Published in final edited form as:

Amino Acids. 2014 September ; 46(9): 2219–2229. doi:10.1007/s00726-014-1762-7.

Metabolomic analysis of amino acid and energy metabolism in rats supplemented with chlorogenic acid

Zheng Ruan,

State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China

Yuhui Yang,

State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China

Yan Zhou,

State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China

Yanmei Wen,

State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China

Sheng Ding,

Institute of Nutrition and Food Safety, Center for Disease Control and Prevention of Jiangxi Province, Nanchang 330029, China

Gang Liu,

Hunan Engineering and Research Center of Animal and Poultry Science, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Xin Wu,

Hunan Engineering and Research Center of Animal and Poultry Science, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Peng Liao,

Hunan Engineering and Research Center of Animal and Poultry Science, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Hunan New Wellful Co., LTD, Changsha 410001, China

Zeyuan Deng,

State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China

Houssein Assaad,

Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA

Correspondence to: Peng Liao, liaopeng@isa.ac.cn; Yulong Yin, yinyulong@isa.ac.cn.

Conflict of interest The authors declare that they have no conflict of interests.

Guoyao Wu, and

Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA

Yulong Yin

State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China

Hunan Engineering and Research Center of Animal and Poultry Science, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Peng Liao: liaopeng@isa.ac.cn; Yulong Yin: yinyulong@isa.ac.cn

Abstract

This study was conducted to investigate effects of chlorogenic acid (CGA) supplementation on serum and hepatic metabolomes in rats. Rats received daily intragastric administration of either CGA (60 mg/kg body weight) or distilled water (control) for 4 weeks. Growth performance, serum biochemical profiles, and hepatic morphology were measured. Additionally, serum and liver tissue extracts were analyzed for metabolomes by high-resolution ^1H nuclear magnetic resonance-based metabolomics and multivariate statistics. CGA did not affect rat growth performance, serum biochemical profiles, or hepatic morphology. However, supplementation with CGA decreased serum concentrations of lactate, pyruvate, succinate, citrate, β -hydroxybutyrate and acetoacetate, while increasing serum concentrations of glycine and hepatic concentrations of glutathione. These results suggest that CGA supplementation results in perturbation of energy and amino acid metabolism in rats. We suggest that glycine and glutathione in serum may be useful biomarkers for biological properties of CGA on nitrogen metabolism in vivo.

Keywords

Chlorogenic acid; Amino acids; Metabolism; Nuclear magnetic resonance spectroscopy

Introduction

Phenolic acids are currently receiving much attention because of their beneficial health effects related to their anti-oxidant, anti-inflammatory and anti-cancer properties (Cai et al. 2004; Trouillas et al. 2003). Chlorogenic acid (CGA) is one of the most abundant hydroxy cinnamic acid (phenolic acids) compounds in the human diet, such as coffee and tea (Algamdi et al. 2011; Meng et al. 2013). Accumulating evidence shows that CGA exhibits many biological effects, including anti-bacterial, anti-oxidant, anti-inflammation, anti-cancer, anti-diabetes, anti-hypertension and anti-obesity actions (Greenberg et al. 2006; Kasai et al. 2000; Lou et al. 2011; Yun et al. 2012; Zhao et al. 2012). There are also reports that CGA modulates glucose and lipid metabolism both in healthy and genetically metabolic disordered conditions (Huang and Chang 2008; Li et al. 2009; Zhang et al. 2011). Furthermore, CGA confers hepato-protective benefits on protecting animals from LPS-induced or chemical injury (Shi et al. 2012; Yun et al. 2012). For example, intraperitoneal administration of CGA (50 mg/kg body weight) suppresses hepatic mRNA levels for toll-like receptor 4 (TLR4), TNF-alpha and NF-kappa B p65 subunit, thereby protecting the liver from LPS-induced injury (Xu et al. 2010). Furthermore, intra-peritoneal administration of

CGA to CCl₄-intoxicated mice (100 mg/kg body weight per day) for 8 days ameliorates lipid peroxidation, hepatic injury, cytochrome P450 (Cyt P450) inactivation, leading to the enhancement of the cellular anti-oxidant defense (Marques and Farah 2009). At present, little is known about effects of oral administration of CGA on anti-oxidant activity or nutrient metabolism in vivo. Wan et al. (2013) found that dietary supplementation with CGA (1 or 10 mg/kg body weight/day) attenuated both the elevated plasma concentrations of total cholesterol and low-density lipoprotein and the decreased plasma concentrations of high-density lipoprotein in rats fed a hypercholesterolemic diet. The hypocholesterolemic effect of CGA may result from altered metabolism of nutrients, including fatty acids, glucose and amino acids.

Previous studies of the biological effects of CGA have been confined only to measurements of a single or several biochemical markers in tissues (Shi et al. 2012; Yun et al. 2012). This approach does not adequately reflect the overall metabolism of nutrients in animals or humans. These parameters are commonly preselected based on a hypothesis. Therefore, the traditional methods cannot be used to perform global screening of physiological perturbations, such that unexpected or novel responses or bio-markers are often not detected. Hence, it is necessary to employ a robust technique to simultaneously quantify and identify a large number (hundreds to thousands) of molecules in blood and other tissues (He et al. 2012; Liu et al. 2011).

Metabolomics has emerged as a biomarker discovery tool for metabolic profiling in nutritional research (He et al. 2011; Lindon et al. 2001). Nuclear magnetic resonance (NMR) spectroscopy is one of the major techniques used in metabolomic studies as the spectra of biofluids or tissues contain a wealth of metabolic information and provide comprehensive biochemical profiles of low-molecular-weight metabolites whose concentrations are precisely regulated in response to various stimuli (Wang et al. 2009). Thus, NMR analysis aids in enhanced understanding of molecular mechanisms and in providing novel insight into the intervention effect or perturbation of diets with regard to nutrient metabolism and health. To capitalize on this advanced technique, the present study was conducted with ¹H NMR to screen the global metabolic profile of the serum and liver of CGA-supplemented rats.

Materials and methods

Dietary treatment and sample collection

This study was approved by the Nanchang University Animal Experiment Ethics Committee, carried out in the Center for Disease Control and Prevention of Jiangxi Province (Nanchang, China), and performed in accordance with the Chinese guidelines for the Laboratory Animals Care. A total of 12 adult female Sprague–Dawley rats (weighting 170–190 g) were obtained from Changsha Tianqin Biotechnology Co., Ltd (Changsha, China). They were housed individually in metabolism cages after acclimation for 7 days in plastic cages, and had free access to the standard rodent diet and drinking water. The animal room was well ventilated and maintained at a cycle of 12 h light and 12 h darkness, 22–25 °C, and the relative humidity of 50 % throughout the study. The experimental diets (Table 1) were formulated to meet nutrient requirements for rats (Xu et al. 2013).

