FOOD & FUNCTION

Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: Results from an intervention study

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Epidemiological and experimental evidence increasingly suggests coffee consumption to be correlated to prevention or delay of degenerative diseases connected with oxidative cellular stress. In an intervention study comprising 33 healthy volunteers, we examined DNA-protective and antioxidative effects exerted in vivo by daily ingestion of 750 mL of freshly brewed coffee rich in both green coffee bean constituents as well as roast products. The study design encompassed an initial 4 wk of wash-out, followed by 4 wk of coffee intake and 4 wk of second wash-out. At the start and after each study phase blood samples were taken to monitor biomarkers of oxidative stress response. In addition, body weight/composition and intake of energy/nutrients were recorded. In the coffee ingestion period, the primary endpoint, oxidative DNA damage as measured by the Comet assay (±FPG), was markedly reduced ($p<0.001$). Glutathione level ($p<0.05$) and GSR-activity ($p<0.01$) were elevated. Body weight ($p<0.01$)/body fat ($p<0.05$) and energy ($p<0.001$)/nutrient ($p<0.001$–0.05) intake were reduced. Our results allow to conclude that daily consumption of 3–4 cups of brew from a special Arabica coffee exerts health beneficial effects, as evidenced by reduced oxidative damage, body fat mass and energy/nutrient uptake.

Keywords:
DNA damage / Fresh coffee brew / Glutathione status/reductase / Intervention study / Weight reduction

Epidemiological evidence suggests coffee consumption to be associated with prevention or delay of degenerative diseases including diabetes type 2, cardiovascular disease and cancer [1]. These beneficial effects have been attributed in part to the antioxidant activity of coffee [2]. Additionally, moderate reduction in body weight was reported to correlate with regular coffee consumption [3, 4].

Coffee brew is a complex mixture of bioactive compounds, representing original coffee constituents such as caffeine, caffeoyl quinic acids (CQAs) and trigonelline, together with compounds formed during roasting, such as *N*-methylpyridinium (NMP), nicotinic acid, nicotinamide and melanoidins [5]. The antioxidant effectiveness of coffee observed in vitro and in vivo has mainly been ascribed to CQAs, NMP and other Maillard products, constituents found to act as radical scavengers, to exhibit metal chelating activity and/or to induce the expression of antioxidant enzymes [6].

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Abbreviations: ACN, acetonitrile; **5-CQA,** 5-caffeoyl quinic acid; **FPG,** formamidopyrimidine-DNA *N*-glycosylase; **GSH,** reduced glutathione; **GSR,** glutathione reductase; **GSSG,** oxidised glutathione; **NMP,** *N*-methylpyridinium; **tGSH,** total glutathione

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In addition, a reduction in body weight was found associated with coffee consumption and the constituents caffeine and CQAs [3, 4]. Moreover, dark roast coffee was found more effective in reducing body weight than light roast (Kotyczka et al., unpublished data). These findings suggested that coffee blends containing high concentrations of green bean as well as roast constituents may be particularly effective in elevating antioxidant defense, supporting body weight control and exerting various further beneficial health aspects relevant to prevention or delay of degenerative diseases (Supporting Information, Introduction).

The aim of the present study therefore was to characterize the DNA-protective and antioxidant potential and beneficial health effects of a coffee brew, rich in both roast products and green coffee bean constituents. In a 12-wk intervention study with 33 healthy probands, biomarkers of DNA damage and redox-sensitive cell response, including glutathione (GSH) level/status and activity of glutathione reductase (GSR) were determined in blood. Additionally, coffee-mediated alteration of body weight/composition and food intake was monitored. To ascertain the bioavailability of NMP, plasma and urine samples were analyzed for NMP and for potential NMP metabolites.

The study was approved by the local ethics committee of Rheinland-Pfalz, no. 837.207.08 (6204). Male healthy non-smoking probands ($n = 33$, age 20–44, BMI 19–32) were recruited. Exclusion criteria were smoking, obesity (BMI > 32), use of medication and chronic diseases. The 12-wk intervention study was designed as follows: weeks 1–4, 1st wash-out; weeks 5–8, coffee uptake; and weeks 9–12, 2nd wash-out. The probands consumed, daily, 750 mL of freshly brewed black filtered coffee (with/without sugar) in three equal portions (morning, noontime, afternoon). In the wash-out phases, the coffee brew was replaced by equal volumes of water. Food records on all intakes during 7-day periods were completed by the participants in the last week of each study phase and in the second week after the end of the study (Supporting Information, subjects and study design). Anthropometric measurements and urine/blood sampling were performed at the beginning of the study and at the last day of each study phase in the morning. Two hours after consumption 250 mL of coffee/water, urine was collected and blood samples were drawn from the participants (Supporting Information, anthropometric measurements and sampling).

The study coffee consisted of a special roasted and blended Arabica coffee rich in both green and roast bean constituents, especially in chlorogenic acids and NMP. Prior to the intervention, the ground coffee was portioned into vacuum packed aliquots of 29.5 g/package each. The coffee was freshly brewed in a conventional coffee filter machine immediately before each individual 250-mL consumption. The coffee brew was analytically characterized using HPLC-DAD and HPLC-MS/MS [7] (Supporting Information, preparation and analysis of the coffee brew studied).

Determination of biomarkers DNA damage was determined by alkaline single cell gel electrophoresis (comet assay) with/without treatment by formamidopyrimidine-DNA *N*-glycosylase (FPG) [8, 9] and was expressed as mean tail intensity (TI%) from two gels (DNA damage ± FPG).

Total glutathione (tGSH: GSH+GSSG) and GSSG (oxidised glutathione) were measured in blood in a kinetic assay by photometric determination of 5-thio-2-nitrobenzoate (TNB) [9]. Results were expressed as μmol GSH and were used to calculate GSH and glutathione status (GSH status = GSH in % of tGSH).

GSR activity in erythrocytes was determined according to [10] as nmol NADPH oxidized/min/mg Hb and expressed in percentage of the value measured at first blood sampling (Supporting Information, determination of biomarkers).

Quantitation of NMP in human spot urine, plasma and in coffee brew was performed by stable isotope dilution analysis and LC-MS/MS. Briefly, an aliquot (100 μL) of plasma or urine (250 μL) was mixed with d_3-NMP iodide (2 nmol/mL), vortexed and diluted with ACN (300 μL). The clear supernatant obtained after centrifugation (12000 rpm, 4°C, 20 min) and a 1/10 dilution with ACN, respectively, was injected (5 μL) into the LC-MS/MS system (Supporting Information Tables 1 and 2, quantitative analysis of NMP).

Intake of nutrients/kcal was assessed (7-day food records) using the nutrition software package PRODI 5 Expert and other sources and presented in percentage of recommended reference values for nutrient intake (D-A-CH, 1st edition; German Nutrition Society, DGE, Bonn, Germany).

Results are reported as means and SD. The Anderson–-Darling test was used for the analysis of normal distribution. Differences of parameters between the study phases (normally distributed results) were analyzed with one-sided paired *t*-test. Differences without normal distribution were analyzed by one-sided paired Wilcoxon test. Independent predictors (Supporting Information Table 3) were analyzed by analysis of variance (ANOVA). The potential influence of study phases or probands was tested by two-way ANOVA. For analyzing the effects of phases, probands and BMI/age, three-way ANOVA was applied.

Study coffee characteristics Concentrations of thermally labile green coffee bean constituents, such as CQAs (580 mg/L) in the study coffee brew were slightly lower than those reported in the literature for commercial filter coffee [7]. The concentrations of NMP (72 mg/L) were in the upper range of dark roasts [5]. The caffeine level (720 mg/L) was in the usual range of conventional coffee brews. Thus, the study coffee is characterized by its specific composition (Supporting information Table 2).

Urine and plasma analysis NMP has been reported to be rapidly eliminated from plasma ($t_{1/2}$ about 2 h), urinary excretion (8 h) accounting for about 62–70% of the ingested dose [11]. After the first wash-out, no NMP was detected in plasma and urine (1 outlier), after the second levels were below the limit of quantitation (10 pmol/mL, 50 fmol absolute amount, 2 outliers). After the coffee phase, NMP was detected

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in plasma (1.1 nmol/mL) and urine (389.3 nmol/mL), demonstrating its high bioavailability (Supporting Information Fig. 1).

Modulation of DNA damage Figure 1 shows DNA damage − FPG and DNA damage + FPG in white blood cells. At the end of the first wash-out phase, both parameters were significantly elevated, as compared to baseline. Coffee intervention resulted in marked decrease in DNA damage − FPG (by 39%) and of DNA damage + FPG (by 44%). After the second wash-out phase, both parameters increased again, without reaching the level at the end of the first wash-out. In all study phases, percentage TI values of DNA damage + FPG were four- to five-fold higher as compared to the respective values of DNA damage − FPG.

Thus, oxidative DNA damage in white blood cells largely accounts for the overall DNA damage observed, in line with earlier findings by our group [9] (Supporting Information Fig. 2). In previous human intervention trials with coffee brew, a decrease in DNA damage + FPG was also reported, whereas DNA damage − FPG was not found modified [12, 13] (Supporting Information, modulation of DNA damage).

