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ORIGINAL CONTRIBUTION

Ellagic acid attenuates high-carbohydrate, high-fat diet-induced metabolic syndrome in rats

Sunil K. Panchal · Leigh Ward · Lindsay Brown

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Abstract
Background Fruits and nuts may prevent or reverse common human health conditions such as obesity, diabetes and hypertension; together, these conditions are referred to as metabolic syndrome, an increasing problem. This study has investigated the responses to ellagic acid, present in many fruits and nuts, in a diet-induced rat model of metabolic syndrome.
Methods Eight- to nine-week-old male Wistar rats were divided into four groups for 16-week feeding with cornstarch diet (*C*), cornstarch diet supplemented with ellagic acid (CE), high-carbohydrate, high-fat diet (*H*) and high-carbohydrate, high-fat diet supplemented with ellagic acid (HE). CE and HE rats were given 0.8 g/kg ellagic acid in food from week 8 to 16 only. At the end of 16 weeks, cardiovascular, hepatic and metabolic parameters along with protein levels of Nrf2, NF-κB and CPT1 in the heart and the liver were characterised.
Results High-carbohydrate, high-fat diet-fed rats developed cardiovascular remodelling, impaired ventricular function, impaired glucose tolerance, non-alcoholic fatty liver disease with increased protein levels of NF-κB and decreased protein levels of Nrf2 and CPT1 in the heart and the liver. Ellagic acid attenuated these diet-induced symptoms of metabolic syndrome with normalisation of protein levels of Nrf2, NF-κB and CPT1.
Conclusions Ellagic acid derived from nuts and fruits such as raspberries and pomegranates may provide a useful dietary supplement to decrease the characteristic changes in metabolism and in cardiac and hepatic structure and function induced by a high-carbohydrate, high-fat diet by suppressing oxidative stress and inflammation.

Keywords Cardiovascular remodelling · Ellagic acid · Metabolic syndrome · Non-alcoholic fatty liver disease · Obesity

S. K. Panchal · L. Brown (✉)
Department of Biological and Physical Sciences,
University of Southern Queensland,
Toowoomba, QLD 4350, Australia
e-mail: Lindsay.Brown@usq.edu.au

S. K. Panchal
School of Biomedical Sciences,
The University of Queensland,
Brisbane, QLD 4072, Australia

L. Ward
School of Chemistry and Molecular Biosciences,
The University of Queensland, Brisbane, QLD 4072, Australia

Introduction

Metabolic syndrome is the presence of multiple risk factors for the development of cardiovascular disease and non-alcoholic fatty liver disease (NAFLD) including hypertension, insulin resistance, obesity and dyslipidaemia [1–4]. The prevalence of metabolic syndrome, cardiovascular disease and NAFLD is increasing throughout the world, in both developed and developing countries [5–7]. Thus, it is important to establish simple therapeutic concepts, such as dietary interventions, that decrease the incidence and symptoms of metabolic syndrome, as well as cardiovascular disease and NAFLD. One of the target strategies in the treatment of NAFLD is to increase the oxidation of fat in the liver, for example, by activation of the mitochondrial regulation of fatty acid oxidation by carnitine palmitoyltransferase 1 (CPT1) [8, 9]. Further targets include the regulators of oxidative stress and inflammation such as Nrf2 and NF-κB, respectively, that lead to cardiac and

hepatic damage [10, 11] associated with cardiovascular disease and NAFLD [12–17]. Normalising the protein levels of these two transcription factors could prevent organ damage in these conditions.

A potential dietary intervention is ellagic acid, a polyphenol found in a wide variety of nuts as well as in fruits such as raspberries, pomegranates, grapes and blackcurrants [18, 19]. Ellagic acid is a dilactone formed from two moieties of gallic acid. Ellagic acid, a radical scavenger [20–22], induced cardioprotection against isoproterenol-induced myocardial infarction [23, 24] and also showed anti-proliferative effects in cancerous cell lines [25]. Further, ellagic acid reduced the damage in a rat model of Crohn's disease, alleviated the oxidative events and returned the levels of pro-inflammatory proteins to basal levels probably through MAPKs and NF-κB signalling pathways [26]. Increased Cu and Zn concentrations in the liver and serum of cholestatic rats were controlled by ellagic acid [27]. Hepatoprotective effects with ellagic acid were measured in vitro and in vivo in models of hepatic damage [28, 29]. We have previously reported that oak ellagitannins, complex esters of ellagic acid with glucose, improved the symptoms of metabolic syndrome in diet-induced obese rats [30].

This study has defined the responses following chronic treatment with ellagic acid on the components of metabolic syndrome and the associated complications including cardiovascular remodelling and fatty liver in a rodent model of diet-induced metabolic syndrome. Rats were fed with either cornstarch or high-carbohydrate, high-fat diets for 16 weeks. These diets were supplemented with ellagic acid for the last 8 weeks of the protocol. At the end of 16 weeks, metabolic parameters and structure and function of the heart and the liver were assessed. Protein levels of CPT1, NF-κB and Nrf2 in the heart and the liver were measured to study potential mechanisms for the action of ellagic acid.