Rats were randomly divided into two groups: the control group (oral administration of distilled water once daily) and the CGA group (oral administration of CGA once daily). CGA with a purity of 98 % (Aladdin Co., Ltd, Shanghai, China) was dissolved in distilled water and orally administered at a dose of 60 mg/kg body weight. This dosage of CGA was based on previous reports (Shi et al. 2009, 2012). Water or CGA was given daily to a respective treatment group for 28 days. After the last administration of water or CGA, rats were euthanized (Ruan et al. 2013) following a 12-h period of food deprivation to avoid a postprandial effect on serum metabolites. Liver (~5 g) and blood samples (~5 mL) were quickly collected. Sera were separated from whole blood by centrifugation at 600×g and 4 °C for 15 min (Ren et al. 2013a). All serum and liver samples were stored at –80 °C until analysis.

Growth performance

The body weight of each rat was recorded every day. The daily weight gain and food intake of the rats were measured. Feed conversion ratio (FCR) was calculated based on the average daily feed intake of rats divided by their body-weight gain (Foo et al. 2003; He et al. 2003).

Serum biochemical parameters

Serum metabolites were determined using a biochemistry analyzer (Beckman, USA) according to the manufacturer's protocol (Nanjing Jiancheng Technology Co., Ltd, Nanjing, China), as previously described (Tan et al. 2012; Ren et al. 2013b). They included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, glucose (GLU), lactate dehydrogenase (LDH), albumin, total protein, cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

Liver index and histological analysis

Liver index was calculated by the following equation: Liver index (%) = (liver weight/body weight) × 100 (Mahaffey et al. 1981). Hematoxylin and eosin staining and histological analysis were performed as previously reported (He et al. 2013; Yao et al. 2011). Liver tissue was fixed by immersion in 10 % neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5 µm thickness, affixed to slides, deparaffinized, stained with hematoxylin and eosin, and examined by light microscopy. Photomicrographs were acquired with 200× magnifications using an Olympus BX51 microscope (Olympus Optical Company, Shanghai, China).

Sample preparation and ¹H NMR measurement

Serum samples were prepared by mixing 350 µL serum and 350 µL D₂O and through centrifugation at 10,000×g for 10 min. The supernatant fluid (550 µL) was collected and transferred into 5-mm NMR tubes. All ¹H NMR spectra were acquired on a Bruker Advance DRX-600 spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at a proton frequency of 600.58 MHz and a temperature of 294 K, using a cryogenic high-resolution probe. ¹H NMR spectra of serum samples were acquired using the Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse sequence to emphasize resonances from low-molecular-weight

metabolites. For each sample, a total of 64 transients were collected into 45-K data points over a spectral width of 9615.4 Hz with a relaxation delay of 4.0 s and an acquisition time of 3.4 s (Ruan et al. 2013).

A pre-weighted liver tissue (0.1 g) was homogenized in 300 μ L buffer solution (0.2 mol/L Na_2HPO_4 and 0.2 mol/L NaH_2PO_4 , pH 7.4) and 400 μ L methanol-d (CH_3OD). The samples were allowed to stand for 20 min prior to centrifugation at $10,000\times g$ at 4 °C for 15 min to remove any precipitates. Aliquots of the supernatant fluid (550 μ L) from each sample were transferred into a 5-mm NMR tube containing 30 μ L of 2 mmol/L TMSP [3-(trimethylsilyl) propionic-(2,2,3,3-d₄) acid sodium salt] (TMSP dissolved in D₂O). Aqueous liver extracts were analyzed using NOESYGPPR-1D pulse sequence (relaxation delay-90°- t_1 -90°- t_m -90°-acquisition) with solvent presaturation (Schnackenberg et al. 2005; Yang et al. 2007). For each sample, a total of 64 transients were collected into 32-K data points over a spectral width of 9,615.4 Hz with a relaxation delay of 4.0 s and an acquisition time of 3.4 s.

Data processing and statistical analysis

All ¹H NMR spectrums from serum and liver extracts were phase-adjusted and baseline-corrected with MNova-6.1.1 (MestreLab, Santiago de Compostela, Spain). For serum samples, NMR spectra were referenced to the lactate-CH₃ signal at 1.33 ppm. The TMSP acted as a chemical shift reference (δ0.0) in spectrum from liver extracts. Each spectrum over the range of δ0.5–9.0 was divided into integrated regions of equal width (0.01 ppm). The region of δ4.60–5.10 and δ4.70–5.20, which included the residual water resonance, was removed from all liver and serum spectra, respectively. In order to avoid any baseline distortion caused by imperfect water suppression, the region of residual methanol δ3.29–3.35 was removed from all liver spectra. Each integral region was then normalized to the sum of total integral regions. The data were imported into Microsoft Excel (version 2003) and included in SIMCA-P 12.0 as variables. The resulting data of principal component analysis (PCA) were demonstrated by the PCA scores' plots and loadings' plots (Wu 2010). Score plots of the first two principal components (PCs) were used to visualize the separation of two groups, and loading plots reflected the NMR spectra regions that were altered as a result of the CGA treatment. The normalized integral values were then input into the software of SPSS 17.0 (SPSS Inc., Chicago, USA) for statistical analysis. The independent-sample *t* test method was used to detect significant differences in signals between two groups (Fu et al. 2010). Probability values ≤ 0.05 were taken to indicate statistical significance (Wei et al. 2012).

Results

General observations

The growth performance of rats is shown in Table 2. Final body weight, ADG, ADFI or FCR did not differ ($P > 0.05$) among all the treatment groups. CGA supplementation had no effect on the serum biochemical profiles of rats, as measured using the conventional methods (Table 3). Overall, CGA supplementation did not affect the liver index or morphology, as compared with the control group (Fig. 1).

¹H NMR spectrum of serum and liver

Typical ¹H NMR spectra of two serum samples taken from the control and CGA groups are illustrated in Fig. 2a and b, respectively. Similarly, typical ¹H NMR spectra of aqueous extracts of two liver samples taken from the control and CGA group rat are shown in Fig. 2c and d, respectively. A number of perturbations in endogenous metabolites are revealed by the ¹H NMR spectra. A total of 35 metabolites in the serum and 36 metabolites in the liver were unambiguously assigned for the ¹H NMR spectra. Assignment of metabolites was made on the basis of comparisons with the published literature (Jiang et al. 2013; Liao et al. 2007; Zira et al. 2013). Their chemical shifts and peak multiplicity are listed in Tables 4 and 5, respectively.

To detect more subtle treatment-related metabolic differences, pattern recognition techniques were applied. PCA was performed on the ¹H NMR spectra of serum and liver samples in the control and CGA groups. The PCA scores plot of the ¹H NMR serum data are summarized in Fig. 3a. This plot shows the first two PCs and accounts for 83.6 % of the variation in the samples. A PCA score plot of the ¹H NMR from liver samples is given in Fig. 3c. This plot displays the first two PCs and explains 59.8 % of the total variations within the data. Figure 3a and c intuitively illustrate the discrete trend of two sets of the data.

