

# EFFECTS OF GREEN COFFEE BEAN EXTRACT IN SOME BIOMARKERS OF ADULT BRAZILIAN SUBJECTS

Mirza Adriana de Assis JÁCOME\*  
Sofia Kimi UEHARA\*\*  
Wânia Lúcia Araújo MONTEIRO\*\*\*  
Ricardo Felipe Alves MOREIRA\*\*\*\*  
Carlos Alberto Bastos DE MARIA\*\*\*\*  
Glorimar ROSA\*\*

■ **ABSTRACT:** The purpose of the present study was to analyze the acute effects of the green coffee extracts consumption in some biomarkers of adult Brazilian subjects. Twenty healthy adult subjects between 18 and 35 years old of different sex and ethnic groups took part in the present study. All participants were submitted a 12 hours overnight fast before experiments. Plasma and serum biochemical parameters were measured in distinct intervals after a breakfast standard ingestion and 0.6 L of green coffee bean extract consumption. No statistically differences (*Wilcoxon test*) on serum lipid profile and plasmatic homocysteine concentration were noted after green coffee beverage intake. Caffeine has been associated with increase of the glycaemia in roasted coffee consumers. In the present study, a significant increase ( $p= 0.03$ ) in glycaemia was observed thirty minutes after the green coffee beverage ingestion and, then, there was a tendency of glycaemia maintenance. The low amount of free caffeine found in green coffee matrix could explain the quick stabilization of the glycaemia. The ingestion of green coffee beverage also significantly reduced uricaemia ( $p= 0.03$ ) (*Wilcoxon test*). It is possible that the polyphenols, present in high amounts in this beverage, could act inhibiting the xanthine oxidase enzyme. Therefore, the consumption of green coffee has to stabilize blood glucose 30 minutes after ingestion of test meal, and reduction of uricaemia.

■ **KEYWORDS:** Green coffee; uric acid; glycaemia; homocysteine; lipid profile.

## INTRODUCTION

Coffee is considered one of the most popular beverages consumed in the world due to its pleasant flavor and pharmacological properties.<sup>8</sup> Prospective and epidemiologic studies of green and especially of roasted

coffee consumption has been carried out to investigate its biological effects on lipids, blood pressure and glycaemia.<sup>6, 7, 25, 29</sup> Scientific evidences have demonstrated that green and regular coffee beverages present high antioxidant properties *in vivo* and *in vitro*.<sup>18, 32</sup> Few recent studies have indicated that soluble extracts of green coffee were effective against the high blood pressure in mice<sup>35</sup> and in human.<sup>20, 24</sup> It is possible that its antihypertensive action be related to vasoreactive factors produced and released from the vascular endothelium.<sup>24</sup>

For several years, regular coffee consumption has been associated with high serum cholesterol concentrations, as an important risk factor for the development of atherosclerosis.<sup>41</sup> However, some of the studies reported in the literature have showed conflicting results. It could be attributed to distinct habits associated to the coffee consumption in each population, making difficult to differentiate coffee effects from other dietetic and environmental factors effects.<sup>15</sup> Other studies have explored the relationship between an increased coffee consumption and a decreased risk to develop *diabetes mellitus* type 2.<sup>30, 39</sup> Isogawa et al.<sup>14</sup> confirmed the inverse association of coffee-drinking with the prevalence of fasting hyperglycaemia. Regular coffee intake was also inversely associated to weight gain and increased energy expenditure.<sup>11</sup> Consequently, it seems possible that coffee consumption may decrease diabetes risk by helping individuals to control their body weight. According to Kiyohara et al.,<sup>19</sup> regular coffee drinking may decrease serum uric acid levels. Hyperuricaemia may be related not only to increased risk of clinical gout but also to an increased risk of cardiovascular disease and hypertension. Additional physiological effects of regular coffee-drinking have prompted a great deal of investigation into health consequences.

\* Program of Pos-Graduated in Nutrition – Master's Degree – Institute of Nutrition Josué de Castro – Federal University of Rio de Janeiro – UFRJ – 21941-902 – Rio de Janeiro – RJ – Brazil.

