

**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 didn't 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] 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 (figure 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 0.5-1 g/day, whereas coffee abstainers consume <100 mg/day. A regular cup of *Arabica* coffee typically contains between 70-200 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

hours 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 10µl aliquots of extract or standards on to a reverse phase column (LiChrospher 100 RP-18, 5µm) with detection at 325nm. 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 270nm. 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 hour 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 hr) 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 placed in aerated ($95\% \text{O}_2$ and $5\% \text{CO}_2$) modified Krebs-Henseleit buffer (4.69 mM KCl, 1.17 mM MgSO_4 , 1.18 mM KH_2PO_4 , 118 mM NaCl, 11.1 mM glucose) containing 8 μM indomethacin (Sigma-Aldrich, Australia) at 37°C to maintain tissue viability. Wires (40 μm diameter) were passed through the lumen of the aortic rings, which were then mounted onto a multi myograph system (620M, DMT Denmark).

Prior to the assessment of vasodilation, the aortic rings were primed with KPSS buffer (13.7 mM KCl, 1.17 mM MgSO_4 , 1.18 mM KH_2PO_4 , 2.50 mM CaCl_2 , 25 mM NaHCO_3 , 0.03 mM EDTA and 5.5 mM glucose) and phenylephrine (1 - 30 μM , Sigma-Aldrich, Australia) to achieve 50% of the pre-determined maximal contractile response.[14] Briefly, the endothelium-dependent relaxation was assessed using increasing doses of acetylcholine (ACh; 10^{-10} to 10^{-5} M, Sigma-Aldrich, Australia),

while endothelium-independent responses to the NO donor DEANO (10^{-10} to 10^{-5} M, Cayman Chemical, USA) were recorded to evaluate smooth muscle viability. The vascular reactivity of the aortic rings to Ach and DEANO following incubation in 100 μ M hypochlorous acid (HOCl) for 60 min were also assessed. All measurements were recorded using the LabChart software (AD Instruments, Australia).

Oxidative stress biomarkers

Blood samples were collected into EDTA and plasma removed after centrifugation at 4°C. After addition of butylated hydroxytoluene (BHT), plasma was stored at -80°C prior to analysis. F₂-isoprostane concentrations were analysed as previously described [24] by monitoring *m/z* 569 and *m/z* 573 on a HP 6890 series gas chromatograph (Hewlett-Packard, USA) couple to a 5973 mass spectrometer (Agilent Technologies, Australia). The identification and quantification of peaks was based on comparison to deuterium labeled internal standards (Cayman Chemical, USA) as previously described.[24]

Statistical analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Inc, Chicago, IL) and SAS 9.2 (SAS institute Inc., Cary, NC, USA). Results are presented as mean and SEM. A type-1 error rate of $P < 0.05$ was the level of significance used for all hypothesis testing. Outcome variables were analyzed with mixed models in SAS using the PROC MIXED command. Mouse number was included as a random factor in all models. Fixed effects included treatment (ND; HFD; HFD+CGA); and time as a categorical variable (from 0 to 12 weeks for % weight gain and from 0 to 120 min for blood glucose) or dose as a categorical variable (from 10^{-10} to 10^{-5} M for Ach and DEANO-mediated relaxation of abdominal aortic rings). The overall effect of treatment was established using the P-value for the effect of treatment. Measures of area under the curve (AUC) and plasma F₂-isoprostanes were analysed using ANOVA with Tukey's HSD for post-hoc comparisons.

Results

HPLC analysis of green coffee bean extract.

The identification of CGA in the green *Arabica* coffee bean extract was based on comparisons with authentic standards and the A_{325nm} traces of both standards and

extract are shown in figure 2. Total CGA content in the extract was 71%, which was predominately 5-*O*-CQA (~55% of total CGA content). Other CGAs: 3-*O*-FQA (10%), 3-*O*-CQA (12%), 5-*O*-FQA (6%), 4,5di-*O*-CQA (6%), 3,4 di-*O*-CQA (6%) 3,5 di-*O*-CQA (5%) were identified by retention times.[22] Small amounts of caffeine (0.03%), were also detected in the coffee extract. A HPLC analysis of the HFD + GCE diet fed to the mice revealed no loss of CGA during feed preparation (data not shown).