Each triangle in the loading plots (Fig. 3b, d) represents a single NMR spectral region or chemical shifts. The loading plots indicated the spectral variables of metabolites. The distance between triangle and origin in the loading plots represented the contribution of the corresponding metabolite to differences between the two groups of rats. The corresponding loading plot (Fig. 3b) showed increases in ¹H NMR peaks for lipoproteins, glycine and unsaturated lipids in the serum of CGA-supplemented rats and decreases in ¹H NMR peaks for β-hydroxybutyrate, lactate, acetoacetate, pyruvate, succinate and citrate (Table 6). They are the major contributors to the separate clustering of the groups. Detailed analysis of the loadings plot from Fig. 3d indicated that the level of hepatic glutathione was increased ($P < 0.05$) in the CGA group, as compared with the control group (Table 7).

Discussion

There is growing interest in phytochemicals as dietary supplements for humans and animals (Kong et al. 2011). In the current work, we sought to assess the effects of CGA supplementation on serum metabolic profiles and hepatic metabolism in rats. Our results support the hypothesis that CGA supplementation can regulate nutrient metabolism and enhance the anti-oxidant function in animals, without negatively affecting growth performance, serum lipid profiles or liver morphology in rats (Tables 2, 3; Fig. 1). Of note, several studies reported that CGA supplementation could protect the liver against stress or pathologic condition-induced injury (Shi et al. 2012; Yun et al. 2012). Similarly, CGA present in coffee is capable of quenching radical species and inhibiting H₂O₂-induced apoptosis via suppressing mitochondrial membrane depolarization and caspase-9 activation in cells (Park 2013). Furthermore, CGA alleviates hepatic ischemia and reperfusion-induced liver injury and that this protection is associated with an inhibition of inflammatory response and enhancement of anti-oxidant defense systems (Yun et al. 2012). Thus, in recent years, CGA has received much attention from biomedical scientists due to its beneficial effects as

an anti-oxidant and hepatoprotective phytochemical. The hypocholesterolemic functions of CGA are probably due to an increase in fatty acid unitization in the liver (Wan et al. 2013), which utilizes long-chain fatty acids as major energy substrates (Dai et al. 2013a; Jobgen et al. 2006). However, the underlying mechanisms responsible for the hepatoprotective effect of CGA in animals remain unknown. Therefore, as an initial step to understand such mechanisms, the present study was conducted to identify biochemical metabolites as biomarkers for protective effects of CGA in rats.

Our metabolomic results indicate effects of CGA on energy and nitrogen metabolism in adult rats. Specifically, dietary supplementation with CGA decreased the concentrations of lactate, pyruvate, succinate, citrate, β -hydroxybutyrate and acetoacetate in the serum, as compared with the control group (Table 4). Pyruvate is a key metabolite in glycolysis, amino acid metabolism, and the tricarboxylic acid (TCA) cycle (Kauppinen and Nicholls 1986). Lactate, which is formed from pyruvate and NADH by lactate dehydrogenase in the cytoplasm, is closely associated with energy metabolism and cellular redox state (Liu et al. 2011). The decreased concentrations of pyruvate and lactate in plasma may be related to an increased rate of hepatic gluconeogenesis and a reduced rate of anaerobic glycolysis in skeletal muscle (Krebs 1970). In addition, dietary supplementation with CGA affects the circulating levels of intermediates in the TCA cycle possibly due to the decreased concentrations of succinate and citrate in serum (Krebs and Lowenstein 1960; Krebs 1970). This suggests that the integrity of cells (e.g., hepatocytes and muscle) is improved by CGA supplementation, thereby reducing the release of intermediates of the TCA cycle into the extracellular space. Both acetoacetate and β -hydroxybutyrate are products of fatty acid oxidation in the liver (Wojtczak 1968; Wu et al. 1991). Interestingly, CGA supplementation decreased concentrations of acetoacetate and β -hydroxybutyrate in serum. This suggests a reduced rate of hepatic ketogenesis and an increased rate of oxidation of ketone bodies by extrahepatic tissues, including skeletal muscle, heart and kidneys (Mubarak et al. 2013). CGA may have a beneficial effect on increasing mitochondrial biogenesis and oxidation, as reported for L-arginine (Satterfield and Wu 2011; Wu et al. 2012). An overall improvement in energy metabolism brought about by CGA supplementation may lead to increased cholesterol transport from peripheral tissues into the liver and the hepatic formation of the lipids beneficial for cardiovascular function and removal from the body. In support of this view, CGA supplementation increased the serum concentrations of unsaturated lipids and lipoproteins, as compared to the control group. Taken together, these results indicate that dietary supplementation with CGA can regulate energy expenditure in rats.

A striking finding from this study is the increased concentration of glutathione in the liver of CGA-supplemented rats, suggesting that CGA can promote the anti-oxidant activity. Glutathione is the major intracellular nonprotein thiol protecting cells against oxidative damage and harmful xenobiotics (Hayes and McLellan 1999; Schulz et al. 2000). Glutathione is synthesized in tissues and cells (e.g., liver and intestine) from glutamate, cysteine and glycine, and hepatocytes are the major producer and exporter of glutathione (Wu et al. 2004, Wu 2010). This finding is consistent with other studies in which CGA exerts a potent anti-oxidant effect in several animal models hepatic injury induced by CCl₄, lipopolysaccharide, or acetaminophen (Ji et al. 2013; Shi et al. 2009, 2013; Xu et al. 2010). In the present study, CGA supplementation increased hepatic concentrations of glutathione,

indicating that CGA can promote glutathione synthesis in the liver. Glutathione also protects the small intestine (Wu et al. 2013a; Hou et al. 2012, 2013) and the large intestine (Wang et al. 2013a) from oxidative damage. We suggest that glutathione (reduced form) in serum may be a useful biomarker for the biological properties of CGA in vivo.

Another novel and unexpected finding from this work is that the concentration of glycine in serum was increased in CGA-supplemented rats (Table 6). This result is in agreement with the increased concentration of hepatic glutathione in the CGA group. In response to malnutrition and chronic diseases (e.g., starvation, diabetes, and obesity), concentrations of glycine in plasma are markedly reduced (Satterfield et al. 2012, 2013; Wu 2013b). Thus, CGA may be beneficial for treating or preventing these metabolic disorders. Available evidence shows that glycine contributes to improved anti-oxidative activity in humans and animals (Wang et al. 2013b). Thus, increasing dietary levels of glycine can enhance glutathione synthesis in tissues and protects tissues (e.g., the vascular bed and the intestine) against oxidative stress (Ruiz-Ramírez et al. 2014; Wang et al. 2014). Furthermore, oral glycine supplement attenuates oxidative damage in patients with metabolic syndrome (Díaz-Flores et al. 2013). Finally, glycine exhibits many biological properties, including metabolic regulation, anti-oxidative, cytoprotective, anti-inflammatory, and immunomodulatory properties (Cruz et al. 2008; El Hafidi et al. 2004; Mauriz et al. 2001).