Materials and methods

Rats and diets

All experimental protocols were approved by The University of Queensland Animal Experimentation Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (8–9 weeks old, 337 ± 2 g, $n = 47$) were obtained from The University of Queensland Biological Resources facility. Rats were randomly divided into four groups: cornstarch diet-fed rats (*C*; $n = 12$), cornstarch diet-fed rats supplemented with ellagic acid (CE; 0.8 g/kg food; $n = 11$; MP Biomedicals, Seven Hills, Australia), high-carbohydrate, high-fat diet-fed rats (*H*; $n = 12$) and high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid (HE; 0.8 g/kg food; $n = 12$). *C* and *H* rats were fed with cornstarch and high-carbohydrate, high-fat diets, respectively, for 16 weeks. CE and HE rats were also fed with cornstarch and high-carbohydrate, high-fat diets, respectively, for 16 weeks with the diets supplemented with ellagic acid (0.8 g/kg food) for the last 8 weeks of the protocol. The cornstarch and high-carbohydrate, high-fat diets have been previously described in detail [31–33]. Drinking water for *H* and HE rats was supplemented with 25 % fructose, whereas *C* and CE rats were given drinking water without any additive. All the rats were individually housed in temperature-controlled 12-h light/dark conditions and were given ad libitum access to food and water.

Physiological measurements

Body weight, food and water intakes were measured daily for all rats. Abdominal circumference and body length were measured at the end of the protocol using a standard measuring tape under light anaesthesia with Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg, i.p.; Virbac, Peakhurst, Australia) [31]. Energy intake, body mass index and feed efficiency were calculated as in previous study [31].

Systolic blood pressure measurements

Systolic blood pressure of rats was measured at the end of the protocol under light anaesthesia with Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg, i.p.), using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia) and inflatable tail cuff connected to a MLT844 Piezo-Electric Pressure Transducer (ADInstruments, Sydney, Australia) and PowerLab data acquisition unit (ADInstruments, Sydney, Australia) [31].

Echocardiography

Echocardiographic examinations (Phillips iE33, 12-MHz transducer) were performed in all rats at the end of protocol as previously described [31]. Briefly, rats were anaesthetised using Zoletil (tiletamine 25 mg/kg and zolazepam 25 mg/kg, i.p.; Virbac, Peakhurst, Australia) and Ilium Xylazil (xylazine 15 mg/kg, i.p.; Troy Laboratories, Smithfield, Australia) and positioned in dorsal recumbency. Electrodes attached to the skin overlying the elbows and right stifle facilitated the simultaneous recording of a lead II electrocardiogram [31].

Body composition measurements

Dual-energy X-ray absorptiometric measurements were performed at the end of the protocol using a Norland XR36

DXA instrument (Norland Corp, Fort Atkinson, WI) under anaesthesia with Zoletil (tiletamine 25 mg/kg and zolazepam 25 mg/kg, i.p.) and Ilium Xylazil (xylazine 15 mg/kg, i.p.). Scans were analysed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1; Norland Corp) as previously described [34]. The precision error of lean mass for replicate measurements, with repositioning, was 3.2 %.

Oral glucose tolerance test

At the end of the protocol, rats were deprived of food for 12 h for oral glucose tolerance testing. During this food deprivation period, fructose-supplemented drinking water in *H* and HE groups was replaced with normal drinking water. Oral glucose tolerance tests were performed after determining basal blood glucose concentrations in tail vein blood using Medisense Precision Q.I.D. glucose meters (Abbott Laboratories, Bedford, MA). Rats were given a glucose load of 2 g/kg body weight as 40 % glucose solution via oral gavage and blood glucose concentrations were measured again 30, 60, 90 and 120 min after oral glucose administration [31]. Blood glucose concentrations over the period of 120 min were used to calculate area under the curve.

Terminal experiments

Rats were euthanised with Lethabarb (pentobarbitone sodium, 100 mg/kg, i.p.; Virbac, Peakhurst, Australia). After euthanasia, heparin (200 IU; Sigma-Aldrich Australia, Sydney, Australia) was injected through the right femoral vein. The abdomen was then opened and blood ($\sim$5 mL) was withdrawn from the abdominal aorta and collected into heparinised tubes. Blood was centrifuged at 5,000$\times g$ for 15 min to obtain plasma. Hearts were removed and were used as an isolated Langendorff heart preparation.

Isolated Langendorff heart preparation

The isolated Langendorff heart preparation assessed left ventricular function of the rats in all the groups as in previous studies [31–33]. Hearts isolated from euthanised rats were perfused with modified Krebs–Henseleit bicarbonate buffer bubbled with 95 % O_2–5 % CO_2 and maintained at 35 °C. Isovolumetric ventricular function was measured by inserting a latex balloon catheter into the left ventricle connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a Maclab system (ADInstruments, Sydney, Australia). All left ventricular end-diastolic pressure values were measured during pacing of the heart at 250 beats per min using an electrical stimulator. End-diastolic pressures were obtained from 0 to 30 mmHg for the calculation of diastolic stiffness constant (κ, dimensionless) as described in previous studies [31–33].

Vascular reactivity

Thoracic aortic rings ($\sim$4 mm in length; n = 11–12 from each group) were suspended in an organ bath filled with Tyrode physiological salt solution bubbled with 95 % O_2–5 % CO_2, maintained at 35 °C and allowed to stabilise at a resting tension of $\sim$10 mN. Cumulative concentration–response curves (contraction) were obtained for noradrenaline (Sigma-Aldrich Australia, Sydney, Australia) and cumulative concentration–response curves (relaxation) were obtained for sodium nitroprusside (Sigma-Aldrich Australia, Sydney, Australia) and acetylcholine (Sigma-Aldrich Australia, Sydney, Australia) following submaximal (70 %) contraction to noradrenaline [31].