Effects of GCE on body weight.

Feeding C57BL6 mice with a HFD resulted in higher body weight gain compared to ND fed mice over the 12 week feeding period (figure 3). Body weight gain in mice receiving the HFD and HFD + GCE were significantly higher than mice fed the normal diet from week 6 onwards ($p < 0.001$). Total end body weight gain of HFD and HFD + GCE fed mice was 33% and 36% higher than normal diet fed mice respectively. There were no significant differences in body weight gain in mice fed the GCE-supplemented HFD compared to mice receiving the HFD alone.

Effects of GCE on glucose intolerance and insulin resistance induced by HFD.

High-fat feeding induced glucose intolerance. This was characterized by impaired glucose clearance of 1g glucose/kg in IPGTTs performed at weeks 5 & 10 in fasting mice (figure 4a, b e & f). HFD and HFD + GCE fed mice had lower glucose tolerance, as evidenced by higher glucose concentrations, at each time point, and significantly higher AUCs compared to mice fed the normal chow ($p < 0.05$). There were no significant differences in IPGTT between mice fed the HFD or HFD + GCE at either week 5 or 10 (figure 4a, b e & f).

Following the i.p administration of 0.5 U insulin/kg during the IPITT, mice receiving the HFD and HFD + GCE had elevated glucose levels and correspondingly higher AUC compared to ND mice fed the normal chow diet at week 11 ($p < 0.05$) (figure 4g & h). This is consistent with a reduced sensitivity to insulin. No significant differences in IPITT were observed between ND and HFD fed mice at 6 weeks, although HFD + GCE fed mice displayed significantly elevated glucose levels and AUC compared to the other two groups ($p < 0.05$) (figure 4c & d).

Effects of GCE on endothelial-dependent vasodilatation.

Abdominal aortic rings isolated from HFD and HFD + GCE fed mice did not display impaired Ach-mediated endothelial-dependent vasodilation, following 12 weeks of diet compared to mice fed the ND. Mean relaxation (expressed as a percentage of phenylephrine pre-constriction) was not significantly different between the three groups (figure 5a). Maximal relaxation (measured at 10^{-5} M Ach) and EC50 were also not significantly different. There were no significant effects on endothelium-independent vasodilatation (figure 6a).

Effects of GCE on systemic oxidative stress.

Systemic oxidative stress was determined by measurement of F₂- isoprostanes in plasma samples. HFD and HFD + GCE fed mice displayed significantly higher levels of F₂- isoprostanes compared to mice fed ND, indicating higher levels of oxidative stress ($p < 0.05$) (figure 7). There was no significant differences in F₂- isoprostane concentrations between HFD and HFD + GCE fed mice.

To assess the effects of GCE diet against oxidative stress-induced ED, aortic rings were incubated in HOCl at a pathophysiological concentration (100 μ M) for 1h prior to testing.[14] All groups displayed significantly impaired Ach-mediated endothelial-dependent relaxation following HOCl treatment (figure 5b), maximal relaxation and EC50 of all animals were not significantly different and there was no protective effect in mice fed the diet enriched in GCE, before or after HOCl incubation (figure 5c).

Endothelial-independent vasodilation to DEANO, a pharmacological NO donor, was assessed before and after HOCl administration to evaluate endothelial smooth muscle viability in all animals. There were no significant differences between the three groups in response to DEANO (figure 6a & b). All aortic rings displayed complete (100%) relaxation before and after HOCl treatment, indicating smooth muscle viability.

Discussion

In the present study we investigated the effects of GCE, rich in CGA, on obesity, glucose intolerance, insulin resistance, systemic oxidative stress and endothelial function in an animal model of the metabolic syndrome. Our results have

demonstrated that the long-term ingestion of 0.5% GCE does not suppress high-fat diet (HFD) induced obesity, glucose intolerance, insulin resistance and oxidative stress in C57BL6 mice. Furthermore, we have shown that GCE does not protect against HOCl-induced ED in mouse abdominal aortas.