\*\* Department of Nutrition and Dietetic – Institute of Nutrition Josué de Castro – Federal University of Rio de Janeiro – UFRJ – 21941-902 – Rio de Janeiro – RJ – Brazil. E-mail: glorimar@nutricao.ufrj.br

\*\*\* Specialist in Clinical Nutrition – Institute of Nutrition Josué de Castro – Federal University of Rio de Janeiro – UFRJ – 21941-902 – Rio de Janeiro – RJ – Brazil.

\*\*\*\* Department of Bioquímica – Federal University of Rio de Janeiro State – UNIRIO – 21941-902 – Rio de Janeiro – RJ – Brazil.

In the medical literature the main topics reported on coffee studies include metabolic and cardiovascular health effects by regular coffee drinking. On the other hand, researches considering green coffee beans consumption and its metabolic effects are scarce. Thus, the purpose of the present study was to analyze the acute effects of the green coffee extracts consumption in some biochemical parameters monitored in a group of adult Brazilian subjects.

## CASUISTIC AND METHODS

### Methods

#### Reagents

The 5-caffeoylquinic acid (5-CQA) and caffeine were obtained from Sigma Company (St Louis, US). HPLC grade solvents were obtained from Carlo Erba (Milano, Italy). All other reagents were of laboratory grade. The Carrez reagent corresponds to two different solutions.<sup>26</sup> The first one (Carrez 1) is an aqueous solution of bi-hydrated zinc acetate (219 mg mL<sup>-1</sup>) containing 3% (v/v) of glacial acetic acid, while the other (Carrez 2) is an aqueous solution of potassium ferrocyanide (106 mg mL<sup>-1</sup>).

#### Coffee analysis

#### Coffee extracts preparation

The sort of green coffee employed in the study was Arabica Coffea from the south region of Minas Gerais, Brazil (Coffee N4. 2/3 Screen – 16/17, software package). The ground green coffee samples (20 g) suffered extraction with 0.25 L of filtrated water in an electric-drip coffee maker (M&C LC-100, Brazil). Each extraction process took 5 minutes and the maximum temperature reached was 70°C.

Each participant received 0.6 L of green coffee extracts, containing 0.75 g of 5-CQA and 0.27 g of caffeine.

#### HPLC analysis

Each coffee extract was clarified with Carrez 1 and Carrez 2 reagents followed by a subsequent agitation of the sample. Each sample was maintained in rest for 10 minutes, then the volume of the volumetric balloon was completed (0.25 L) with water and the sample was agitated and filtered (Whatmann n° 1) under gravity. To finish, this first solution was filtered again using a Millipore filter (0.45 µm). The filtrates were injected (20 µl) in a reversed-phase column (C-18, 250 mm x 4 mm d.i.; Lichrocart Merck, Darmstadt, Germany) HPLC system with UV detector (Shimadzu LC-10 AD, Kyoto, Japan). The 5-CQA analysis were carried out using a mobile phase (1 mL min<sup>-1</sup>) composed of an aqueous solution of trissodium citrate (0.01 M, pH 2.5) and methanol 60:40 (v/v). The 5-CQA was monitored in a

wave-length of 325 nm. In the caffeine analysis, the mobile phase employed was methanol and water 40:60 (v/v), also with a flow rate of 1 mL min<sup>-1</sup>, under a wave-length of 272 nm. Caffeine and 5-CQA analysis were based on methods previously published.<sup>28,37,38</sup> Peak identification was achieved by comparing the retention times of the compounds in the sample chromatograms with the retention times of the available standards obtained in its chromatograms. Quantification was based on area measurement and comparison with the 5-CQA and caffeine standards.

### Casuistic and Subjects

The current controlled clinical experimental protocol was reviewed and approved by the Clementino Fraga Filho University Hospital Human Ethics Committee. Written consent was obtained from each subject before participation in this study. Different ethnic groups from UFRJ were eligible as follows: they usually drank coffee daily; were between 18 and 35 years of age; had a body mass index < 30 kg/m<sup>2</sup>; were not consuming alcoholic beverage, had no smoking habits, had no history of cardiovascular and gastrointestinal disease, and were not consuming a prescribed diet and were not using medicines of continuous use.