Glycine is synthesized from threonine, serine, and hydroxyproline via inter-organ metabolism of amino acids (Wu 2013a; Wu et al. 2011). At present, the relative contributions of these pathways to endogenous glycine provision in animals remain unknown and should be quantified using isotopic techniques. An important role for intestinal microbes in providing glycine for absorption into the portal circulation should also be taken into consideration (Dai et al. 2011). This view is based on the recent findings that bacteria in the small intestine play an important role in the metabolism of amino acids, including glycine (Dai et al. 2010, 2012a, b, 2013b). It is possible that the increased level of glycine in the blood circulation may be explained by an ability of CGA to promote the endogenous synthesis of glycine in mammalian cells and intestinal microbes, as well as by enhanced absorption of glycine from the lumen of the gut. Traditionally, little attention has been paid to dietary requirement of glycine by animals (Wu et al. 2013b, 2014). Compared with many amino acids that are abundant in animal proteins, the content of glycine is relatively low in plant-based diets (Li et al. 2011; Dai et al. 2014). Under normal feeding conditions, the amount of glycine synthesized in vivo is insufficient to meet metabolic demands in birds and young mammals, particularly in a diseased state (Rezaei et al. 2013a, b; Wang et al. 2014; Wu et al. 2014). Although mild insufficiency of glycine is not threatening to survival of animals, a chronic shortage may result in impaired immune responses and other adverse effects on nutrient metabolism and health (Li et al. 2007; Matilla et al. 2002). Therefore, an increased concentration of glycine in serum or plasma can be another useful biomarker for the biological properties of CGA in vivo.

In summary, our results support the hypothesis that supplementation of CGA to a normal diet will regulate energy metabolism and enhance anti-oxidant function of rats. Dietary supplementation with CGA increased the concentrations of glycine in the serum and the

concentrations of glutathione in the liver, thus promoting the anti-oxidant activity in the whole body. CGA also beneficially modulates energy metabolism in rats. We suggest that glycine and glutathione in the blood circulation are useful biomarkers for the biological effects of CGA in vivo. Further studies may be warranted to elucidate the underlying mechanisms and possible links between CGA and amino acid metabolism in animals.

Acknowledgments

This research was financially supported by National Natural Science Foundation of China (Grant No. 31001014), the Research Foundation (SKLF-TS-201108 and SKLF-TS-200817) and the Open Project Program (SKLF-KF-201005 and SKLF-KF-201216) of State Key Laboratory of Food Science and Technology at Nanchang University, and Texas A&M AgriLife Research H82000. H. Assaad was supported by a postdoctoral training grant (R25T-CA090301) from the National Cancer Institute.

Abbreviations

CGA	Chlorogenic acid
PC	Principal components
PCA	Principal component analysis
NMR	Nuclear magnetic resonance

References

- Algami N, Mullen W, Crozier A. Tea prepared from *Anastatica hierochuntica* seeds contains a diversity of antioxidant flavonoids, chlorogenic acids and phenolic compounds. *Phytochemistry*. 2011; 72:248–254. [PubMed: 21176927]
- Cai Y, Luo Q, Sun M, et al. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*. 2004; 74:2157–2184. [PubMed: 14969719]
- Cruz M, Maldonado-Bernal C, Mondragon-Gonzalez R, et al. Glycine treatment decreases proinflammatory cytokines and increases interferon-gamma in patients with type 2 diabetes. *J Endocrinol Invest*. 2008; 31:694–699. [PubMed: 18852529]
- Dai ZL, Zhang J, Wu G, et al. Utilization of amino acids by bacteria from the pig small intestine. *Amino Acids*. 2010; 39:1201–1215. [PubMed: 20300787]
- Dai ZL, Wu G, Zhu WY. Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. *Front Biosci*. 2011; 16:1768–1786.
- Dai ZL, Li XL, Xi PB, et al. Metabolism of select amino acids in bacteria from the pig small intestine. *Amino Acids*. 2012a; 42:1597–1608. [PubMed: 21344175]
- Dai ZL, Li XL, Xi PB, et al. Regulatory role for l-arginine in the utilization of amino acids by pig small-intestinal bacteria. *Amino Acids*. 2012b; 43:233–244. [PubMed: 21928075]
- Dai ZL, Wu ZL, Yang Y, et al. Nitric oxide and energy metabolism in mammals. *BioFactors*. 2013a; 39:383–391. [PubMed: 23553707]
- Dai ZL, Li XL, Xi PB, et al. l-Glutamine regulates amino acid utilization by intestinal bacteria. *Amino Acids*. 2013b; 45:501–512. [PubMed: 22451274]
- Dai ZL, Wu ZL, Jia SC, et al. Analysis of amino acid composition in proteins of animal tissues and foods as pre-column o-phthalaldehyde derivatives by HPLC with fluorescence detection. *J Chromatogr B*. 2014
- Díaz-Flores M, Cruz M, Duran-Reyes G, et al. Oral supplementation with glycine reduces oxidative stress in patients with metabolic syndrome, improving their systolic blood pressure. *Can J Physiol Pharmacol*. 2013; 91:855–860. [PubMed: 24144057]