The extract of unroasted green *Arabica* coffee beans was predominantly composed of 5-*O*-CQA, the main isomer of CGA present in coffee beans.[10] While some of the beneficial effects of coffee may be mediated by caffeine, [25] only small traces of caffeine were detected in our decaffeinated extract.[6] The major components GCE were CGAs and there were no changes in content or composition following incorporation into the diet. Hence, 5-*O*-CQA was the predominant isomer in the HFD + GCE consumed by the animals. Based on diet ingested, it was estimated that the mice consumed the equivalent of 5 cups coffee/day.

HFD feeding in mice has previously been described as effective for inducing symptoms resembling those of the metabolic syndrome in humans.[26] Mice receiving a HFD developed marked obesity with subsequent glucose intolerance and insulin resistance, over 12 weeks of feeding. Systemic oxidative stress levels were also elevated compared to mice fed the normal chow diet. This reflects previous findings describing a correlation between the metabolic syndrome and oxidative stress levels in human.[27] Oxidative stress plays a major role in promoting vascular diseases including ED.[28] However, we did not observe any impairment of endothelial-dependent vasodilation in the abdominal aortas of HFD fed mice, consistent with previous observations in mice.[29] It is possible that our animal model of the metabolic syndrome was not severe enough to induce ED. Previous studies using Apo E deficient mice fed a HFD, a model exhibiting severe hypercholesterolaemia and atherosclerosis, do show ED.[29] Furthermore the duration of our study may not have been sufficient to allow the development of ED, despite the development of features of the metabolic syndrome.[30]

It has recently been reported that CGA supplementation may reduce fat accumulation in numerous tissues via inhibitory actions of CGA on enzyme regulators involved in lipogenesis.[18] In the present study, we found that CGA supplementation did not suppress body weight gain or attenuate obesity in HFD fed mice. A possible

explanation for this discrepancy is a difference in the source or dose of CGA. Unlike Murase et al, we extracted CGA from unroasted green coffee beans, rather than roasted beans. Significant differences in CGA content and composition has previously been described between roasted and unroasted coffee beans.[13] Our results are consistent with a previous study carried out by our group, using pure CGA.[31]

Obesity predisposes to glucose intolerance and insulin resistance as excess free fatty acids inhibit glucose uptake in liver and muscle tissues, thereby resulting in elevated glycemia and insulin levels.[32] Consistent with its lack of effects against obesity, we found that GCE did not prevent the development of glucose intolerance or insulin resistance in HFD fed mice. Unexpectedly, we found that mice receiving the HFD + GCE displayed significantly greater insulin resistance compared to mice receiving the HFD alone at week 6, although this was not observed at week 11. One limitation pertaining to the IPGTTs and IPITTs in our study, was determining glucose and insulin injection doses on the basis of total body mass of the mice, rather than the lean body mass. Skeletal muscle and liver tissues account for a larger proportion of glucose uptake compared to adipose tissues.[33] Due to the excess fat, HFD fed animals were injected with higher doses of glucose and insulin, despite their lean body mass being feasibly similar to mice fed the normal chow diet. It has previously been suggested that CGA could lower glycemia, by directly inhibiting hepatic glucose release.[34] However these findings were observed in liver perfusion experiments. It is not surprising our study could not corroborate these previous reports as evidence suggests that CGA undergoes intestinal metabolism upon ingestion, which indicates that only a portion of intact CGA reaches the liver.[35, 36]

Alleviation of oxidative stress has been shown to improve ED.[37] CGA has been found to act as an antioxidant *in vitro*; by directly scavenging a number of reactive oxygen species (ROS).[12] However, experiments in cultured cells do not necessarily mimic the effects of CGA *in vivo*. Indeed, we found that GCE did not protect against increased oxidative stress due to the HFD, implying a lack of antioxidant activity.