### Study Methods

Height (m) and weight (kg) were obtained according to the technique described by Gibson.<sup>10</sup> Body Mass Index (BMI) was calculated as weight divided by height in meters square (Kg/m<sup>2</sup>). The nutritional diagnosis was carried out accordance to the World Organization of Health.<sup>45</sup> A food ingestion register of three alternate days was used to evaluate the dietetic ingestion of each volunteer. The evaluation of the chemical composition of the dietetic inquiries was carried out through the computational program Food Processor (version 12.0, ESHA Corporation, Oregon, US).<sup>9</sup> Caffeine-containing products (chocolate, chocolate drinks, cola, tea, coffee, mate and certain painkillers) and polyphenolic compounds present in fruits and vegetables were prohibited one day before clinical tests. They also were advised to drink 1.5 L of water, to notify in case of diarrhea, colds and fever to cancel the experiments.

After a 12 hours overnight fast, the samples were collected in tubes containing gel and ethylene diamine tetracetic acid (EDTA) to separate serum or plasma after centrifugation. After collection, samples were centrifuged for 15 minutes at 1209 x g. Kits from Katal® (Minas Gerais, Brazil) and then employed in the analysis of serum uric acid, triglycerides, High Density Lipoprotein cholesterol (HDL-cholesterol) and Total-Cholesterol. In the case of plasmatic glucose, a Celm® kit (São Paulo, Brazil) was used. The above mentioned analysis were based on colorimetric enzymatic methods,<sup>36</sup> except for the HDL-cholesterol analysis that was in agreement with the method developed by Warnick et al.<sup>44</sup> Plasmatic insulin was analyzed by an electrochemiluminescence immunoassay<sup>21</sup>

of the Roche® Laboratory (Tarrytown, US) and a competitive immunoassay method,<sup>40</sup> was employed for the plasmatic homocysteine analysis. After fasting blood collection, participants consumed a standard breakfast consisting of 2 slices of whole-meal bread with low-fat margarine and 0.6 L of a green coffee bean extract with artificial sweeteners (sacarine and cyclamate). For the serum uric acid, triglyceride, HDL-cholesterol, Total-Cholesterol and plasmatic homocysteine analysis, the blood collection was carried out from the fasting point to 180 minutes after the standard breakfast ingestion. Only the fasting point was considered in the plasmatic insulin analysis. The plasmatic glucose analysis was developed using the blood material collected in the fasting point, 30 and 60 minutes after the standard breakfast ingestion.

### Statistical Analysis

Analysis were performed with the statistical program SPSS (Statistical Package for Social Sciences, version 11.0, California State University, US)<sup>33</sup> and *Statistica* (version 6.0 Oklahoma, US).<sup>34</sup> Initially, a descriptive analysis of the data was carried out. All values were expressed as mean  $\pm$  standard deviation (SD). It was employed *Wilcoxon* order marked and *Friedman tests* in order to verify the influence of green coffee beans extracts in the biochemical parameters determined in the above mentioned intervals. Any p-value less than 0.05 was considered significant.

## RESULTS AND DISCUSSION

According to the clinical study, the group was composed of 20 participants, 65% women (n=13) and 35% (n = 7) men. Twelve participants (60%) were caucasian. The mean familiar income presented by the participants was established as 6.3 ( $\pm$  5.3) minimum wages. As observed in Table I, dietetic variables were analyzed and macronutrients have been shown to be in agreement with the dietetic recommendations that prevent cardiovascular risk. The mean values of protein ingestion were larger than 15% of the total calories consumed; the ingestion of carbohydrates corresponded to 50 - 60% of the total calories consumed; the lipid ingestion was from 25 to 35% of the total calories and the amount of total fibers consumed ranged from 20 to 30 g d<sup>-1</sup>. Regarding the biochemical variables, the mean ( $\pm$ SD) concentrations of lipids in the nonfasting serum samples presented adherence to the normal values proposed by the IV Diretriz Brasileira para Dislipidemia e Diretriz para Prevenção para Aterosclerose.<sup>3</sup> It was also observed that the mean ( $\pm$ SD) concentrations of plasmatic glucose was under the normal limits (<110 mg dL<sup>-1</sup>) established by the Brazilian Society of Diabetes (2002). No abnormal uric acid concentrations (< 7.0 mg dL<sup>-1</sup>)<sup>16</sup> and homocysteine concentrations (<15  $\mu$ mol L<sup>-1</sup>)<sup>17</sup> were noted in the subjects of the studied group. In respect to caffeine products, the mean daily consumption of caffeine observed in the participants of the present study (see Table I) was lower than the *per capita* consumption noted in the US (200 mg d<sup>-1</sup>) and Holland (400 mg d<sup>-1</sup>).<sup>2</sup>