Organ weights

After isolated heart perfusion studies, hearts (n = 8–9 from each group) were separated into left ventricles (with septum) and right ventricles and weighed. Livers (n = 8–9 from each group) were isolated and weighed. Retroperitoneal, epididymal and omental abdominal fat pads were removed separately and weighed. These organ weights were normalised against the tibial length (48.2 $\pm$ 0.1 mm, n = 35) at the time of organ removal and expressed as mg/mm of tibial length [31].

Histology

Histology of the heart

Hearts were removed from the rats (n = 3 from each group) soon after euthanasia and these hearts were fixed in 10 % neutral buffered formalin for 3 days. The samples were then dehydrated and embedded in paraffin wax. Thin sections (5 μm) of left ventricle were cut and stained with haematoxylin and eosin to study infiltration of inflammatory cells and picrosirius red to study collagen deposition [31].

Histology of the liver

Liver portions were isolated (n = 3) and fixed in 10 % neutral buffered formalin for three days. These tissue samples were dehydrated and then embedded in paraffin wax. Thin sections (5 μm) of these tissues were cut and stained with haematoxylin and eosin for the determination

of inflammatory cell infiltration (20×) and for determining the fat vacuoles (40×) in liver. Liver sections were also stained with Milligan's trichrome stain to determine portal fibrosis (20×) [31].

Plasma biochemistry

Plasma concentrations of total cholesterol and triglycerides were determined using kits and controls supplied by Olympus using an Olympus analyser (AU 400, Tokyo, Japan) [31]. Non-esterified fatty acids (NEFA) in plasma were determined using a commercial kit (Wako, Osaka, Japan) [31]. Plasma activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and the concentrations of albumin, total bilirubin, urea and uric acid were determined using kits and controls supplied by Olympus using an Olympus analyser (AU 400, Tokyo, Japan) [31]. Plasma C-reactive protein (BD Bioscience, Brisbane, Australia) concentrations were measured using commercial kits according to manufacturer-provided standards and protocols.

Regulatory protein levels in the heart and the liver

Heart and liver samples isolated from rats ($n = 3$ from each group) were stored at −80 °C. These samples were homogenised and sonicated after adding cell lysis buffer followed by ultracentrifugation at 100,000×g for 30 min at 4 °C. Supernatants were used to measure protein concentration in each sample by bicinconinic acid method (Thermo Scientific). Supernatants with equal protein amounts (40 μg) from each group were used in Western blot analysis to study the protein levels of CPT-1, Nrf2 (antibodies from Santa Cruz Biotechnology, Santa Cruz, CA), NF-κB (antibody from Cell Signaling Technology, Danvers, MA) and β-actin (antibody from Sigma-Aldrich Corp., St. Louis, MO) in the heart and the liver.

Statistical analysis

Values are presented as mean ± SEM. Results were tested for variance using Bartlett's test and variables that were not normally distributed were transformed (using log 10 function) prior to statistical analyses. All the groups were tested for effects of diet (D), treatment (E) and their interactions ($D \times E$) by two-way ANOVA. When interaction and/or the main effects were significant, means were compared using Newman–Keuls multiple comparison post-test. Mean ellagic acid intakes between CE and HE groups were compared using Student's t-test. $P < 0.05$ was considered significant. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows.

Results

Physiological and metabolic parameters

Table 1 presents the effects of ellagic acid on physiological and metabolic parameters in rats. Mean ellagic acid intake was higher in CE rats (63.4 ± 1.8 mg/kg/day) compared with HE rats (35.6 ± 1.0 mg/kg/day) due to differences in food intake and body weights. Food intake and water intake were decreased in *H* rats compared with *C* rats; ellagic acid reduced food intake in HE rats without changing water intake, whereas it reduced water intake in CE rats without changing food intake. Energy intake was increased due to higher energy content of *H* diet and H rats showed higher feed efficiency than *C* rats. Energy intake was unaffected by ellagic acid supplementation in both CE and HE rats. Feed efficiency was lower in HE rats than in *H* rats. *H* rats gained more weight than *C* rats over the period of 16 weeks. The whole-body fat mass was almost doubled in *H* rats compared to *C* rats without any change in lean mass. The increase in abdominal fat deposition, as indicated by increases in abdominal circumference and body mass index, included the increases in the retroperitoneal, epididymal and omental fat deposits. Body weight and lean mass were lowered with ellagic acid supplementation in HE rats without any change in whole-body fat mass. Body weight and lean mass were unchanged in CE rats. Retroperitoneal and omental fat were normalised in HE rats, while epididymal fat was lowered. Retroperitoneal and omental fat were lowered in CE rats compared to *C* rats without any change in epididymal fat. Total abdominal fat deposition was lowered in both HE and CE rats compared with *H* and *C* rats, respectively. Basal blood glucose concentrations were increased in *H* rats compared with *C* rats. During oral glucose tolerance test, area under the curve was increased for *H* rats compared with *C* rats. During oral glucose tolerance test, ellagic acid supplementation lowered fasting blood glucose concentrations along with area under the curve in HE rats compared with *H* rats. Plasma concentrations of triglycerides, total cholesterol and NEFA were increased in *H* rats compared with *C* rats. Plasma concentrations of triglycerides, total cholesterol and NEFA were decreased in HE rats. Plasma concentrations of total cholesterol were decreased in CE rats without any changes in plasma concentrations of triglycerides and NEFA. Plasma uric acid concentrations were increased, whereas plasma urea concentrations were decreased in *H* rats. Plasma uric acid concentrations were decreased, whereas plasma urea concentrations were increased in HE rats; these parameters were unaffected in CE rats. Plasma C-reactive protein concentrations were higher in *H* rats than in *C* rats and were decreased in HE rats compared with *H* rats.

