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Green coffee polyphenols do not attenuate features of the metabolic syndrome and improve endothelial function in mice fed a high fat diet

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ABSTRACT

We have investigated the effects of the major polyphenol in coffee, chlorogenic acid (CGA), on obesity, glucose intolerance, insulin resistance, systemic oxidative stress and endothelial dysfunction in a mouse model of the metabolic syndrome. Thirty C57BL6 mice were randomly divided into (n = 10/group) (i) normal diet (ND), (ii) high fat diet (HFD), or (iii) high fat diet supplemented with 0.5% w/w green coffee bean extract (GCE) rich in chlorogenic acid (HFD + GCE). The high fat diet consisted of 28% fat and all animals were maintained on their diets for 12 weeks. The mice fed a HFD and HFD + GCE displayed symptoms of the metabolic syndrome compared to their normal fed counterparts, although no endothelial dysfunction was detected in the abdominal aortas after 12 weeks. GCE did not attenuate HFD-induced obesity, glucose intolerance, insulin resistance or systemic oxidative stress. Furthermore, GCE did not protect against *ex vivo* oxidant (hypochlorous acid)-induced endothelial dysfunction.

Introduction

The endothelium is essential for maintaining vascular homeostasis. It regulates smooth muscle tone and blood pressure via the synthesis and release of regulatory mediators such as nitric oxide (NO)[1] [1]. Reduction in the bioavailability and/or bioactivity of endothelial-derived NO is thought to result in endothelial dysfunction (ED), a condition characterised by impaired endothelial-dependent vasodilation [1]. ED contributes to the pathogenesis of atherosclerosis and hypertension, and is considered an independent risk factor for cardiovascular disease (CVD) [2]. Previous studies have indicated that oxidative stress plays a prominent role in the development of ED, as potent physiological oxidants such as hypochlorous acid (HOCl), were shown to reduce NO bioavailability through the formation of reactive oxygen species (ROS), in particular, superoxide [3]. Risk factors associated with the metabolic syndrome and Type-2 diabetes mellitus (T2DM), including obesity, glucose intolerance and insulin resistance have been associated with both increased systemic oxidative stress and ED [4].

Coffee is one of the most widely consumed beverages in the world. Several epidemiological studies have associated coffee consumption with a reduced risk of chronic diseases including, T2DM and liver diseases [5,6]. Studies looking at the effects of coffee on ED and CVD risk remain inconclusive due the association of coffee consumption with confounding factors such as smoking and sedentary lifestyle [7]. Whilst caffeine may impart some of the beneficial effects of coffee, a meta-analysis involving >500,000 subjects correlated lower risk of T2DM risk with higher decaffeinated coffee consumption, suggesting the presence of bioactive components other than caffeine [6]. Over the past decade, coffee polyphenols have attracted considerable interest. Consumption of polyphenol-rich foods and beverages (such as certain fruits, vegetables and tea) have been associated with lower CVD [8] and improved endothelial function in healthy subjects [9].

The main polyphenols (phenolic acid) present in coffee are the chlorogenic acids (CGA) (esters of *trans*-cinnamic acid and quinic acid), which can be classified into the caffeoylquinic (CQA), feruloylquinic (FQA) and dicaffeoylquinic (diCQA) acids, in decreasing order of abundance [10]. The principal CGA, 5-*O*-CQA and its isomers 3- and 4-*O*-CQA (Fig. 1) constitute almost 86% of total polyphenol content [11]. Coffee represents a major source of CGA in the diet of regular drinkers with daily intake reaching

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[1] *Abbreviations used:* NO, nitric oxide; ED, endothelial dysfunction; CVD, cardiovascular disease; HOCl, hypochlorous acid; ROS, reactive oxygen species; T2DM, type-2 diabetes mellitus; CGA, chlorogenic acids; CQA, caffeoylquinic acid; FQA, feruloylquinic acid; diCQA, dicaffeoylquinic acid; BP, blood pressure; GCE, green coffee bean extract; HFD, high-fat diets; IPGTT, intraperitoneal glucose tolerance test; IPITT, insulin tolerance test; AUCs, area under concentration–time curves; BHT, butylated hydroxytoluene.

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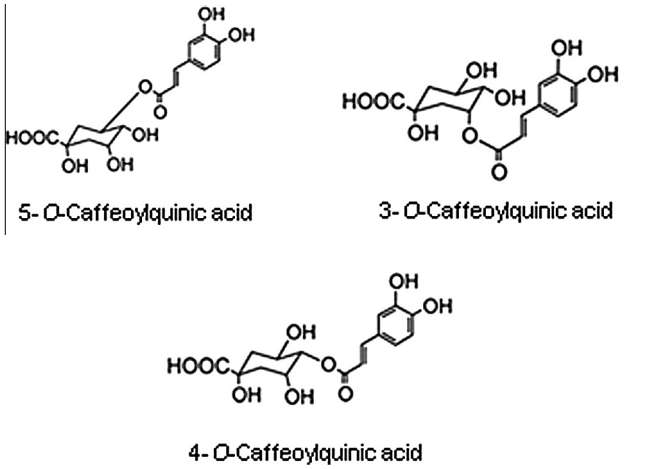

Fig. 1. The major chlorogenic acids present in coffee.