- El Hafidi M, Pérez I, Zamora J, et al. Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats. *Am J Physiol Regulatory Integrative Comp Physiol*. 2004; 287:1387–1393.
- Foo HL, Loh TC, Lai PW, et al. Effects of adding *Lactobacillus plantarum* I-UL4 metabolites in drinking water of rats. *Pakistan J Nutr*. 2003; 2:283–288.
- Fu WJ, Stromberg AJ, Viele K, et al. Statistics and bioinformatics in nutritional sciences: analysis of complex data in the era of systems biology. *J Nutr Biochem*. 2010; 21:561–572. [PubMed: 20233650]
- Greenberg JA, Boozer CN, Geliebter A. Coffee, diabetes and weight control. *Am J Clin Nutr*. 2006; 84:682–693. [PubMed: 17023692]
- Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radical Res*. 1999; 31:273–300. [PubMed: 10517533]
- He ML, Wang YZ, Xu ZR, et al. Effect of dietary rare earth elements on growth performance and blood parameters of rats. *J Anim Physiol Anim Nutr*. 2003; 87:229–235.
- He QH, Ren PP, Kong XF, et al. Metabolomics and its role in amino acid nutrition research. *Front Biosci*. 2011; 16:2451–2460.
- He QH, Ren PP, Kong XF, et al. Comparison of serum metabolite compositions between obese and lean growing pigs using an NMR-based metabonomic approach. *J Nutr Biochem*. 2012; 23:133–139. [PubMed: 21429726]
- He LQ, Yang HS, Li TJ, et al. Effects of dietary l-lysine intake on the intestinal mucosa and expression of CAT genes in weaned piglets. *Amino Acids*. 2013; 45:383–391. [PubMed: 23722415]
- Hou YQ, Wang L, Zhang W, et al. Protective effects of *N*-acetylcysteine on intestinal functions of piglets challenged with lipopolysaccharide. *Amino Acids*. 2012; 43:1233–1242. [PubMed: 22180025]
- Hou YQ, Wang L, Yi D, et al. *N*-acetylcysteine reduces inflammation in the small intestine by regulating redox, EGF and TLR4 signaling. *Amino Acids*. 2013; 45:513–522. [PubMed: 22532030]
- Huang Z, Chang C. Advances of study on glucose and lipids metabolism of chlorogenic acid regulating. *J Hyg Res*. 2008; 37:637–639.
- Ji L, Jiang P, Lu B, et al. Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism. *J Nutr Biochem*. 2013; 24:1911–1919. [PubMed: 24011717]
- Jiang CY, Yang KM, Yang L, et al. A ¹H NMR-based metabonomic investigation of time-related metabolic trajectories of the plasma, urine and liver extracts of hyperlipidemic hamsters. *PLoS One*. 2013; 8:e66786. [PubMed: 23840531]
- Jobgen WS, Fried SK, Fu WJ, et al. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. *J Nutr Biochem*. 2006; 17:571–588. [PubMed: 16524713]
- Kasai H, Fukada S, Yamaizumi Z, et al. Action of chlorogenic acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxyguanosine formation in vitro and in a rat carcinogenesis model. *Food Chem Toxicol*. 2000; 38:467–471. [PubMed: 10762733]
- Kauppinen RA, Nicholls DG. Synaptosomal bioenergetics. The role of glycolysis, pyruvate oxidation and responses to hypoglycaemia. *Eur J Biochem*. 1986; 158:159–165. [PubMed: 2874024]
- Kong XF, Wu G, Yin YL. Roles of phytochemicals in amino acid nutrition. *Front Biosci*. 2011; S3:372–384.
- Krebs HA. Rate control of the tricarboxylic acid cycle. *Adv Enzym Regul*. 1970; 8:335–353.
- Krebs HA, Lowenstein JM. The tricarboxylic acid cycle. *Metab Pathw*. 1960; 1:129–203.
- Li P, Yin YL, Li DF, et al. Amino acids and immune function. *Br J Nutr*. 2007; 98:237–252. [PubMed: 17403271]
- Li SY, Chang CQ, Ma FY, et al. Modulating effects of chlorogenic acid on lipids and glucose metabolism and expression of hepatic peroxisome proliferator-activated receptor- α in golden hamsters fed on high fat diet. *Biomed Environ Sci*. 2009; 22:122–129. [PubMed: 19618689]
- Li XL, Rezaei R, Li L, et al. Composition of amino acids in feed ingredients for animal diets. *Amino Acids*. 2011; 40:1159–1168. [PubMed: 20842395]

- Liao PQ, Wei L, Zhang XY, et al. Metabolic profiling of serum from gadolinium chloride-treated rats by ^1H NMR spectroscopy. *Anal Biochem.* 2007; 364:112–121. [PubMed: 17386919]
- Lindon JC, Holmes E, Nicholson JK. Pattern recognition methods and applications in biomedical magnetic resonance. *Prog NMR Spectrosc.* 2001; 39:1–40.
- Liu G, Wang Y, Wang Z, et al. Nuclear magnetic resonance (NMR)-based metabolomic studies on urine and serum biochemical profiles after chronic cysteamine supplementation in rats. *J Agric Food Chem.* 2011; 59:5572–5578. [PubMed: 21476594]
- Lou Z, Wang H, Zhu S. Antibacterial activity and mechanism of action of chlorogenic acid. *J Food Sci.* 2011; 76:M398–M403. [PubMed: 22417510]
- Mahaffey KR, Capar SG, Gladen BC, et al. Concurrent exposure to lead, cadmium, and arsenic. Effects on toxicity and tissue metal concentrations in the rat. *J Lab Clin Med.* 1981; 98:463–481. [PubMed: 7288264]
- Marques V, Farah A. Chlorogenic acids and related compounds in medicinal plants and infusions. *Food Chem.* 2009; 113:1370–1376.
- Matilla B, Mauriz JL, Culebras JM, et al. Glycine: a cell-protecting anti-oxidant nutrient. *Nutrición Hospitalaria.* 2002; 17:2–9. [PubMed: 11939124]
- Mauriz JL, Matilla B, Culebras JM, et al. Dietary glycine inhibits activation of nuclear factor kappa B and prevents liver injury in hemorrhagic shock in the rat. *Free Radic Biol Med.* 2001; 31:1236–1244. [PubMed: 11705702]
- Meng S, Cao J, Feng Q, et al. Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. *Evid Based Complement Alternat Med.* 2013
- Mubarak A, Hodgson JM, Considine MJ, et al. Supplementation of a high-fat diet with chlorogenic acid is associated with insulin resistance and hepatic lipid accumulation in mice. *J Agric Food Chem.* 2013; 61:4371–4378. [PubMed: 23586419]
- Park JB. Isolation and quantification of major chlorogenic acids in three major instant coffee brands and their potential effects on H_2O_2 -induced mitochondrial membrane depolarization and apoptosis in PC-12 cells. *Food Funct.* 2013; 4:1632–1638. [PubMed: 24061869]
- Ren WK, Luo W, Wu MM, et al. Dietary l-glutamine supplementation improves pregnancy outcome in mice infected with type-2 porcine circovirus. *Amino Acids.* 2013a; 45:479–488. [PubMed: 22086211]
- Ren WK, Zou LX, Ruan Z, et al. Dietary l-proline supplementation confers immuno-stimulatory effects on inactivated *Pasteurella multocida* vaccine immunized mice. *Amino Acids.* 2013b; 45:555–561. [PubMed: 23584431]
- Rezaei R, Wang WW, Wu ZL, et al. Biochemical and physiological bases for utilization of dietary amino acids by young pigs. *J Anim Sci Biotech.* 2013a; 4:7.
- Rezaei R, Knabe DA, Tekwe CD, et al. Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. *Amino Acids.* 2013b; 44:911–923. [PubMed: 23117836]
- Ruan Z, Lv Y, Fu X, et al. Metabolomic analysis of amino acid metabolism in colitic rats supplemented with lactosucrose. *Amino Acids.* 2013; 45:877–887. [PubMed: 23963536]
- Ruiz-Ramírez A, Ortiz-Balderas E, Cardozo-Saldaña G, et al. Glycine restores glutathione and protects against oxidative stress in vascular tissue from sucrose-fed rats. *Clin Sci (Lond).* 2014; 126:19–29. [PubMed: 23742196]
- Satterfield MC, Wu G. Growth and development of brown adipose tissue: significance and nutritional regulation. *Front Biosci.* 2011; 16:1589–1608.
- Satterfield MC, Dunlap KA, Keisler DH, et al. Arginine nutrition and fetal brown adipose tissue development in diet-induced obese sheep. *Amino Acids.* 2012; 43:1593–1603. [PubMed: 22327565]
- Satterfield MC, Dunlap KA, Keisler DH, et al. Arginine nutrition and fetal brown adipose tissue development in nutrient-restricted sheep. *Amino Acids.* 2013; 45:489–499. [PubMed: 22130738]
- Schnackenberg L, Beger RD, Dragan Y. NMR-based metabolomic evaluation of livers from rats chronically treated with tamoxifen, mestranol, and phenobarbital. *Metabolomics.* 2005; 1:87–94.
- Schulz JB, Lindenau J, Seyfried J, et al. Glutathione, oxidative stress and neurodegeneration. *Eur J Biochem.* 2000; 267:4904–4911. [PubMed: 10931172]