Table 1 Effects of ellagic acid on physiological and metabolic variables in *C*, CE, *H* and HE rats

Variables	*P* value						
	C	CE	*H*	HE	*D*	*E*	*D* × *E*
Physiological variables							
Initial body weight (g)	338 ± 2	339 ± 2	342 ± 2	336 ± 2	0.77	0.14	0.06
Final body weight (g)	421 ± 4^{c}	427 ± 8^{c}	515 ± 8^{a}	473 ± 11^{b}	<0.0001	0.031	0.005
Food intake (g/day)	30.9 ± 0.7^{a}	31.6 ± 0.5^{a}	23.2 ± 0.6^{b}	21.0 ± 0.4^{c}	<0.0001	0.19	0.014
Water intake (mL/day)	32.1 ± 0.7^{a}	30.1 ± 0.5^{b}	20.2 ± 0.7^{c}	19.0 ± 0.4^{c}	<0.0001	0.01	0.50
Energy intake (kJ/day)	359 ± 8^{b}	355 ± 5^{b}	466 ± 8^{a}	447 ± 6^{a}	<0.0001	0.11	0.29
Feed efficiency (g/kJ)	0.23 ± 0.01^{c}	0.25 ± 0.02^{c}	0.39 ± 0.01^{a}	0.31 ± 0.02^{b}	<0.0001	0.06	0.003
Body mass index (g/cm^{2})	0.63 ± 0.01^{b}	0.66 ± 0.01^{b}	0.72 ± 0.01^{a}	0.72 ± 0.02^{a}	<0.0001	0.27	0.27
Abdominal circumference (cm)	19.6 ± 0.4^{c}	19.6 ± 0.2^{c}	23.3 ± 0.4^{a}	21.3 ± 0.3^{b}	<0.0001	0.005	0.005
Retroperitoneal fat (mg/mm tibial length)	231 ± 13^{b}	146 ± 12^{c}	378 ± 31^{a}	247 ± 13^{b}	<0.0001	<0.0001	0.24
Epididymal fat (mg/mm tibial length)	120 ± 10^{c}	91 ± 7^{c}	240 ± 12^{a}	157 ± 11^{b}	<0.0001	<0.0001	0.012
Omental fat (mg/mm tibial length)	103 ± 9^{b}	67 ± 5^{c}	199 ± 10^{a}	116 ± 7^{b}	<0.0001	<0.0001	0.006
Total abdominal fat (mg/mm tibial length)	454 ± 20^{b}	303 ± 24^{c}	817 ± 32^{a}	520 ± 29^{b}	<0.0001	<0.0001	0.009
Whole-body fat mass (g)	81 ± 7^{b}	113 ± 12^{b}	151 ± 11^{a}	156 ± 16^{a}	<0.0001	0.13	0.27
Whole-body lean mass (g)	307 ± 8^{ab}	298 ± 8^{b}	326 ± 9^{a}	281 ± 4^{b}	0.89	0.001	0.023
Metabolic variables							
Basal blood glucose (mmol/L)	4.1 ± 0.1^{b}	4.3 ± 0.1^{b}	5.1 ± 0.1^{a}	3.9 ± 0.2^{b}	0.03	0.0005	<0.0001
Area under the curve (mmol/L·min)	685 ± 16^{b}	666 ± 14^{b}	776 ± 12^{a}	696 ± 11^{b}	<0.0001	0.0006	0.028
Plasma triglyceride (mmol/L)	0.5 ± 0.1^{b}	0.7 ± 0.1^{b}	1.2 ± 0.1^{a}	0.5 ± 0.1^{b}	0.017	0.017	<0.0001
Plasma total cholesterol (mmol/L)	1.5 ± 0.1^{a}	0.8 ± 0.1^{c}	2.1 ± 0.2^{a}	1.5 ± 0.1^{b}	<0.0001	<0.0001	0.71
Plasma NEFA (mmol/L)	1.3 ± 0.2^{b}	1.4 ± 0.1^{b}	2.9 ± 0.4^{a}	1.7 ± 0.2^{b}	0.0006	0.037	0.014
Plasma uric acid (μmol/L)	36.5 ± 3.3^{b}	40.5 ± 3.9^{b}	58.9 ± 6.2^{a}	44.3 ± 3.8^{b}	0.005	0.24	0.043
Plasma urea (mmol/L)	6.0 ± 0.3^{a}	6.9 ± 0.3^{a}	3.3 ± 0.2^{b}	6.2 ± 0.4^{a}	<0.0001	<0.0001	0.002
Plasma C-reactive protein (μmol/L)	2.22 ± 0.09^{b}	2.35 ± 0.07^{b}	2.69 ± 0.08^{a}	2.40 ± 0.07^{b}	0.002	0.31	0.011

Values are mean ± SEM and $n = 8$–12 for each group. Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$)

C cornstarch diet-fed rats, *CE* cornstarch diet-fed rats supplemented with ellagic acid, *H* high-carbohydrate, high-fat diet-fed rats, *HE* high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid

Cardiovascular structure and function

H rats showed cardiovascular abnormalities as increases in systolic blood pressure, left ventricular internal diameter during diastole and systole (LVIDd and LVIDs, respectively), systolic volume and diastolic stiffness along with increased infiltration of inflammatory cells and increased collagen deposition (Table 2; Fig. 1c, g) compared to *C* rats (Fig. 1a, e). These changes were associated with decreases in left ventricular function including fractional shortening and ejection fraction (Table 2). Ellagic acid reduced systolic blood pressure in both CE and HE rats compared with *C* and *H* rats, respectively (Table 2), whereas it improved structural and functional parameters in HE rats without changing those parameters in CE rats. Left ventricular wet weights (with septum) and right ventricular wet weights were unchanged between the groups (Table 2). Infiltration of inflammatory cells and collagen deposition were reduced in left ventricle of HE rats (Fig. 1d, h).