We found no effect of GCE on endothelial-dependent vasodilation following 12 weeks of feeding in the present study. However, there was no significant difference in relaxation between the HFD fed mice and those fed the normal chow diet. This

suggests that the HFD did not cause ED, so supplementation with CGA could be expected to have a minimal effect on this. To assess the effects of GCE against oxidative-stress induced ED, we incubated the aortic rings in HOCl. HOCl has previously been shown to induce ED in rabbit aortas, by reducing NO production through a superoxide-dependent reduction in the stability of eNOS (endothelial nitric oxide synthase); the enzyme responsible for endothelial NO production.[3] Consistent with previous observations, we found that incubation of aortic rings from all three groups, with HOCl, significantly impaired endothelial-dependent vasodilation. It is unlikely that this impairment was due to a time effect, as previous studies have reported no difference in endothelial-dependent vasodilation in time controls.[3] While previous studies have shown that other polyphenols (such as quercetin) can protect against HOCl-induced ED[38], our study suggests that the phenolic acid CGA has no such protective effects against oxidative stress-induced ED.

When assessing the findings of this study, a few limitations should be considered. Firstly our study was performed in an animal model, and the effects of GCE observed in animals may not necessarily replicate the effects seen in humans. Secondly, the duration of this study may not have not been sufficient to allow any effects of GCE to be observed. Thirdly, in this study, we characterised the effects of CGA, which was extracted from unroasted green *Arabica* coffee beans. However, most coffee beverages consumed are brewed from roasted coffee beans, which together with brewing methods, may have effects on CGA composition. However, CGA supplements extracted from unroasted green coffee beans are currently being marketed as weight-reducing agents in the alternative medicine industry. It should be noted that the effects of GCE on features of the metabolic syndrome and ED, as described in this study, may not represent the effects of coffee consumption. Coffee is a complex mixture of bioactive substances, and interaction between different components is likely to alter the bioactivity of CGA. Indeed, a recent paper has highlighted that high coffee consumption (>28 cups per week) is associated with increase in all-cause mortality and CVD. [39]

In conclusion, while our study has demonstrated no positive effects of GCE supplementation on features of high-fat diet induced metabolic syndrome in mice, it cannot be ruled out that coffee or CGA will have beneficial effects in humans. To

date, evidence supporting the beneficial effects of CGA remains sparse. Future studies addressing the limitations outlined in this study, as well as examining these effects in humans are warranted.

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Figure Legends:

Figure 1: The major chlorogenic acids present in coffee.

Figure 2: HPLC analysis of the major coffee polyphenols present in unroasted green *Arabica* coffee bean extract (C). The arrow denotes the main peak (peak 2) which was identified as 5-*O*-CGA, via comparison to previously tested standards purchased from (A) Sigma-Aldrich and (B) Qingdao International. Remaining peaks were identified as; 3-*O*-CGA (peak 1), 3-*O*-FGA (peak 3), 5-*O*-FGA (peak 4), 3,4di-*O*-CGA (peak 5), 3,5di-*O*-CGA (peak 6), 4,5di-*O*-CGA (peak 7), through the comparison of peak retention times from published studies.[22]

Figure 3: Percentage body weight gain for C57BL6 mice (6-8 weeks, 10/group) fed a normal diet, HFD and HFD+CGA for 12 weeks. Mice were weighed weekly and body weight gain expressed as a percentage of initial body weight (week 0). Data is expressed as mean \pm SEM, ** $p < 0.001$ compared to normal diet.

Figure 4: Intraperitoneal glucose tolerance (IPGTT) and insulin tolerance (IPITT) test, with corresponding area under the curve (AUC) of C57BL6 mice, fed a normal diet, HFD and HFD+CGA for 12 weeks. (A) IPGTT and (B) AUC at 5 weeks and (E) IPGTT and (F) AUC at 10 weeks. (C) IPITT and (D) AUC at 6 weeks and (G) IPITT and (H) AUC at 11 weeks. Values are expressed as mean \pm SEM, * $p < 0.05$ compared to normal diet and # $p < 0.05$ compared with HFD, $n = 9-10$ /group.

Figure 5: Acetylcholine (ACh) mediated relaxation of abdominal aortic rings isolated from mice fed (A) normal diet, HFD and HFD+CGA and (B) then following subsequent incubation with 100 μ M HOCl for 1 hr. (C) illustrates relaxation curves from mice fed HFD+CGA, before and after HOCl. Rings were pre-constricted with phenylephrine to 50% of the maximal contraction, and relaxation is expressed as % pre-constriction. Values are mean \pm SEM ($n = 6-9$ /group), * $p < 0.05$ compared to before HOCl incubation.