Table 1 – Baseline characteristics of the healthy subjects who participated in trial.

Characteristics n=20	Mean	SD ( $\pm$ )
Age (years)	25.30	3.69
BMI (kg/m <sup>2</sup> )	22.98	2.99
<b>Dietary variables</b>		
Coffee (mL)	152.23	25.75
Caffeine (mg d <sup>-1</sup> )	75.56	54.91
Energy (kcal)	2129.91	286.54
Proteins (g d <sup>-1</sup> )	97.44	15.58
Carbohydrates (g d <sup>-1</sup> )	293.73	20.98
Lipid (g d <sup>-1</sup> )	69.83	20.83
Total Fibers (g d <sup>-1</sup> )	22.35	3.71
Soluble Fibers (g d <sup>-1</sup> )	5.03	0.64
Cholesterol (mg d <sup>-1</sup> )	260.79	77.69
Polyunsaturated Fatty acid (mg d <sup>-1</sup> )	8.68	3.89
Monounsaturated Fatty acid (mg d <sup>-1</sup> )	23.63	9.32
Saturated Fatty acid (mg d <sup>-1</sup> )	74.45	10.73
<i>Trans</i> Fatty acid (mg d <sup>-1</sup> )	3.05	2.44
<b>Biochemical variables</b>		
Serum uric acid (mg dL <sup>-1</sup> )	5.10	1.15
Serum total cholesterol (mg dL <sup>-1</sup> )	158.00	32.41
Serum HDL-cholesterol (mg dL <sup>-1</sup> )	46.94	11.04
Serum triglyceride (mg dL <sup>-1</sup> )	66.24	25.07
Plasmatic Insulin ( $\mu$ U mL <sup>-1</sup> )	8.76	5.4
Plasmatic Glucose (mg dL <sup>-1</sup> )	68.35	14.96
Plasmatic Homocysteine ( $\mu$ mol L <sup>-1</sup> )	7.62	2.44

Summary consumption frequency score of daily products.

The occurrence in the literature of researches concerning with coffee intake and biochemical parameters in humans is uncommon. In the present study, no statistically significant difference (*Wilcoxon test*) on serum lipid profile was noted after green coffee beverage intake. On the other hand, scientific literature data have shown conflicting results regarding to regular coffee consumption and lipid profile changes.<sup>5, 12, 15, 46</sup> Differences in the coffee beverage preparation appear to be instrumental in these conflicting outcomes.

Clinical trials have demonstrated that elevated plasmatic concentrations of total homocysteine could be associated to an increased risk of cardiovascular disease.<sup>31</sup> In the present study, no significant differences in the homocysteine concentrations after green coffee intake were found (*Wilcoxon test*). Otherwise, conflicting results have been found with regular coffee intake. Both short and long term studies demonstrated that regular coffee intake increased homocysteine concentrations.<sup>42,43</sup> However, a short term study, using also regular coffee extracts, carried out by Natella et al.<sup>23</sup> did not confirm these results. The studies above mentioned have applied distinct experimental approaches with relevant differences in the coffee species, roasting process and coffee beverage concentrations. For instance, the roasting process could produce either new

compounds or cause the rupture of complex macromolecular structures, releasing compounds that could influence the plasmatic homocysteine concentration. The lack of a standard experimental model could justify the conflicting outcomes observed.