H rats showed reduced aortic contractile responses to noradrenaline and relaxation responses to sodium nitroprusside and acetylcholine. Ellagic acid improved these responses in HE rats without changing responses in CE rats (Fig. 2a–c).

Hepatic structure and function

Livers from *H* rats showed infiltration of inflammatory cells, steatosis and portal fibrosis (Fig. 3c, g, k). These livers also showed higher wet weights along with increased plasma activities of ALT, AST, ALP and LDH indicating the liver damage caused by *H* diet (Table 2). Ellagic acid supplementation in the diet attenuated the high-carbohydrate, high-fat diet-induced changes in the liver and plasma activities of the enzymes from HE rats (Fig. 3d, h, l; Table 2). Plasma concentrations of albumin and total bilirubin were unchanged between the groups (Table 2).

Table 2 Effects of ellagic acid on cardiovascular and hepatic variables in *C*, CE, *H* and HE rats

Variables	*P* value						
	C	CE	*H*	HE	*D*	*E*	*D* × *E*
Cardiovascular variables							
Systolic blood pressure (mmHg)	129 ± 1^{c}	112 ± 1^{d}	145 ± 1^{a}	133 ± 2^{b}	<0.0001	<0.0001	0.07
LVIDd (mm)	6.62 ± 0.14^{b}	6.28 ± 0.14^{b}	7.39 ± 0.10^{a}	6.66 ± 0.12^{b}	<0.0001	0.0001	0.13
LVIDs (mm)	3.35 ± 0.20^{b}	3.46 ± 0.15^{b}	4.84 ± 0.14^{a}	3.70 ± 0.26^{b}	<0.0001	0.012	0.003
Systolic volume (μL)	42.5 ± 7.4^{b}	45.3 ± 5.5^{b}	109.2 ± 8.3^{a}	59.3 ± 6.3^{b}	<0.0001	0.002	0.0005
Fractional shortening (%)	51.9 ± 1.2^{a}	53.1 ± 2.4^{a}	38.6 ± 1.5^{b}	48.4 ± 2.3^{a}	<0.0001	0.006	0.029
Ejection fraction (%)	86.6 ± 1.8^{a}	82.5 ± 2.0^{a}	71.5 ± 1.3^{b}	81.1 ± 2.7^{a}	0.0002	0.18	0.002
Estimated left ventricular mass (g)	0.67 ± 0.02^{c}	0.66 ± 0.02^{c}	0.91 ± 0.03^{a}	0.79 ± 0.03^{b}	<0.0001	0.015	0.038
Left ventricular + septum wet weight (mg/mm tibial length)	20.5 ± 0.6	21.1 ± 0.7	22.1 ± 0.7	21.0 ± 0.4	0.23	0.68	0.17
Right ventricular wet weight (mg/mm tibial length)	4.63 ± 0.21	4.58 ± 0.16	4.48 ± 0.25	4.56 ± 0.19	0.68	0.94	0.76
Left ventricular diastolic stiffness constant, κ	20.5 ± 0.8^{b}	21.2 ± 1.1^{b}	27.3 ± 1.3^{a}	23.1 ± 0.9^{b}	0.0002	0.10	0.025
Hepatic variables							
Liver wet weight (mg/mm tibial length)	259 ± 11^{b}	270 ± 8^{b}	302 ± 10^{a}	272 ± 5^{b}	0.015	0.29	0.025
Plasma ALT activity (U/L)	35.3 ± 2.7^{b}	34.4 ± 2.1^{b}	57.6 ± 3.5^{a}	34.5 ± 2.9^{b}	0.0003	0.0001	0.0004
Plasma AST activity (U/L)	77 ± 6^{b}	87 ± 4^{b}	105 ± 8^{a}	86 ± 5^{b}	0.007	0.22	0.04
Plasma ALP activity (U/L)	177 ± 14^{b}	174 ± 16^{b}	279 ± 18^{a}	149 ± 18^{b}	0.026	0.0002	0.0004
Plasma LDH activity (U/L)	272 ± 33^{b}	283 ± 23^{b}	417 ± 31^{a}	287 ± 27^{b}	0.014	0.047	0.019
Plasma albumin concentration (g/L)	28.1 ± 0.5	28.6 ± 0.3	28.5 ± 0.5	28.5 ± 0.4	0.73	0.57	0.57
Plasma total bilirubin (μmol/L)	2.3 ± 0.1	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.1	0.75	0.34	0.34

Values are mean ± SEM and $n = 8$–12 for each group. Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$)

C cornstarch diet-fed rats, *CE* cornstarch diet-fed rats supplemented with ellagic acid, *H* high-carbohydrate, high-fat diet-fed rats, *HE* high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid

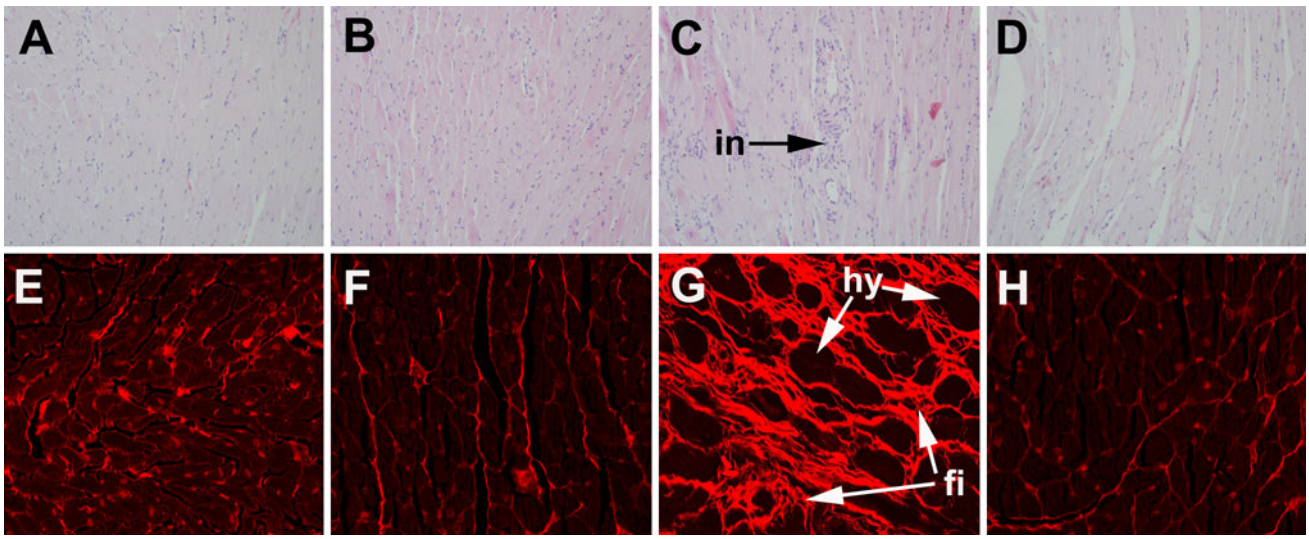
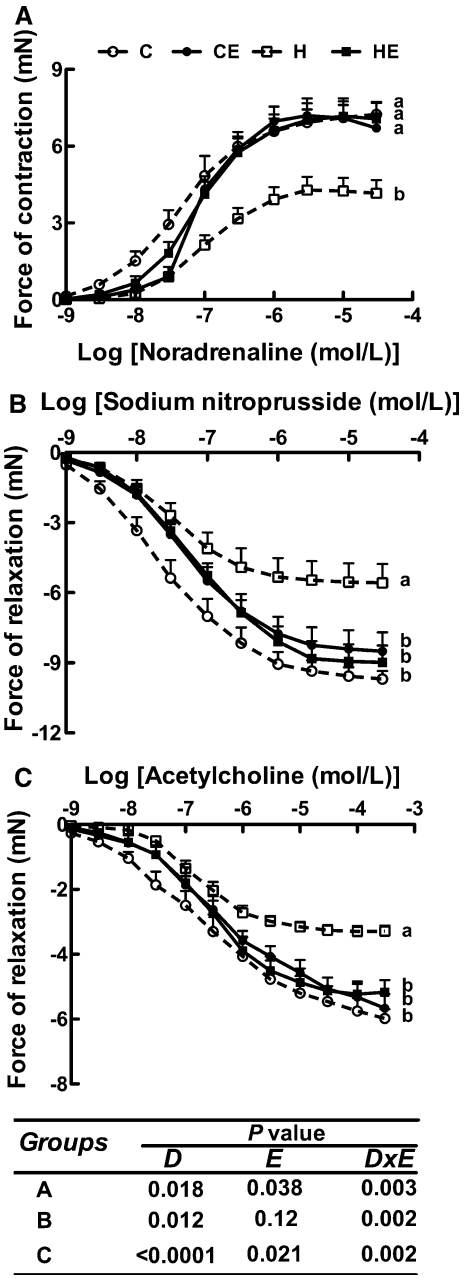


Fig. 1 Effects of ellagic acid supplementation on inflammation and fibrosis in the heart ($n = 3$ per group). Haematoxylin and eosin staining of left ventricle showing infiltration of inflammatory cells (**a**–**d**, inflammatory cells marked as 'in'; ×20) from cornstarch diet-fed rats (**a**), cornstarch diet-fed rats supplemented with ellagic acid (**b**), high-carbohydrate, high-fat diet-fed rats (**c**) and high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid (**d**). Picrosirius red staining of left ventricle showing collagen deposition and hypertrophy (**e**–**h**, fibrosis marked as 'fi' and hypertrophied cardiomyocytes as 'hy'; ×40) from cornstarch diet-fed rats (**e**), cornstarch diet-fed rats supplemented with ellagic acid (**f**), high-carbohydrate, high-fat diet-fed rats (**g**) and high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid (**h**)



Groups	P value		
	D	E	DxE
A	0.018	0.038	0.003
B	0.012	0.12	0.002
C	<0.0001	0.021	0.002

Fig. 2 Effects of ellagic acid supplementation on vascular responses. Noradrenaline-induced contraction (**a**), sodium nitroprusside-induced relaxation (**b**) and acetylcholine-induced relaxation (**c**) in thoracic aortic preparations from *C*, CE, *H* and HE rats. Values are mean ± SEM, $n = 10$–12. End-point means without a common letter differ, $P < 0.05$. *C*, cornstarch diet-fed rats; *CE*, cornstarch diet-fed rats supplemented with ellagic acid; *H*, high-carbohydrate, high-fat diet-fed rats; *HE*, high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid

Regulatory protein levels in the heart and the liver

In the hearts of *H* rats, protein levels of NF-κB were higher than in *C* rats, whereas the protein levels of CPT1 were lower than in *C* rats. These changes were normalised in HE rats. There was no difference in the cardiac protein levels of Nrf2 between the groups (Fig. 4a, c). In the liver from *H* rats, protein levels of Nrf2 and CPT1 were decreased, whereas NF-κB protein levels were enhanced compared with *C* rats. Protein levels of Nrf2 and CPT1 in the liver were increased in both CE and HE rats compared with *C* and *H* rats, respectively, whereas NF-κB protein levels were lowered in both CE and HE rats compared with *C* and *H* rats, respectively (Fig. 4b, d).