Figure 6: DEANO mediated relaxation of abdominal aortic rings isolated from mice fed (A) normal diet, HFD and HFD+CGA for 12 weeks, and (B) following

subsequent incubation with 100 μ M HOCl for 1 hr. Rings were pre-constricted with phenylephrine to 50% of the maximal contraction, and relaxation is expressed as % pre-constriction. Values are mean \pm SEM (n = 6-9/group).

Figure 7: Plasma F₂-isoprostane concentrations in mice fed normal diet, HFD and HFD+CGA for 12 weeks. Values are presented as mean \pm SEM (n = 6-9/group), *p < 0.05 compared with normal diet.

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Figure 1:

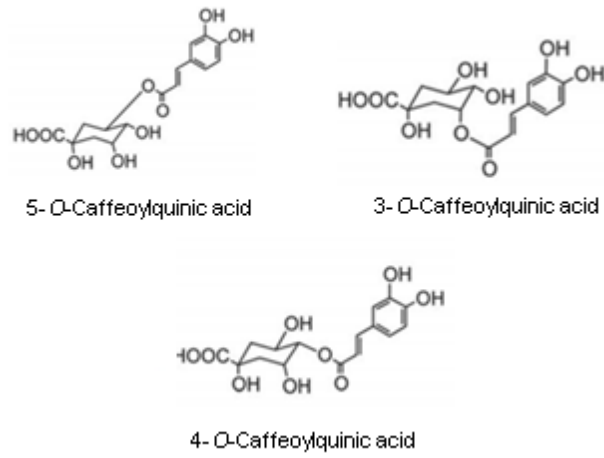


Figure 2:

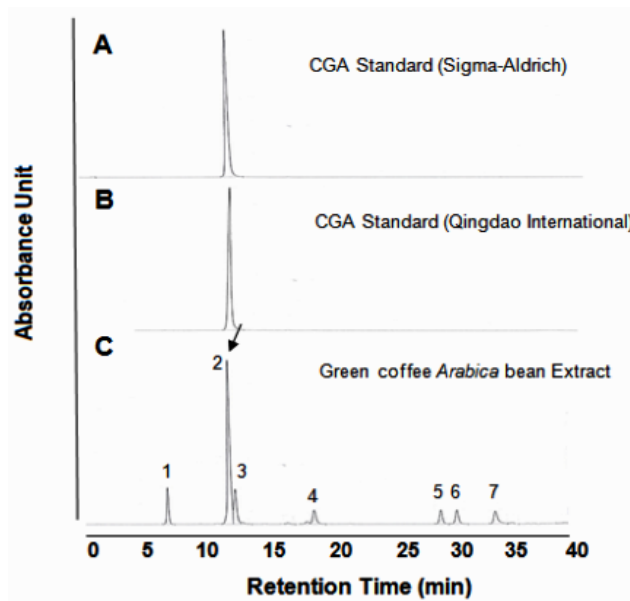


Figure 3:

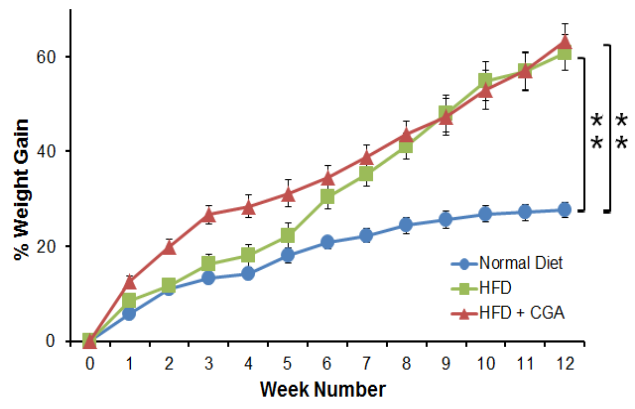


Figure 4:

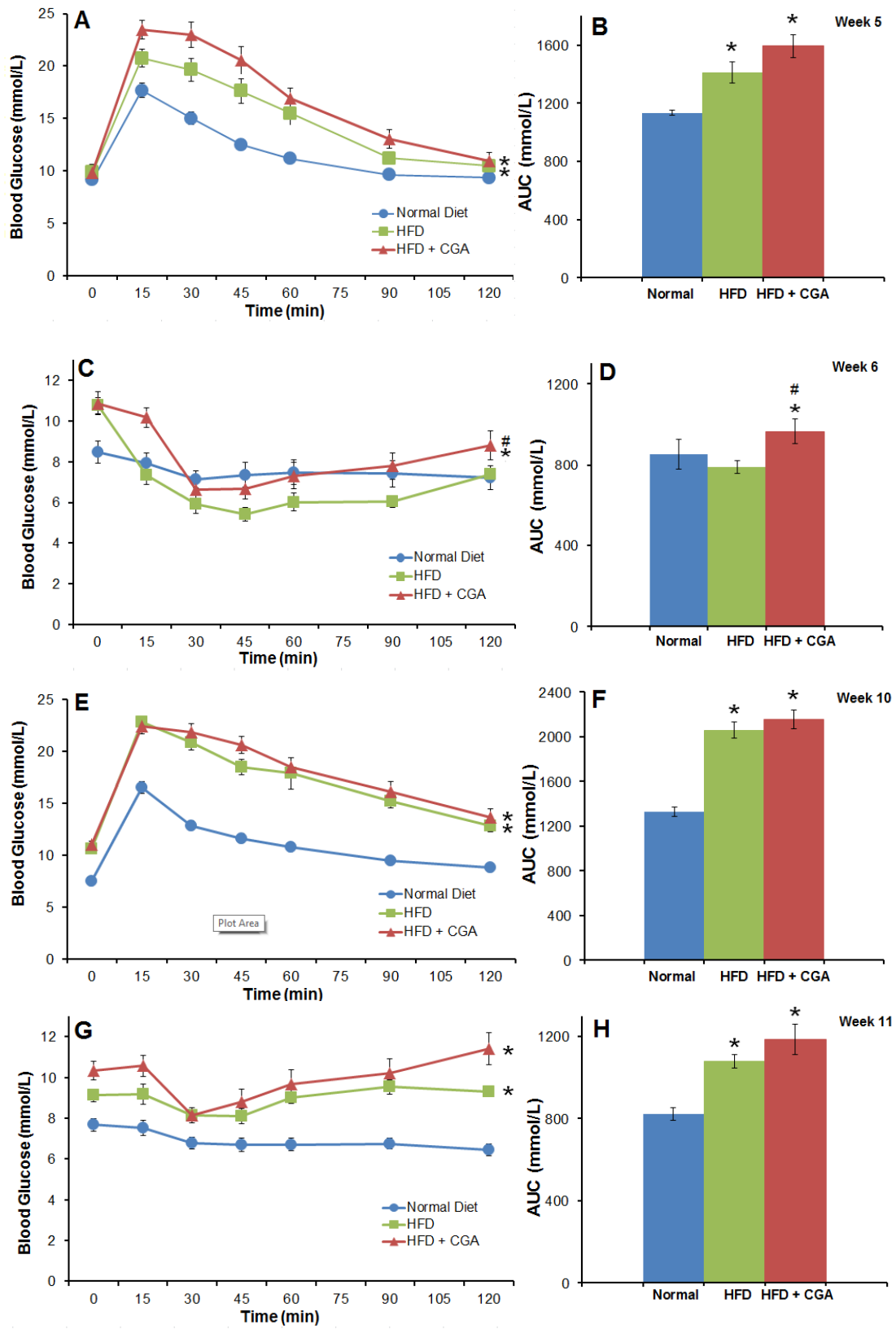


Figure 5:

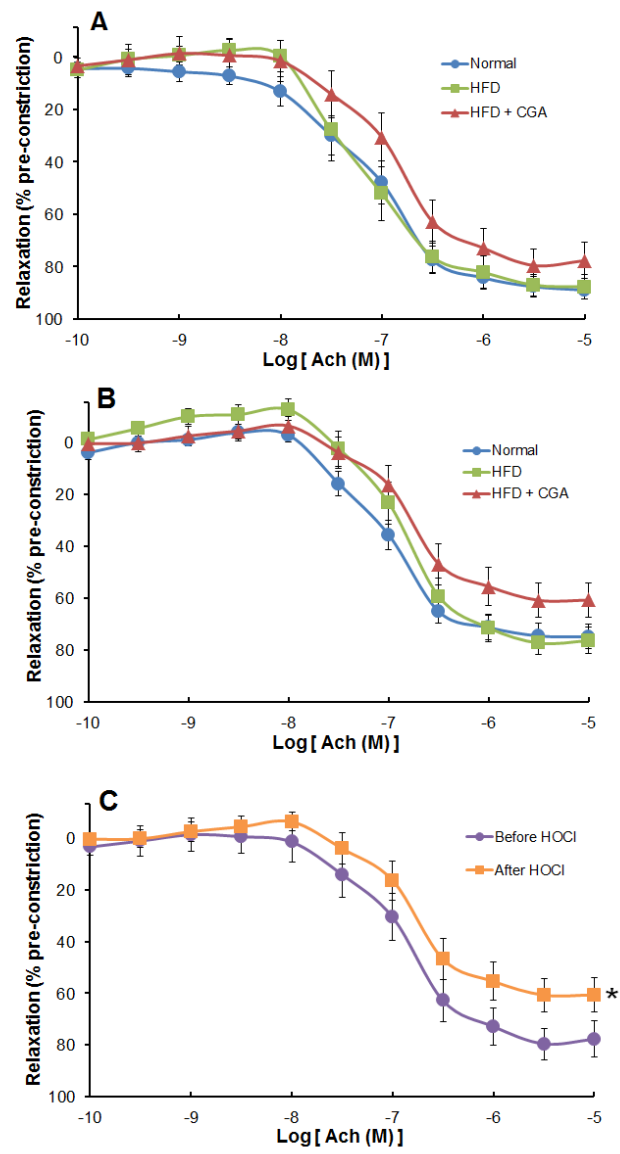


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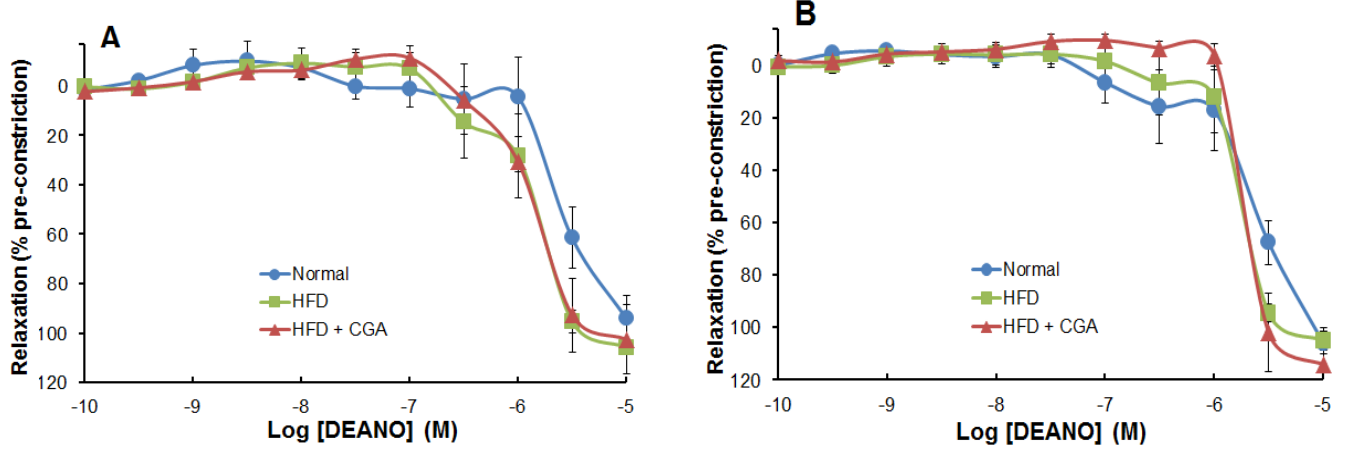


Figure 7:

