$) (Fig. 1B) and with no changes in total eNOS expression (Fig. 1C). Regarding nNOS, its expression was higher in F with respect to C (65%, $p < 0.05$) (Fig. 1D). iNOS expression was higher in both, F and FEC, compared to C (60%, $p < 0.05$) (Fig. 1E).

Superoxide anion production was higher in F with respect to C (26%, $p < 0.05$), while FEC showed values even lower than C (39% and 43% lower than C and FE, respectively, $p < 0.05$) (Fig. 2A). In line with these results, $p47^{phox}$ expression was higher in F compared to C and FEC (29% and 34%,

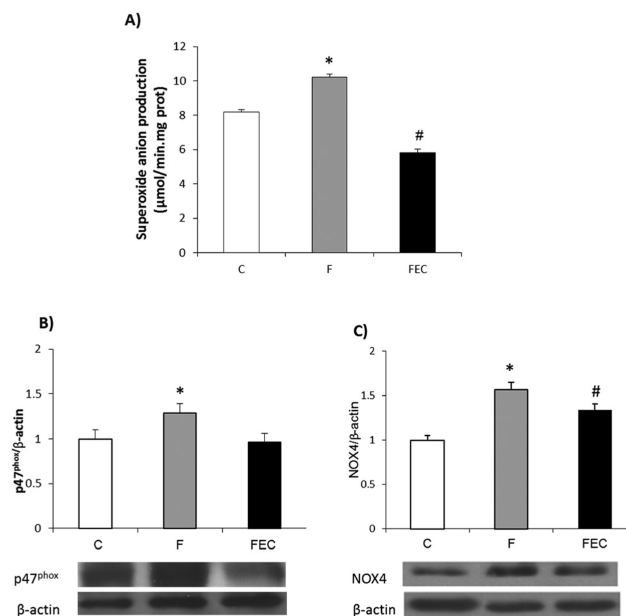

Fig. 2 Effect of dietary (−)-epicatechin in heart NADPH dependent superoxide anion production and the $p47^{phox}$ and NOX4 subunit expressions. (A) Superoxide anion production measured using lucigenin chemiluminescence in heart homogenates; (B) western blot for $p47^{phox}$ and (C) NOX4 in heart homogenates. The β-Actin levels were used as loading controls. The determinations was done after 8 weeks of the corresponding treatment as described in Materials and methods. Values are expressed as mean ± SEM (n = 9 per group). *$p < 0.05$ *vs.* C and FEC; $^{\#}p < 0.05$ *vs.* C.

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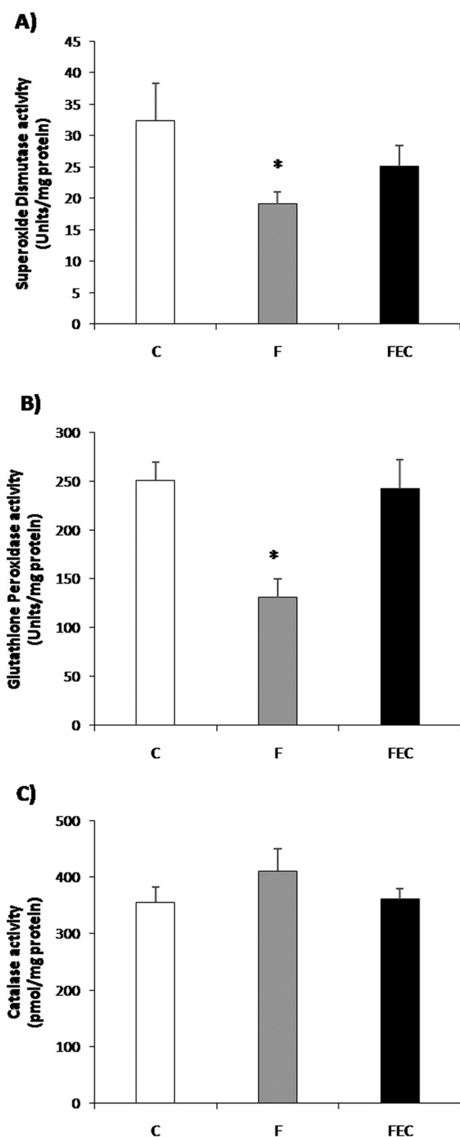