Discussion

Metabolic syndrome is the clustering of risk factors for cardiovascular disease, NAFLD and type 2 diabetes. These risk factors, such as hypertension and dyslipidaemia, are also responsible for the increased morbidity and mortality in humans. Thus, it is important to establish biological targets for the reduction of risk factors and treatment of this syndrome. Natural products that are rich in phytochemicals may be effective against this syndrome. Our previous studies with natural products including oak bark extract (rich in ellagitannins) [30], purple carrot juice (rich in anthocyanins) [35], olive leaf extract (rich in oleorupein) [32], chia seeds (rich in α-linolenic acid) [33] and studies with pure phytochemicals from natural products including rutin [36] and piperine [37] in diet-induced obese rats have shown promising results against metabolic syndrome. These studies have also defined the in vivo antioxidant and anti-inflammatory effects of these natural products. Ellagic acid is a phytochemical found in nuts and fruits and is part of the human diet. Thus, we investigated the responses to dietary ellagic acid supplementation on the targets in metabolic syndrome.

Rats fed with *H* diet for 16 weeks presented with the symptoms as well as the associated complications of metabolic syndrome, including central obesity, dyslipidaemia, hypertension and impaired glucose tolerance. The cardiovascular complications included inflammation, cardiac hypertrophy, fibrosis, increased diastolic stiffness, increased ventricular dimensions, decreased ventricular function and decreased vascular responses. The hepatic complications included steatosis, inflammation and portal fibrosis along with increased plasma activities of transaminases. These results have previously been characterised in detail [31]. These changes were accompanied by decreased protein levels of Nrf2 and CPT1 and increased protein levels of NF-κB in the heart and the liver together with increased plasma C-reactive protein concentrations.

Ellagic acid improved hepatic and cardiovascular structure and function, and normalised metabolic parameters such as glucose tolerance, blood lipid components, central obesity and physiological parameters such as body