- Shi H, Dong L, Bai Y, et al. Chlorogenic acid against carbon tetrachloride-induced liver fibrosis in rats. *Eur J Pharmacol.* 2009; 623:119–124. [PubMed: 19786014]
- Shi H, Dong L, Jiang J, et al. Chlorogenic acid reduces liver inflammation and fibrosis through inhibition of toll-like receptor 4 signaling pathway. *Toxicology.* 2012; 303:107–114. [PubMed: 23146752]
- Shi H, Dong L, Dang X, et al. Effect of chlorogenic acid on LPS-induced proinflammatory signaling in hepatic stellate cells. *Inflamm Res.* 2013; 62:581–587. [PubMed: 23483217]
- Tan BE, Li XG, Wu G, et al. Dynamic changes in blood flow and oxygen consumption in the portal-drained viscera of growing pigs receiving acute administration of l-arginine. *Amino Acids.* 2012; 43:2481–2489. [PubMed: 22660901]
- Trouillas P, Calliste CA, Allais DP, et al. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. *Food Chem.* 2003; 80:399–407.
- Wan CW, Wong CNY, Pin WK, et al. Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene expression of PPAR- α in hypercholesterolemic rats induced with a high-cholesterol diet. *Phytother Res.* 2013; 27:545–551. [PubMed: 22674675]
- Wang J, Wu G, Zhou H, et al. Emerging technologies for amino acid nutrition research in the post-genome era. *Amino Acids.* 2009; 37:177–186. [PubMed: 19011733]
- Wang QJ, Hou YQ, Yi D, et al. Protective effects of *N*-acetylcysteine on alleviating acetic acid-induced colitis in a porcine model. *BMC Gastroenterol.* 2013a; 13:133. [PubMed: 24001404]
- Wang WW, Wu ZL, Dai ZL, et al. Glycine metabolism in animals and humans: implications for nutrition and health. *Amino Acids.* 2013b; 45:463–477. [PubMed: 23615880]
- Wang W, Dai Z, Wu Z, et al. Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs. *Amino Acids.* 2014
- Wei JW, Carroll RJ, Harden KK, et al. Comparisons of treatment means when factors do not interact in two-factorial studies. *Amino Acids.* 2012; 42:2031–2035. [PubMed: 21547361]
- Wojtczak AB. Control of acetoacetate and β -hydroxybutyrate production in rat liver mitochondria. *Biochem Biophys Res Commun.* 1968; 31:634–640. [PubMed: 5656251]
- Wu G. Functional amino acids in growth, reproduction and health. *Adv Nutr.* 2010; 1:31–37. [PubMed: 22043449]
- Wu, G. Amino acids: biochemistry and nutrition. Boca Raton: CRC Press; 2013a.
- Wu G. Functional amino acids in nutrition and health. *Amino Acids.* 2013b; 45:407–411. [PubMed: 23595206]
- Wu GY, Gunasekara A, Brunengraber H, et al. Effects of extracellular pH, CO₂, and HCO₃[–] on ketogenesis in perfused rat liver. *Am J Physiol.* 1991; 261:E221–E226. [PubMed: 1908188]
- Wu GY, Fang YZ, Yang S, et al. Glutathione metabolism and its implications for health. *J Nutr.* 2004; 134:489–492. [PubMed: 14988435]
- Wu G, Bazer FW, Burghardt RC, et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids.* 2011; 40:1053–1063. [PubMed: 20697752]
- Wu ZL, Satterfield MC, Bazer FW, et al. Regulation of brown adipose tissue development and white fat reduction by l-arginine. *Curr Opin Clin Nutr Metab Care.* 2012; 15:529–538. [PubMed: 23075933]
- Wu X, Zhang YZ, Yin YL, et al. Roles of heat-shock protein 70 in protecting against intestinal mucosal damage. *Front Biosci.* 2013a; 18:356–365.
- Wu G, Wu ZL, Dai ZL, et al. Dietary requirements of “nutritionally nonessential amino acids” by animals and humans. *Amino Acids.* 2013b; 44:1107–1113. [PubMed: 23247926]
- Wu G, Bazer FW, Dai ZL, et al. Amino acid nutrition in animals: protein synthesis and beyond. *Annu Rev Anim Biosci.* 2014; 2:387–417. [PubMed: 25384149]
- Xu Y, Chen J, Yu X, et al. Protective effects of chlorogenic acid on acute hepatotoxicity induced by lipopolysaccharide in mice. *Inflammation.* 2010; 59:871–877.
- Xu XD, Zhang LH, Shao B, et al. Safety evaluation of mesozeaxanthin. *Food Control.* 2013; 32:678–686.

- Yang Y, Li C, Nie X, et al. Metabonomic studies of human hepatocellular carcinoma using high-resolution magic-angle spinning ^1H NMR spectroscopy in conjunction with multivariate data analysis. *J Proteome Res.* 2007; 6:2605–2614. [PubMed: 17564425]
- Yao K, Guan S, Li T, et al. Dietary l-arginine supplementation enhances intestinal development and expression of vascular endothelial growth factor in weanling piglets. *Br J Nutr.* 2011; 105:703–709. [PubMed: 21219670]
- Yun N, Kang JW, Lee SM. Protective effects of chlorogenic acid against ischemia/reperfusion injury in rat liver: molecular evidence of its antioxidant and anti-inflammatory properties. *J Nutr Biochem.* 2012; 23:1249–1255. [PubMed: 22209001]
- Zhang LT, Chang CQ, Liu Y, et al. Effect of chlorogenic acid on disordered glucose and lipid metabolism in db/db mice and its mechanism. *Acta Academiae Medicinae Sinicae.* 2011; 33:281–286. [PubMed: 21718611]
- Zhao Y, Wang J, Ballevre O, et al. Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertens Res.* 2012; 35:370–402. [PubMed: 22072103]
- Zira A, Kostidis S, Theocharis S, et al. ^1H NMR-based metabonomics approach in a rat model of acute liver injury and regeneration induced by CCl_4 administration. *Toxicology.* 2013; 303:115–124. [PubMed: 23146765]

Fig. 1.
a, b Effects of CGA on liver weight and liver index of rats in the control group and CGA group. Values are mean $\pm$ SEM, $n = 6$. **c, d** Histological features of liver sections stained with H&E, where typical images were chosen from the control group and CGA group (original magnification $\times 200$); **c** the liver of rats in the control group; **d** the liver of rats in the CGA group

Fig. 2.

Typical ^1H NMR spectra (600 MHz) of serum obtained from rats in the control group (A) and the CGA group (B), as well as aqueous soluble liver extracts obtained from rats in the control group (C) and the CGA group (D)

Fig. 3.