Fig. 3 Effect of dietary (−)-epicatechin on heart total SOD (A), GPx (B) and catalase (C) activities, in total homogenates from the C, F and FEC groups. The determination was done after 8 weeks of the corresponding treatment as described in Materials and methods. Values are expressed as mean ± SEM (n = 8 per group). *$p < 0.05$ *vs.* C and FEC groups.

respectively, $p < 0.05$) (Fig. 2B). In addition, NOX4 protein expression evaluated in F was elevated with respect to C and FEC (Fig. 2C, $p < 0.05$).

To investigate the possible changes in heart enzymes involved in the metabolism of superoxide anion and hydrogen peroxide, the SOD, GPx, and catalase activities were evaluated. The SOD and GPx activities were lower in F with respect to C and FEC (SOD: 41% and 34%, respectively; GPx: 48% and 46%, respectively) (Fig. 3A and B). No significant differences were found for the catalase activity (Fig. 3C).

Heart oxidative stress

To evaluate the oxidative stress in cardiac tissue, we determined both the oxidation levels of glutathione as the

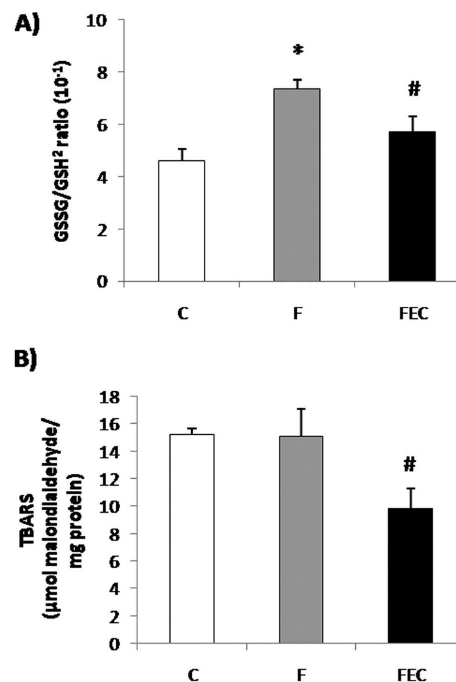

Fig. 4 Effect of dietary (−)-epicatechin on heart oxidative stress markers. (A) $GSSG/GSH^2$ ratio; and (B) TBARS content in the C, F and FEC groups. The determination was done after 8 weeks of the corresponding treatment as described in Materials and methods. Values are expressed as mean ± SEM (n = 10 per group). *$p < 0.05$ with respect to C; $^{\#}p < 0.05$ with respect to C and F.

$GSSG/GSH^2$ ratio and lipid oxidation as the TBARS content. The increase in $GSSG/GSH^2$ observed in F, due to a higher GSSG level and a slightly lower GSH level, was prevented by the administration of (−)-epicatechin ($p < 0.05$ F *vs.* C and *vs.* FEC) (Fig. 4A). The TBARS content was similar in C and F, but was reduced to 50% in FEC (Fig. 4B).

Discussion

Dietary fructose overload led to biochemical and molecular changes associated to NO and superoxide anion metabolism in the heart of rats. These changes could represent the onset of a progression to physiological modifications, and were mostly prevented by (−)-epicatechin administration.

One interesting characteristic of the used model of fructose overload is the absence of heart hypertrophy. In this regard, it should be considered that heart hypertrophy development in fructose overloaded rats depends on the amount of fructose provided and the treatment length. Diets providing high fructose, *i.e.* 60% (w/w) for 8 weeks, led to heart hypertrophy.[31–34] However, lower fructose administration as was used in our study (10% w/v in the beverage) was reported to proceed without heart hypertrophy especially during the initial weeks, even with increases in BP.[35] Longer periods, *i.e.* 8 months with fructose 10% (w/v) have shown to induce heart hypertrophy in rats.[36]

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In terms of physiological variables, (−)-epicatechin supplementation was associated with the prevention of increased systolic BP and the improvement in plasma lipid profile, both, induced by fructose overload, as it was previously reported in this rat model.[37–39]

Concerning biochemical changes, in terms of NO production, NOS activity was higher in the heart of fructose-overloaded respect to control rats independently of (−)-epicatechin administration. Then, the observed positive effects of (−)-epicatechin prevention of high BP development and other metabolic parameters cannot be associated to a direct effect of (−)-epicatechin increasing NO generation. In addition, the overall response in NOS activity seems to be associated to the activation of eNOS and the expression of iNOS, but not to nNOS.

