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Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects

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SUMMARY

Background: The consumption of unfiltered coffee, containing bioactive diterpenes, causes an increase in plasma homocysteine concentration. A slight increase in plasma homocysteine is also caused by large quantities of filtered coffee. Coffee terpenes also raise plasma glutathione in mice.
Aim: To verify the effect of Italian-style coffee consumption on the plasma concentration of glutathione and homocysteine in healthy subjects.
Methods: Twenty-two volunteers consumed five cups of coffee per day for 1 week and maintained their usual diet. Five subjects were enrolled as controls. The intervention trial was preceded and followed by seven coffee-free days.
Results: Plasma glutathione increased by 16% ($P < 0.05$) on coffee consumption, and returned to the original concentration after the washout period. The increase in plasma homocysteine concentration (13% after 1 week of coffee intake) was not significant. No differences in glutathione or homocysteine concentration were observed in the control group. No variation of plasma hydroperoxide concentration was detectable.
Conclusions: A coffee intake regimen, representing the average consumption of coffee drinkers in Italy, increased the plasma concentration of glutathione, but no significant increase in the plasma homocysteine concentration was detected.

INTRODUCTION

Coffee consumption is very popular in Europe, as well as in the USA and Japan, but the type of coffee beverage is strictly associated with the social habits and culture of individual countries. Differences in raw bean composition, roasting conditions and extraction procedures used to prepare coffee result in a great diversity of chemical compositions of the final product. In addition, the size of a single serving is enormously variable, and can derive from the brewing of an amount of roasted coffee ranging from as little as 5 g up to 15 g or more.[1]

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Little is known with certainty about the physiological effects of coffee, but a bewildering array of health and mood effects are attributed to it. In addition to the action of caffeine, it has been demonstrated that the lipid fraction of coffee, which includes two bioactive diterpenes, kafestol and kahweol, is able to increase low-density lipoprotein and total serum cholesterol.[2] The presence of these substances depends on both the variety of coffee and the extraction procedure used for preparation. Soluble coffee and beverages obtained by percolation contain a small amount of diterpenes, espresso and moka a moderate amount, and Turkish and boiled coffee a large amount.[3] Recent studies have suggested that a 10% higher concentration of homocysteine (Hcy) is associated with unfiltered coffee consumption, but it is not clear whether the lipid fraction is

the only factor responsible for the increase in Hcy concentration.[4] A recent paper has correlated the increase in Hcy to the intake of chlorogenic acids, the main phenolic compounds present in green coffee beans.[5] Chlorogenic acids are strongly degraded during roasting, and probably become part of the brown, water-soluble polymer, called coffee melanoidin, which constitutes up to 30% of coffee dry matter. Urgert *et al.* observed an increase in Hcy concentration on heavy intake (1 L/day) of filtered coffee.[6]

The stimulation of glutathione transferase (GST) activity by coffee components has been reported since 1983,[7] and recently it has been shown that kafestol and kahweol can increase the glutathione (GSH) concentration in mice.[8] Molecular evidence has been provided of the ability of some phenolic compounds to activate γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis.[9] Abraham and Singh have suggested that the increase in GSH concentration induced by plant bioactive secondary metabolites contributes to the chemo-prevention against environmental carcinogens.[10]

Several authors have reported that coffee intake decreases the concentration of γ-glutamyltransferase, an enzyme which is a marker of liver damage usually induced by alcohol abuse.[11, 12] Moreover, epidemiological data have confirmed a negative association between coffee consumption and the incidence of liver disease in alcoholics.[13] The effect is probably related to an increase in GSH concentration, which is dramatically reduced in alcoholics.[14]

This study aims to verify the effects of coffee intake on the plasma concentrations of Hcy and GSH in healthy subjects. An 'Italian-style' coffee regime was analysed, preparing coffee as moka (usual form of domestic coffee intake) or espresso.