According to Pizziol et al.,<sup>27</sup> caffeine was positively associated to the acute effects on glycaemia during 2 weeks. An appreciable increase in the glycaemia of 2 hours was noted after the consumption of 50 mL of decaffeinated coffee added by 200 mg of caffeine (amount equivalent to 4 cups of coffee). No effect in the glycaemia was observed when volunteers ingested only the decaffeinated coffee. In

the present study, a significant increase in the glycaemia of 30 minutes ( $p= 0.03$ ) (*Friedman test*) was found after green coffee ingestion with the standard breakfast (see Figure 1). After 30 minutes, there was a tendency to glycaemia maintenance. There are two caffeine types in coffee: a free and bound form. The major form of caffeine found in the green coffee beans is the one linked to other compounds like polysaccharides, potassium chlorogenate and proteins.<sup>13</sup> The low amount of free caffeine found in this matrix could explain the quick stabilization of the glycaemia. In spite of the glycaemia variations, the volunteers showed a normal insulin secretion pattern during the trials.

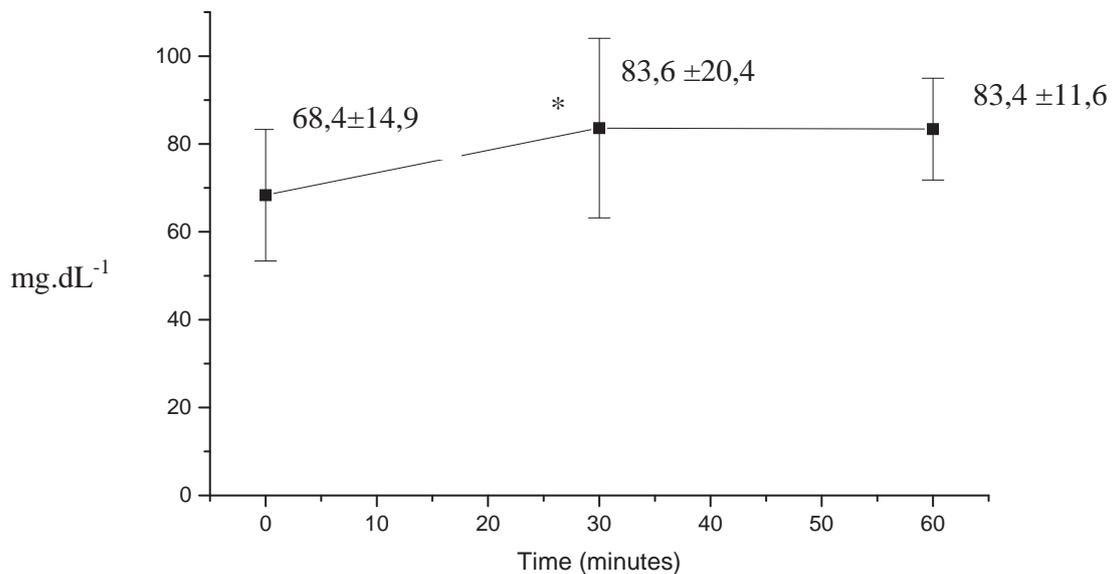


FIGURE 1 – Mean (± SD) plasma glucose concentrations after green coffee ingestion ( $p = 0.03^*$ ).