In a context of a similar capacity of NO production with or without (−)-epicatechin supplementation, superoxide anion levels become a key factor to define NO bioavailability.[40] In this work, fructose overload installed a condition of increased superoxide anion production and decreased SOD activity in the heart, which would determine the high superoxide anion steady state levels. In line with these results, mice receiving a high-fructose diet showed increased myocardial superoxide anion production.[41] (−)-Epicatechin supplementation prevented such modifications contributing to the normalization of NO bioavailability. Changes in superoxide anion production in the heart observed in this work were paralleled with modifications in the expression of p47phox, the regulatory subunit of NOX2, and of NOX4. Similar (−)-epicatechin effects lowering superoxide anion levels by modulating NOX subunits, and consequently increasing NO bioavailability were observed in the kidney and aorta of fructose overloaded rats,[38,39] and kidney, aorta and heart of L-NAME-treated[19,42,43] and DOCA-salt rats.[44]

The SOD, GPx, and catalase enzymes are responsible for metabolizing the superoxide anion and its dismutation product, hydrogen peroxide. Lower SOD and GPx activities found in fructose overloaded rats were restored to control values when (−)-epicatechin was present in the diet. This indicates the recovery in the ability of the heart to cope with augmented levels of superoxide anion and potentially, hydrogen peroxide. The increased levels of these oxidants as a consequence of fructose overload led to oxidative stress establishment in the heart that was prevented by (−)-epicatechin, as indicated by the return of the GSSG/GSH^2 to control values. The same effect of (−)-epicatechin on the glutathione redox state was reported in other tissues or cell types, *i.e.* cortical astrocyte cell cultures[45] and human erythrocytes.[46] Moreover, previous results from our group have shown that (−)-epicatechin improves the glutathione redox state in the plasma and heart of L-NAME-treated rats.[42,43]

In terms of (−)-epicatechin as an antioxidant, it could decrease oxidative stress by acting as a direct antioxidant by scavenging free radicals, or as an indirect antioxidant by interacting with specific proteins. The fact that the (−)-epicatechin tissue levels, even after supplementation, are very low as compared to other compounds that can act as physiological antioxidants[47–50] underscores an indirect antioxidant action. This could be either the modulation of the expression of the p47phox NOX subunit and/or a regulation of the SOD and GPx activities. It is also possibly the inhibition of NOX activity by *O*-methylated metabolites of (−)-epicatechin as has been shown to occur in cultured endothelial cells.[51]

In summary, (−)-epicatechin was able to ameliorate fructose induced biochemical modifications in the heart through modulating the expression and/or activity of specific proteins, thus resulting in a controlled oxidant metabolism favoring NO bioavailability. Given the ubiquitous role of NO in maintaining heart physiology, the observed effects of (−)-epicatechin appear as highly relevant. The fact that (−)-epicatechin is commonly present in human diets stresses its actions, as a reasonable reason for the positive cardiovascular effects of a high consumption of fruits and vegetables. In addition, it opens the possibility for proposing the preferential consumption of flavanol-rich foods that can share some of these health benefits.

Abbreviations

BP	Blood pressure
eNOS	Endothelial nitric oxide synthase
ERK	Mitogenic extracellular signal-regulated protein kinase
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
HDL	High density lipoprotein
iNOS	Inducible nitric oxide synthase
LDL	Low density lipoprotein
MMP	Matrix metalloproteases
nNOS	Neuronal nitric oxide synthase
NOS	Nitric oxide synthase
NOX	NADPH oxidase
PBS	Phosphate buffered saline
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TG	Triglycerides

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

Acknowledgements

This work was supported by grants from the Universidad de Buenos Aires (UBACyT 20020120100177, 20020130100760BA, and 20020100300012); Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) PIP0612; and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT BID PICT 2012-0765, and BID PICT 2011-2409).

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