SUBJECTS AND METHODS

Study design

The study was conducted according to Good Clinical Practice Guidelines at the Department of Food Science of the University of Napoli 'Federico II' and at the diagnostic centre 'Sanciro' in Portici, Napoli. Subjects were recruited from the students of the Faculties of Agriculture and Biotechnology. The study protocol was fully explained to the volunteers and they gave written informed consent.

The study population included 27 healthy subjects (22 treated and five controls) who had not taken any medication over the previous 4 weeks. Of the 22 treated subjects, three had an Hcy concentration above 20 μmol/L and were eliminated from the study, and one did not complete the experimental plan for personal reasons unrelated to coffee consumption. Therefore, all data refer to 18 treated and five control subjects. The characteristics of the subjects are illustrated in Table 1.

Throughout the 3 weeks of the study, subjects were asked not to change their dietary habits and to maintain an average coffee consumption of five cups per day for 7 days. A coffee-free period of 1 week was observed before and after the week of coffee intake. During the 3 weeks of the intervention trial, subjects were asked to record the intake of barley brew and chocolate, which was negligible for all subjects. Tea and cola were prohibited throughout the 3 weeks of the study.

Blood was taken at noon, a minimum of 4 h and a maximum of 5 h after breakfast and the last intake of coffee.

The overall intake of vegetable antioxidants was monitored by alimentary questionnaires. The average consumption of fruit and vegetables was 3.5 ± 1.4 servings/day, with no significant difference between the treated and control groups.

Analytical methods

High performance liquid chromatography (HPLC) determination of Hcy and GSH. The total plasma concentrations of Hcy and GSH were determined by HPLC with fluorimetric detection, according to Atsushi and Sako.[15] The total thiol compounds in plasma (free and bound to proteins) were reduced by treatment with tri-n-butylphosphine and derivatized by a thiol-specific fluorogenic reagent, namely ammonium 7-fluoro-benzo-2-oxa-1, 3-diazole-4-sulphonate (Wako, Germany). The

Table 1. Characteristics of the subjects investigated

	Treated subjects	Controls
n	18	5
Male/female	7/11	2/3
Age range (years)	19–25	20–27
Body mass index*	Male: 24.7 ± 2.9 Female: 22.8 ± 5.4	Male: 23.0 ± 1.9 Female: 22.2 ± 2.4
Smokers/ non-smokers	9/9	2/3

* Body mass index $= \frac{\text{weight (kg)}}{h^2\,\text{(mt)}}$

derivatives were analysed by reversed-phase HPLC. Chromatography was performed at room temperature using the following solvent system: (A) 0.1 M acetate buffer, pH 4.0, containing 2% methanol (v/v); (B) 0.1 M phosphate buffer, pH 6.0, containing 5% methanol (v/v). HPLC separation was carried out at a flow rate of 1 mL/min using a Prodigy ODS2 5 μm (250 × 4.6 mm i.d.) column. Elution was carried out by a linear gradient from 0% solvent B to 100% solvent B in 15 min.

The quantification of single compounds was achieved by a calibration curve obtained using pure Hcy and GSH as external standards (Sigma Chemicals, St. Louis, MO, USA).

The results are expressed in micromoles of Hcy and micromoles of GSH per litre of plasma.

Oxidative status. The oxidative status was evaluated by measuring plasma hydroperoxides by the D-Roms test (Diacron, Italy). The assay was carried out as described previously.[16, 17] Briefly, 5 μL of plasma was added to an *N*,*N*-diethyl-*p*-phenylenediamine solution in acid medium (acetate buffer, pH 4.8). In these conditions, serum hydroperoxides react through the Fenton reaction with the iron released by serum transport proteins, developing a purple colour due to the formation of the radical cation of this chromogen.[18] The purple colour resulting from this reaction was then monitored at 505 nm in an ECOM F 6124 Eppendorf photometer (purchased from Diacron, Italy). The kinetic of colour development is proportional to the amount of hydroperoxides in the 5 μL serum sample used in the assay and provide a measure of the oxidative status.[16]

The results are expressed in arbitrary units (Carr units, U. Carr), each corresponding to a concentration of 0.08% of hydrogen peroxide. The normal range of this method, tested over a large population of healthy controls, is 250–300 U. Carr.[16]

Data analysis and statistics

Data were expressed as means ± s.d., unless otherwise indicated. A *t*-test was performed to compare the mean plasma concentrations of Hcy and GSH in the 23 healthy volunteers (18 subjects and five controls) at the end of both the coffee and coffee-free periods. Differences in plasma oxidative status were calculated per subject and also analysed using *t*-test. All statistical analyses were performed using the SPSS (version 8) package for Windows. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

The intake of five cups of coffee per day was tolerated well by all subjects. There was no discontinuation because of side-effects caused by coffee intake. The 18 subjects completing the study had an average intake of 5.1 ± 0.4 cups of coffee per day (3.6 moka and 1.5 espresso; moka, 40–50 mL/cup; espresso, 25–35 mL/cup). Decaffeinated coffee intake was 24% of the total. The total serum protein concentration did not change in subjects in either group during the trial.

In Table 2 and Figure 1, the data obtained on the plasma concentration of GSH are shown. A significant increase after the week of coffee intake was observed. The average concentration of GSH changed from 4.1 ± 1.4 μmol/L at the end of the first coffee-free period to 5.0 ± 1.2 μmol/L after 7 days of coffee consumption, that is an increase of 16%. The GSH concentration decreased to 4.3 ± 1.1 μmol/L at the end of the second coffee-free week. No variation in plasma GSH concentration was detectable in the control group. The trend was fully confirmed when the GSH concentration was corrected for the total serum protein concentration.

Table 2. Effect of a habitual regime of Italian-style coffee consumption (espresso and moka) on the plasma concentration of glutathione (GSH)

	Plasma total GSH of treated subjects ($n = 18$)		Plasma total GSH of control subjects ($n = 5$)	
	μmol/L	nmol/g protein	μmol/L	nmol/g protein
First coffee-free period	4.1 ± 1.4*	66 ± 22*	4.1 ± 0.8	65 ± 13
Coffee period	5.0 ± 1.2	81 ± 19	4.2 ± 0.6	69 ± 10
Second coffee-free period	4.3 ± 1.1*	71 ± 18*	4.2 ± 0.7	71 ± 12

Data represent the mean of three values ± s.d.
* $P < 0.05$ vs. coffee period.