PCA **a** scores plot and **b** loadings plot from ^1H NMR spectra of serum obtained from rats in the control group (*filled triangle*) and the CGA group (*filled square*). PCA **c** scores plot and **d** loadings plot from ^1H NMR spectra of aqueous soluble liver extracts obtained from rats in the control group (*filled triangle*) and the CGA group (*filled square*)

Table 1

Composition of the experiment diet

Ingredients	Content (%)	Chemical composition	Content
Wheat	14	Digestible energy (Mcal/kg)	3.40
Corn	43	Crude protein (%)	21.0
Soybean meal	24	Crude fat (%)	4.5
Full fat soybean extruded	8	Dry matter	90.0
Soybean oil	1.4	Calcium (%)	1.0
Whey powder	3	Total phosphate (%)	0.7
Fish meal	3.2	Sodium (%)	0.3
Limestone	1.3	Met + Cys (%)	0.78
Dicalcium phosphate	1.1	Lys (%)	1.35
Vitamin-mineral premix ^a	1.0	Thr (%)	0.88

The nutrient levels of the diet were based on China General Quality Standards for Animal Feed (GB14924.1-2001)

^aThe vitamin-mineral premix provided (per kilogram feed): vitamin A, 14,000 IU; vitamin D₃, 1,500 IU; vitamin E, 5 mg; vitamin K, 5 mg; thiamine, 13 mg; riboflavin, 12 mg; pyridoxine, 12 mg; vitamin B₁₂, 0.022 mg; niacin 60 mg; pantothenic acid, 24 mg; biotin, 0.2 mg; folic acid, 6 mg; choline chloride, 350 mg; Fe (as iron sulfate), 120 mg; Cu (as copper oxide), 10 mg; Mn (as manganous oxide), 75 mg; Zn (as zinc oxide), 30 mg; I (as ethylenediamine dihydroiodide), 0.5 mg; and Se (as sodium selenite), 0.2 mg

Table 2

Effects of CGA on growth performance in rats

Group	Initial body weight (g)	Finish body weight (g)	ADG (g/day)	ADFI (g/day)	FCR (g/g)
Control	179.00 ± 3.19	255.80 ± 3.69	2.74 ± 0.10	18.50 ± 1.09	6.74 ± 0.23
CGA	187.00 ± 3.10	265.33 ± 6.58	2.80 ± 0.24	19.59 ± 0.62	7.01 ± 0.37

Values are mean ± SEM, $n = 6$ *ADG* average daily gain, *ADFI* average daily feed intake, *FCR* feed conversion rate (average daily feed intake/body weight gain)

Table 3

Effects of CGA on serum biochemical profiles in rats

Items	Control group	CGA group
ALT (IU/L)	73.0 ± 3.4	74.0 ± 0.9
AST (IU/L)	175 ± 7.3	184 ± 2.5
ALP (U/L)	115 ± 13	111 ± 4.4
TBIL (mg/L)	0.50 ± 0.03	0.52 ± 0.04
Glucose (mmol/L)	4.68 ± 0.20	4.63 ± 0.26
LDH (U/L)	1502 ± 62	1418 ± 57
Albumin (g/L)	20.5 ± 0.9	22.6 ± 0.5
Total protein (g/L)	79.4 ± 1.7	81.3 ± 2.2
Cholesterol (mmol/L)	2.14 ± 0.09	2.30 ± 0.17
Triglyceride (mmol/L)	0.91 ± 0.07	1.01 ± 0.06
LDL (mmol/L)	0.20 ± 0.02	0.18 ± 0.01
HDL (mmol/L)	2.02 ± 0.06	2.21 ± 0.13

Values are mean ± SEM, $n = 6$

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *TBIL* total bilirubin, *LDH* lactate dehydrogenase, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

Table 4

Relative changes in concentrations of serum metabolites in CGA-supplemented rats on the basis of the chemical shift of the methyl group of lactate at δ 1.33

Key	Metabolites	Moieties	$\delta^1\text{H}$ (ppm) and multiplicity	CGA VS control
1	Lipoproteins	$\text{CH}_3, \text{CH}_2\text{CH}_2\text{C}=\text{}$	0.88(m), 1.28(m)	↑
2	L-Leucine	$\alpha\text{CH}, \delta\text{CH}_3, \delta\text{CH}_3$	0.91(d), 0.96(d), 3.72(t)	—
3	L-Valine	$\alpha\text{CH}_3, \beta\text{CH}, \gamma\text{CH}_3$	0.99(d), 1.04(d)	—
4	L-Isoleucine	$\gamma\text{CH}_3, \delta\text{CH}_3$	0.94(t), 1.01(d)	—
5	β -Hydroxybutyrate	γCH_3	1.22(d)	↓
6	L-Threonine	$\alpha\text{CH}, \beta\text{CH}, \gamma\text{CH}_3$	1.32(d), 4.25(m), 3.58(d)	—
7	Lactate	$\alpha\text{CH}, \beta\text{CH}_3$	1.33(d), 4.11(q)	↓
8	L-Alanine	$\alpha\text{CH}, \beta\text{CH}_3$	3.77(q), 1.48(d)	↓
9	Lipids	$\text{CH}_2^*\text{CH}_2\text{CO}, \text{CH}_2-\text{C}=\text{C}$	1.58(m), 2.04(m)	—
		$\text{CH}_2-\text{C}=\text{O}, \text{CH}-\text{O}-\text{CO}$	2.24(m), 2.75(m)	—
10	L-Lysine	$\alpha\text{CH}, \beta\text{CH}_2, \gamma\text{CH}_2, \delta\text{CH}_2$	3.77(t), 1.89(m), 1.73(m)	↓
11	Acetate	$\text{CH}_2-\text{C}=\text{O}$	1.92(s)	—
12	Glycoprotein	$\text{CH}_3-\text{C}=\text{O}$	2.05(s), 2.08(m), 2.15(s)	—
13	Acetoacetate	CH_3, CH_2	2.29(s), 3.49(s)	↓
14	L-Glutamate	$\alpha\text{CH}, \beta\text{CH}_2, \gamma\text{CH}_2$	3.75(m), 2.08(m), 2.37(m)	↓
15	Pyruvate	CH_3	2.37(s)	↓
16	Succinate	$\alpha, \beta\text{CH}_2$	2.41(s)	↓
17	L-Glutamine	$\alpha\text{CH}, \beta\text{CH}_2, \gamma\text{CH}_2$	3.68(t), 2.15(m), 2.45(m)	↑
18	Citrate	CH_2	2.52(d), 2.70(d)	↓
19	Dimethylamine	CH_3	2.71(s)	↑
20	Trimethylamine	CH_3	2.92(s)	↑
21	Albumin	Lysyl- CH_2	3.02(s)	↓
22	Creatine	$\text{N}-\text{CH}_3, \text{CH}_2$	3.04(s), 3.93(s)	↓
23	Choline	$\text{N}-(\text{CH}_3)_3, \alpha\text{CH}_2, \beta\text{CH}_2$	3.20(s), 4.05(t), 3.51(t)	↑
24	GPC	$\text{N}-(\text{CH}_3)_3, \text{OCH}_2, \text{NCH}_2$	3.22(s), 4.32(t), 3.51(t)	↑
25	TMAO	CH_3	3.26(s)	↑
26	Betaine	CH_3, CH_2	3.28(s), 3.90(s)	—
27	Taurine	$\text{N}-\text{CH}_2, \text{S}-\text{CH}_2$	3.27(t), 3.43(t)	—
28	Glycine	CH_2	3.56(s)	↑
29	L-Ornithine	$\text{CH}_2, \alpha\text{CH}$	3.80(s), 3.79(t)	↑
30	β -Glucose	2-CH, 1-CH	3.25(dd), 4.65(d)	—
31	α -Glucose	1-CH	5.24(d)	—
32	Unsaturated lipids	$=\text{C}-\text{CH}_2-\text{C}=\text{}, -\text{CH}=\text{CH}-$	5.19(m), 5.31(m)	↑
33	L-Tyrosine	$\alpha\text{CH}, \text{CH}_2$	7.20(d), 6.91(d)	↑
34	1-Methylhistidine	4-CH, 2-CH	7.05(s), 7.77(s)	↑