0.5–1 g/day, whereas coffee abstainers consume <100 mg/day. A regular cup of *Arabica* coffee typically contains between 70 and 200 mg CGA, depending on brewing methods [12]. In addition, the roasting of coffee beans will have a significant impact on both CGA content and composition, as previously described [13].

Whilst the beneficial effects of other polyphenols, such as quercetin, on ED have been shown [14], considerably less is known of the effects of CGA. CGA was found to reduce oxidative stress in cell culture studies by scavenging a variety of ROS. This antioxidant ability was attributed to the presence of phenolic hydroxyl groups [15]. Recent work by our group has demonstrated the antioxidant activity of CGA *in vivo* [16]. Animal studies have suggested that CGA may also modulate lipid and glucose metabolism in both healthy and obese animals [17–19] and reduce blood pressure (BP) in spontaneously hypertensive rats [20]. Similar reductions in BP have been observed following ingestion of a polyphenol-rich coffee extract in mildly hypertensive subjects [21].

In this study, we aimed to investigate the effects of green coffee bean extract (GCE), rich in CGA on obesity, glucose intolerance, insulin resistance, systemic oxidative stress and endothelial function in a mouse model of the metabolic syndrome. We hypothesized that supplementation with 0.5% GCE in high-fat diets (HFD) in C57BL6 mice for 12 weeks would reduce body weight gain, improve glucose and insulin sensitivity, reduce oxidative stress levels and improve *ex vivo* endothelial-dependent vasodilation of abdominal aortas. Furthermore, we proposed that GCE would also confer protection against oxidative stress-induced ED. Overall the aim of this study was to provide insight into the potential benefits of coffee phenolic acid components, on features of the metabolic syndrome and ED.

Methods

Extraction of coffee polyphenols

Coffee phenolic acids were extracted from unroasted commercial *Arabica* green coffee beans (BioBean, Perth Australia). The ground coffee beans were soaked for 24 h and repeatedly washed with ethyl acetate to remove neutral lipids and caffeine. Phenolic acids were then extracted from the treated coffee beans with 70:30 water:ethanol solution. The extraction solvent was removed at 70 °C under reduced pressure using a rotary evaporator (Buchi Rotavapor-R, Switzerland), and a residual CGA-containing coffee extract was recovered, that contained ~70% CGA.

HPLC analysis of GCE extract

Prior to incorporation into the diet, the green coffee extract was analysed by HPLC using an Agilent 1100 series HPLC system (Agilent Technologies, Mulgrave, Australia). Compounds were detected by injecting 10 μl aliquots of extract or standards onto a reverse phase column (LiChrospher 100 RP-18, 5 μm) with detection at 325 nm. A mobile phase of methanol:water (80:20) was employed and separation was achieved using an initial gradient of 80% methanol, which was increased to 100% after 5 min. The total run time was 15 min at a flow rate of 1 ml/min. Identification of CGA was achieved via comparison of peak retention times with authentic CGA standards (Sigma Aldrich, Australia & Qingdao International, China). The different isomers of CGA present in the extract were identified using previously described methods [22]. Identification of caffeine in the coffee extract was achieved using an eluent composed of methanol:water (10:90) with detection at 270 nm. Separation was achieved with an initial gradient of 90% water, which was increased to 100% after 5 min. Total run time was 15 min at 1 ml/min [23].

Animals and diets

Male C57BL6 mice (6–8 weeks) were purchased from the Animal Resource Centre (Perth, Australia) and maintained at 23 ± 2 °C under a 12 h light–dark cycle. Following a week of acclimatization, the mice were randomly divided into one of three groups (n = 10, 5 mice/cage); (i) normal diet (ND), (ii) high fat diet (HFD), or (iii) high fat diet supplemented with 0.5% weight/weight (wt/wt) GCE (HFD + CGA). The ND was commercial rodent chow consisting of 4.8% wt/wt fat, while the HFD contained 23.5% wt/wt fat (clarified butter). Mice were allowed ad libitum access to water and all diets were prepared by Specialty Feeds (Glen Forrest, Australia). The mice were maintained on their respective diets for 12 weeks. Body weight of all animals was measured weekly and weight gain was expressed as a percentage of initial body weight to eliminate variability between animals.

The use of animals was approved by the Royal Perth Hospital Animal Ethics Committee (R510/11-12). All animal experiments were compliant with National Health and Medical Research Council (NHMRC) guidelines for the Care and Use of Laboratory Animals in Australia.

Metabolic testing

Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (IPITT) were performed on fasting mice at weeks 5 and 10 and weeks 6 and 11, respectively. To measure blood glucose levels, blood samples were taken from the tail of fasting mice (5 h) before (t = 0 min) and at subsequent time intervals of t = 15, 30, 45, 60, 90 and 120 min following intraperitoneal administration of 1 g glucose/kg and 0.5 U insulin/kg body weight for IPGTT and IPITT respectively. Blood glucose levels were measured using Accu-Chek Performa Strips and Glucometer (Roche Diagnostics, Australia). Area under concentration–time curves (AUCs) for the IPGTTs and IPITTs of all 30 mice were calculated using the trapezoidal method.

Ex vivo vessel function studies

At the end of the 12 week feeding period, non-fasting animals were anaesthetized via inhalation of methoxyflurane (Medical Developments International). A blood sample was taken via cardiac puncture and 0.9% saline solution at near physiologic pressure was perfused through the heart. The descending abdominal aorta was subsequently harvested, freed of adipose and connective tissue and cut into rings of equal length (2 mm) before being place in