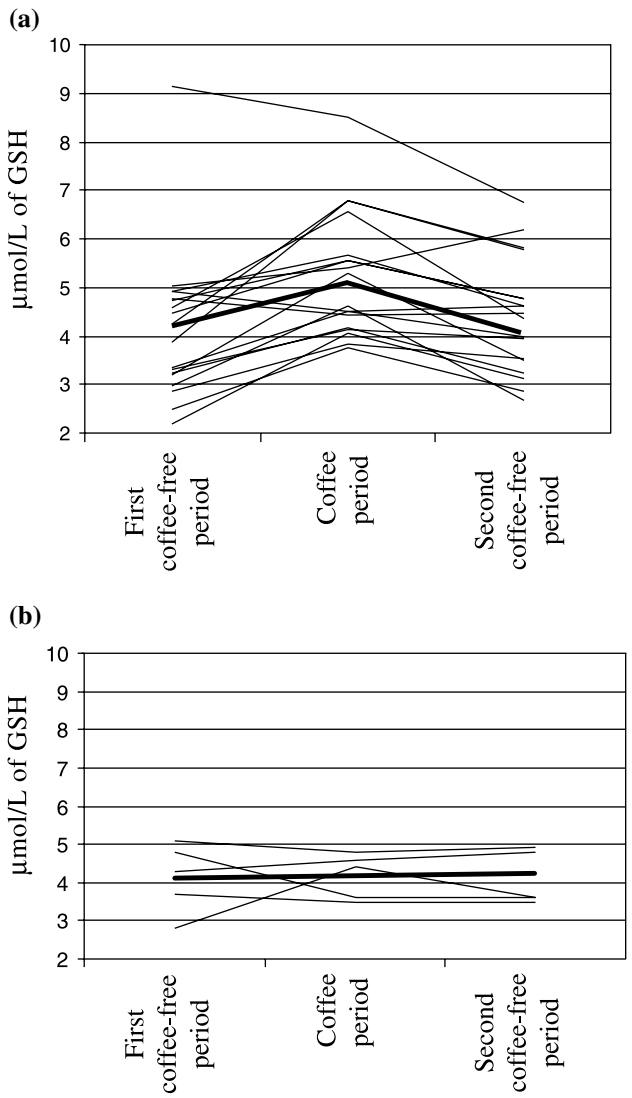

Figure 1. Time course of glutathione (GSH) concentration in the 23 subjects completing the study. The mean values are shown by the bold line. (a) Treated subjects. (b) Controls.

The plasma concentration of Hcy is reported in Table 3. The ingestion of coffee caused a slight increase in the plasma concentration of Hcy. The average Hcy concentration changed from 10.1 ± 2.6 μmol/L for the first blood sample to 11.4 ± 3.3 μmol/L after 7 days of coffee consumption, but this increase was not significant. The concentration of plasma Hcy was still 11.4 ± 3.9 μmol/L after the second coffee-free period. No variations in plasma Hcy were observed in the control group ($n = 5$), whose average values during the observation period were 11.8 ± 2.0, 12.2 ± 1.9 and 12.0 ± 1.6 μmol/L, respectively.

Table 3. Effect of a habitual regime of Italian-style coffee consumption (espresso and moka) on the plasma concentration of homocysteine (Hcy)

	Plasma total Hcy (μmol/L)	
	Treated subjects ($n = 18$)	Controls ($n = 5$)
First coffee-free period	10.1 ± 2.6	10.8 ± 1.4
Coffee period	11.4 ± 3.3	10.8 ± 1.9
Second coffee-free period	11.4 ± 3.9	10.2 ± 1.5

The oxidative status of plasma was monitored by measuring the plasma hydroperoxide. The average values were 276 ± 74 U. Carr before coffee intake, 265 ± 68 U. Carr after 1 week of coffee consumption and 257 ± 49 U. Carr after the washout period. No significant variation of this parameter was observed during the whole trial.

DISCUSSION

More than 90% of the research carried out on the physiological properties of coffee has been devoted to caffeine. However, coffee is much more than a caffeine solution, and it has been demonstrated that many physiological effects related to the intake of coffee are absolutely independent of caffeine.[19]

The aim of this study was to verify the effect of a moderate intake of Italian-style coffee on the plasma concentration of GSH and Hcy. According to D'Amicis *et al.*, the average intake of Italian coffee drinkers is 5.5 cups per day, with 70% domestic consumption (i.e. moka) and 30% espresso coffee.[20] We performed a 3-week trial consisting of the consumption of five cups of coffee per day for 1 week, preceded and followed by a 7-day coffee-free period. Olthof *et al.* reported that 7 days were sufficient to observe an increase in plasma Hcy due to the ingestion of chlorogenic acid,[5] whereas Wattemberg and Lam showed that 5 days were sufficient to detect GST induction by coffee terpenes in rats.[21]

The results demonstrate that 1 week of coffee intake, in an amount representing the average consumption for Italian coffee drinkers, significantly increases (16%, $P < 0.05$) the plasma concentration of GSH. This effect was also observed when the values were normalized for serum protein content, thus ruling out a confounding action due to the possible diuretic effect of caffeine.[22]

The increase in GSH was not coupled with a corresponding increase in oxidative stress, measured as plasma hydroperoxide concentration. This finding suggests that the activation of the GSH synthetic pathway does not occur as a consequence of an increased production of free radicals.