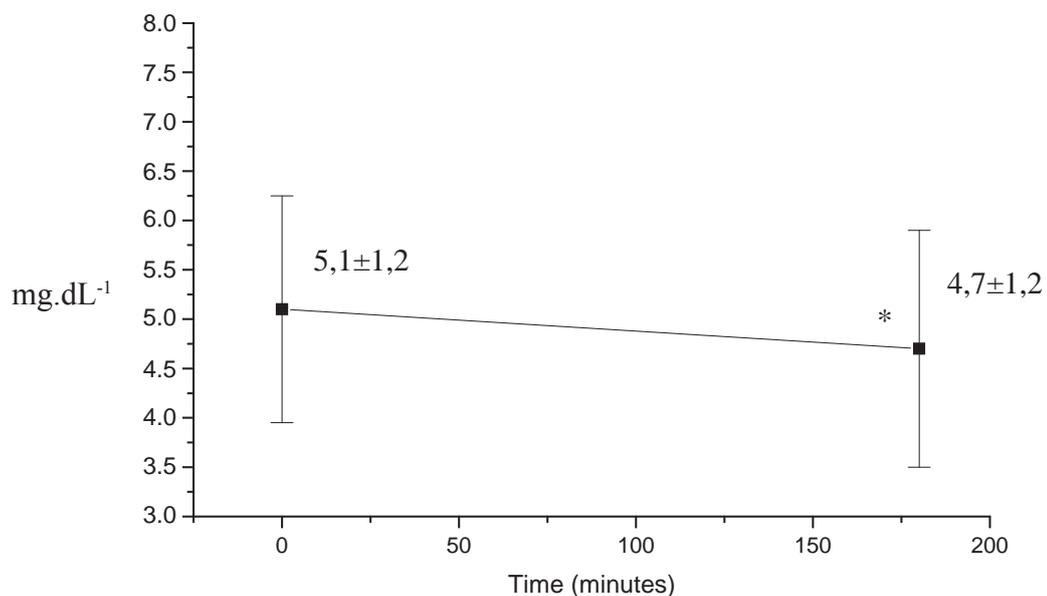


FIGURE 2 – Mean (± SD) serum uric acid concentrations after green coffee ingestion ( $p = 0.03^*$ ).

Uric acid is a product of the catabolism of purines and its overproduction can lead to gout, increased oxidative stress, mutagenesis and probably cancer.<sup>1</sup> Few literature data were described concerning with coffee consumption and serum uric acid concentrations. Reports from literature showed that the serum uric acid concentration in human decreased with instant, regular and decaffeinated coffee intake.<sup>4,19</sup> In the present study, the ingestion of green coffee beverage also reduced uricaemia ( $p= 0.03$ ) (*Wilcoxon test*) (see Figure 2). In previous studies, xanthine oxidase, the enzyme that converts hypoxanthine to uric acid, was inhibited *in vitro* by polyphenols.<sup>1,22</sup> It is possible that the polyphenolic compounds (e.g.: chlorogenic acid) present in high amounts in the coffee matrix could act *in vivo* inhibiting the same enzyme. Further studies are needed to confirm this possible physiological effect of the green coffee beverage.

## CONCLUSIONS

Our results showed that acute consumption of green coffee provides the stabilization of blood glucose 30 minutes after ingestion of the test meal, which would be interesting for the cases of diabetes mellitus. Additionally we observed reduced uricaemia, whereas the high concentrations of uric acid has been identified as cardiovascular risk factor, the green coffee would have an important role in controlling this biomarker.

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■RESUMO: O objetivo deste estudo foi avaliar o efeito do consumo agudo do extrato de café verde em biomarcadores em brasileiros adultos. Participaram do presente estudo 20 indivíduos adultos entre 18 e 35 anos, de ambos os sexos e diferentes etnias. Todos os participantes estavam em jejum de 12 horas antes do experimento. Foram obtidos parâmetros bioquímicos séricos e plasmáticos em intervalos distintos após a ingestão de um lanche padrão e o consumo de 0,6 L de café verde. Não houve diferença estatística (Teste de *Wilcoxon*) no perfil lipídico e nas concentrações de homocisteína plasmática, após o consumo de café verde. Na literatura, a cafeína tem sido associada ao aumento da glicemia em consumidores de café torrado. No presente estudo, foi observado um aumento significativo ( $p= 0,03$ ) da glicemia, 30 min após a ingestão da bebida. A partir de 30 min, houve uma tendência na estabilização da glicemia. A pequena quantidade de cafeína encontrada na matriz do café verde poderia explicar a rápida estabilização da glicemia. A ingestão de café verde também reduziu significativamente a uricemia ( $p= 0,03$ ) (Teste de *Wilcoxon*). Portan-

to, o consumo de café verde proporcionou a estabilização da glicemia após 30 minutos da ingestão da refeição-teste e redução da uricemia.

■PALAVRAS-CHAVE: Café verde; ácido úrico; glicemia; homocisteína; perfil lipídico.

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