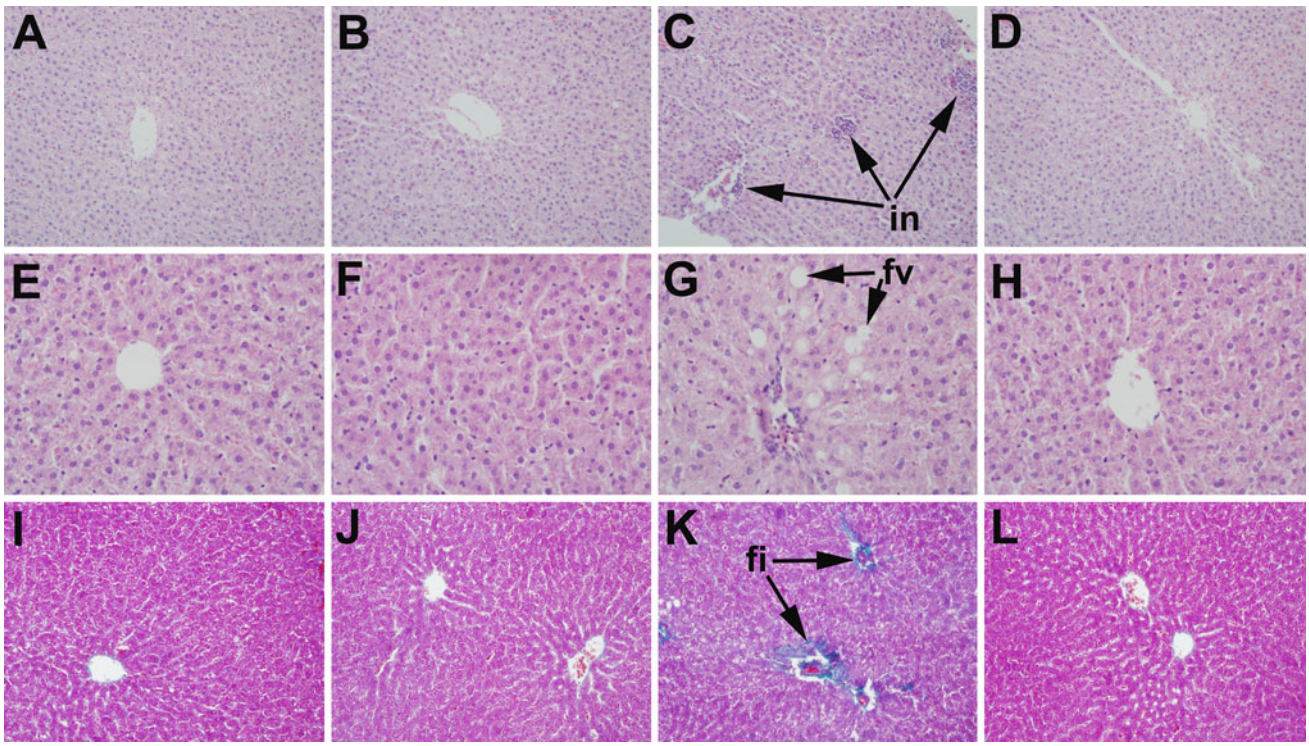


Fig. 3 Effects of ellagic acid supplementation on inflammation, fat deposition and fibrosis in the liver ($n = 3$ per group). Haematoxylin and eosin staining of the liver showing inflammatory cells (**a**–**d**, marked as 'in'; ×20) and enlarged fat vacuoles (**e**–**h**, marked as 'fv'; ×40) from cornstarch diet-fed rats (**a**, **e**), cornstarch diet-fed rats supplemented with ellagic acid (**b**, **f**), high-carbohydrate, high-fat diet-fed rats (**c**, **g**) and high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid (**d**, **h**) rats. Milligan's Trichrome staining of the liver showing fibrosis in the hepatic portal region (**i**–**l**, marked as 'fi'; ×20) from cornstarch diet-fed rats (**i**), cornstarch diet-fed rats supplemented with ellagic acid (**j**), high-carbohydrate, high-fat diet-fed rats (**k**) and high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid (**l**)

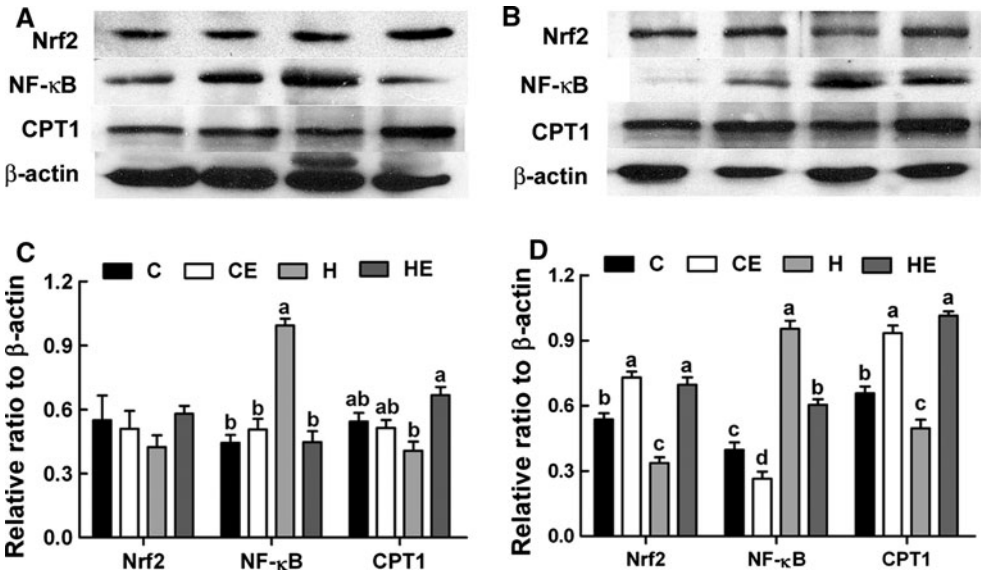


Fig. 4 Effects of ellagic acid supplementation on protein levels of Nrf2, NF-κB and CPT1 in the heart (**a**) and the liver (**b**). For quantitative analysis, the protein levels of these proteins were normalised against the protein levels of β-actin in the heart (**c**) and the liver (**d**). Values are mean ± SEM, $n = 3$. Means without a common letter differ, $P < 0.05$. *C*, cornstarch diet-fed rats; *CE*, cornstarch diet-fed rats supplemented with ellagic acid; *H*, high-carbohydrate, high-fat diet-fed rats; *HE*, high-carbohydrate, high-fat diet-fed rats supplemented with ellagic acid

weight. Reduced abdominal fat deposition without change in whole-body fat indicates lipid redistribution as seen with α-linolenic acid-rich chia seeds [33]. This redistribution of fat was accompanied by reduction in the blood lipid components and hepatic steatosis. The increased CPT1 protein levels in both the heart and the liver indicate that the redistribution of fat was accompanied by increased fatty acid oxidation.

Oxidative stress and inflammation, causing damage to the heart and the liver, were also targeted with ellagic acid

in this study. Nrf2 and NF-κB are important regulators of the oxidative stress and inflammation pathways, respectively [10, 38]. *H* diet decreased the protein levels of Nrf2 in the liver and increased NF-κB protein levels in the heart and the liver. Supplementation with ellagic acid reversed these changes and attenuated oxidative stress and inflammation in the heart and the liver.

The rats were given 0.8 g ellagic acid/kg of food for a dose of $\sim$50 mg/kg body weight/day. This dose corresponds to $\sim$1 g ellagic acid/day in a 70-kg human according to scaling equation [39] or $\sim$0.6 g/day according to body surface area comparisons between rats and humans [40]. Although the average daily human intake of ellagic acid is not known, the total intake of polyphenols is $\sim$1 g/day [41]. If the majority of polyphenols in the diet are taken from the fruits and nuts containing ellagic acid, then the above-mentioned dose is realistic in humans. One viable approach could be to provide this required amount through nutraceutical products containing partly purified ellagic acid from fruits and nuts as the major constituent.

In conclusion, high-carbohydrate, high-fat diet-induced symptoms of metabolic syndrome in rats were reversed by ellagic acid, accompanied by changes in protein levels of Nrf2, CPT1 and NF-κB. These results indicated that these proteins play important roles in the damage associated with metabolic syndrome and targeting these proteins with natural products can attenuate the complications in metabolic syndrome. Since the prevalence of metabolic syndrome, NAFLD and cardiovascular disease is still increasing in the population, the use of either purified ellagic acid as a complementary medicine or an increased dietary intake of fruits and nuts could be potentially effective intervention strategies.

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Conflict of interest No conflict of interest.

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