The concentration of plasma GSH reflects the intrahepatic concentration,[23] and, for this reason, dietary compounds that can increase the plasma GSH concentration may be of great importance to human health. It has been reported that agents increasing the pool of GSH in cells act as inhibitors of carcinogenesis.[7] An increase in GSH concentration may be useful for the therapeutic treatment of chronic hepatitis C and cirrhosis, where, in some cases, a shortage of GSH is observed.[23–26]

The ability of coffee to increase GSH and GST activity in the liver of mice was observed by Abraham and Singh.[10] These authors demonstrated that coffee constituents other than caffeine are responsible for the marked anti-carcinogenic effect of coffee, and their results confirmed the importance of coffee in the chemoprevention of environmental carcinogenesis.

An increase in GSH concentration was observed in rats exposed for 10 days to the coffee bioactive terpenes kafestol and kahweol.[8] The authors correlated the increase in GSH with the activation of γ-glutamylcysteine synthetase, which is the rate-limiting enzyme of GSH synthesis. Interestingly, in these animals, the increase in GSH concentration was independent of the oxidative stress, as observed in our study. Grubben *et al.*, in a study aimed to verify the protective effect of unfiltered coffee consumption against colon cancer, found that the intake of unfiltered coffee increased the GSH concentration in plasma by 15% and in colorectal mucosa by 8%.[27] However, it is unlikely that the effect on GSH synthesis was attributable only to kafestol and kahweol. Data from Wattemberg and Lam have demonstrated that bioactive diterpenes only account for 40% of the GST-inducing activity in mouse liver.[21] In the same paper, they showed that the roasting procedure, which causes a severe degradation of chlorogenic acid, does not affect GST activation.

Corrao *et al.* found a strong epidemiological relationship between coffee consumption and liver disease in alcoholics.[13, 28] A heavy intake of coffee reduced the incidence of cirrhosis. Also, in this case, data have shown that the effect is not due to caffeine, but rather to other factors which probably include different ingredients of coffee and lifestyle factors correlated with coffee consumption.

It is of great interest to ascertain which is the main coffee component responsible for the increase in GSH concentration in our experimental conditions. The amount of kahweol and kafestol present in espresso and moka preparations is quite low (between 1.0 and 2.3 mg/cup according to Urgert and Katan[3]), and therefore their relevance in our experimental trial must be marginal. Moreover, bioactive terpenes are almost absent in filtered coffee; therefore, their relevance to coffee consumers in the majority of the population is negligible.

Coffee has an exceptionally high antioxidant activity.[29] The antioxidant activity is due to melanoidins and phenolic compounds, which are probably responsible for the anti-genotoxic and anti-mutagenic effects of coffee.[30, 31]

Green beans are extremely rich in phenolic compounds (mainly chlorogenic acids), which are largely degraded during processing. During roasting, some of the phenolic compounds are incorporated into the brown, water-soluble polymer, called coffee melanoidin.[32, 33]

Melanoidins, which are formed through the Maillard reaction between protein and carbohydrates, are largely present in roasted coffee and are the major component of coffee beverages (up to 30% of dry matter). They have a polysaccharide backbone which serves as a carrier for several potential bioactive chemical groups, such as phenolic rings,[34, 35] reductones[36] and stable radicals.[37] Questions have been raised about whether coffee melanoidins influence physiological parameters, as their rate of intestinal absorption has been estimated to be very low.[38]

The plasma Hcy concentration did not show a significant variation during this study. An increase of Hcy was detectable (13%), but not significant. In addition, the 7-day coffee-free period did not restore the average value observed at the beginning of the experiment. Our data do not confirm those reported by Urgert *et al.*[6] The amount of coffee intake was lower in our study (five cups instead of 1 L) and the length of the study was shorter (1 week vs. 4 weeks); however, an increase in plasma Hcy concentration close to the borderline of statistical significance was observed.

There is considerable evidence indicating the importance of phenolic compounds in the human diet. The possibility that fundamental parameters, such as GSH concentration or GST activity, are related to their intake, represents an attractive perspective for nutrition

and biochemical research. Coffee components, because of the world-wide consumption of coffee, may have a pivotal role, in particular in human diseases such as chronic hepatitis, where a dramatic depletion of endogenous GSH occurs.

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