Key	Metabolites	Moieties	$\delta^1\text{H}$ (ppm) and multiplicity	CGA VS control
35	Formate	CH	8.45(s)	–

↓ and ↑, the metabolite levels are lower or higher, respectively, compared with the control group; –, the metabolite levels are the same as the control group

CGA VS control the CGA group compared to control group, *s* singlet, *d* doublet, *t* triplet, *q* quartet, *m* multiplet, *dd* doublet of doublets, *TMAO* trimethylamine-N-oxide, *GPC* glycerophosphoryl choline

Table 5

Changes in relative concentrations of endogenous metabolites in aqueous soluble extracts from liver in CGA-supplemented rats on the basis of chemical shifts relative to TMSP at 80.00

Key	Metabolites	Moieties	$\delta^1\text{H}$ (ppm) and multiplicity	CGA VS control
1	Leucine	αCH , δCH_3 , δCH_3	3.72(t), 0.91(d), 0.96(d)	—
2	Isoleucine	γCH_3 , δCH_3	1.01(d), 0.94(t)	↑
3	Valine	αCH_3 , βCH , γCH_3	3.61(d), 2.26(m), 0.99(d), 1.04(d)	—
4	β -Hydroxybutyrate	γCH_3	1.22(d)	—
5	Lactate	αCH , βCH_3	1.33(d), 4.11(q)	↑
6	Threonine	αCH , βCH , γCH_3	1.32(d), 4.25(m), 3.58(d)	—
7	Alanine	αCH , βCH_3	1.48(d), 3.77(q)	—
8	Acetate	$\text{CH}_2\text{—C=O}$	1.92(s)	—
9	Glutamate	αCH , βCH_2 , γCH_2	2.08(m), 2.37(m), 3.75(m)	—
10	Glutamine	αCH , βCH_2 , γCH_2	3.68(t), 2.15(m), 2.45(m)	—
11	Succinate	α , βCH_2	2.41(s)	↑
12	Glutathione	αCH , αCH_2 , βCH_2 , γCH_2	2.55(m), 2.16(m)	↑
13	Methylamine	CH_3	2.61(s)	—
14	Dimethylamine	CH_3	2.72(s)	—
15	Creatine	N—CH_3 , CH_2	3.02(s), 3.93(s)	—
16	Creatinine	CH_3 , CH_2	3.05(s), 4.05(s)	—
17	Choline	$\text{N—(CH}_3)_3$, αCH_2 , βCH_2	3.2(s), 4.05(t), 3.51(t)	—
18	Phosphocholine	$\text{N—(CH}_3)_3$, βCH_2 , αCH_2	3.21(s)	—
19	GPC	$\text{N—(CH}_3)_3$, OCH_2 , NCH	3.23(s)	↓
20	TMAO	CH_3	3.27(s)	↓
21	Betaine	CH_3 , CH_2	3.90(s), 3.28(s)	↓
22	Glycine	CH_2	3.56(s)	—
23	Taurine	N—CH_2 , S—CH_2	3.41(t), 3.26(t)	—
24	β - Glucose	2-CH, 1-CH	3.25(dd), 4.65(d)	—
25	α -Glucose	1-CH	5.22(d)	—
26	Glycogen	CH	5.38–5.45(m), 3.35–3.4(m)	—
27	Uracil	N—CH	7.54(d), 5.81(d)	↓
28	Uridine	CH_2	7.87(d), 5.89(d), 3.92(dd)	↓
29	COP-choline	$\text{N—(CH}_3)_3$, αCH_2 , βCH_2	7.95(s), 5.98(dd), 6.11(dd)	—
30	Adenosine	CH_2	8.22(s), 8.33(s), 6.06(d)	↓
31	Fumarate	CH, CH_3	6.53(s)	—
32	Tyrosine	αCH , βCH_2	7.18(d), 6.88(d)	—
33	Histidine	αCH , βCH_2	7.83(s), 7.08(s)	—
34	Hypoxanthine	N—CH , CH	8.19(s), 8.22(s)	—
35	Formate	CH	8.46(s)	↓

Key	Metabolites	Moieties	$\delta^1\text{H}$ (ppm) and multiplicity	CGA VS control
36	Nicotinate	CH_2	7.53(dd), 8.26(dt), 8.62(dd), 8.95(d)	↑

↓ and ↑, the metabolite levels are lower or higher, respectively, compared with the control group; –, the metabolite levels are the same as the control

CGA VS control the CGA group compared to control group, *s* singlet, *d* doublet, *t* triplet, *q* quartet, *m* multiplet, *dd* doublet of doublets, *TMAO* trimethylamine-N-oxide, *GPC* glycerophosphoryl choline

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 6

Relative changes in concentrations of serum metabolites in CGA-supplemented rats as compared to the control group

Metabolites	NMR chemical shift (δ)	Change in direction	<i>P</i> values
Lipoproteins	0.88, 1.28	↑	0.042
β -Hydroxybutyrate	1.22	↓	0.049
Lactate	1.33, 4.11	↓	0.030
Acetoacetate	2.29	↓	0.037
Pyruvate	2.37	↓	0.015
Succinate	2.41	↓	0.027
Citrate	2.52	↓	0.011
Glycine	3.56	↑	0.017
Unsaturated lipids	5.31	↑	0.029

↓ Indicates a relative decrease, and ↑ indicates a relative increase in the integral value for the region containing the identified metabolite

Significant differences are set at $P < 0.05$

Table 7

Relative changes in concentrations of metabolites in aqueous soluble extracts from liver samples in CGA-supplemented rats as compared to the control group

Metabolites	NMR chemical shift (δ)	Change in direction	<i>P</i> value
Glutathione (reduced form)	2.55, 2.16	↑	0.023

↓ Indicates a relative decrease, and ↑ indicates a relative increase in the integral value for the region containing the identified metabolite

Significant differences are set at $P < 0.05$