Glutathione level and GSR activity Blood samples showed large inter-individual variations in levels of tGSH, GSSG and GSH at the start of study (range: 556–1057 μM tGSH, 53.5–123.5 μM GSSG). Participants with BMI > 25 exhibited distinctly lower tGSH levels, as compared to the group with BMI < 25 (790.9 μM ± 137.1, $n = 17$, versus 901.2 μM ± 130.2, $n = 16$: $p < 0.01$). Thus, tGSH levels were inversely correlated to BMI values (linear regression analysis: $R = -0.448$). This is in line with previous reports on lower erythrocyte glutathione levels in obese subjects, compared to non-obese controls [14] and with an inverse correlation between GSH levels and body weight/BMI [15].

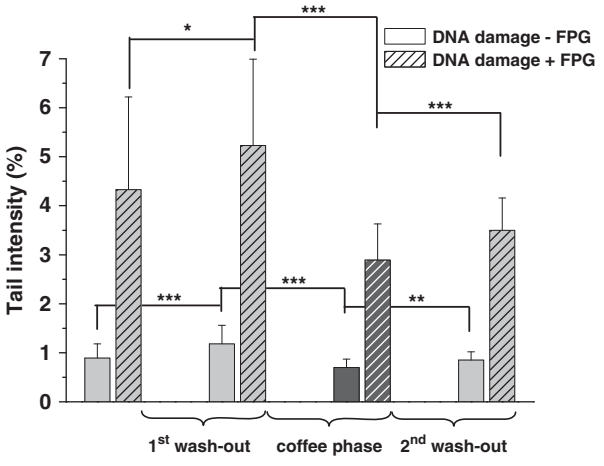

Figure 1. DNA damage ± FPG in lymphocytes at start of study and after the study phases. Data are expressed as TI% (tail intensity in percentage) showing means and SD; significantly different DNA damage − FPG: $^{**}p < 0.01$, $^{***}p < 0.001$(one-sided, paired Wilcoxon test); significantly different DNA damage + FPG: $^{*}p < 0.05$, $^{***}p < 0.001$(one-sided, paired *t*-test).

After the first wash-out, levels of tGSH and GSH were found slightly decreased, those of GSSG elevated (Fig. 2). In the coffee phase, levels of tGSH and GSH increased significantly, whereas the GSSG level decreased slightly (not significantly).

Similar to our results, in a trial with healthy subjects, consumption of espresso and mocha was found to result in elevated blood GSH levels [16]. Of note, in the present study, participants with BMI < 25 ($n = 16$) exhibited significantly higher tGSH levels after coffee ingestion than the group with BMI > 25 ($n = 17$): $p < 0.01$. The activity of GSR showed a significant increase in the coffee phase (Fig. 2). The GSH status (GSH in % of tGSH) was also elevated (from 80.0 to 81.1%), but this did not reach significance. Underlying causes for the differential responses of probands with BMI < 25 and those > 25 with respect to tGSH have yet to be uncovered.

Cellular levels of GSH are influenced by multiple factors, including the activities of γ-glutamyl cysteine ligase (γ-GCL). Coffee constituents (e.g. CQAs, NMP) and coffee extracts have been found to increase the expression/activity of γ-GCL, GSR, glutathione transferase (GST) and other antioxidant response element (ARE)-dependent enzymes [6, 17]

Body weight and body composition Results of anthropometric measurements showed a slight reduction of body weight and body fat in the first wash-out phase (Table 1). In the coffee phase, these were found more distinctly diminished (by 0.62 ± 1.2 kg and 0.68 ± 1.9 kg, respectively), followed by a slight re-increase in the second wash-out. In the group of lean probands (BMI < 25), the loss of body weight and body fat was significant and more distinct ($p < 0.001$ and $p < 0.05$, respectively) than in the group with BMI > 25.

Our results are in line with previous reports on reduction of body weight following consumption of caffeinated, decaffeinated and chlorogenic acid enriched coffee brews [3, 4]. Since body water and fat-free mass remained

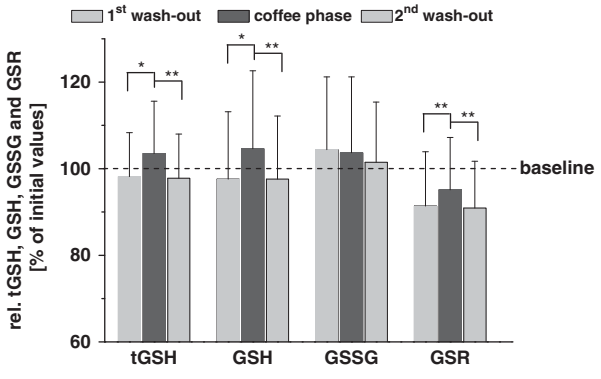

Figure 2. tGSH/GSH/GSSG levels and GSR activity in blood samples after study phases; data are expressed in percentage of initial values (start) as means and SD. Significantly different: $^{*}p < 0.05$, $^{**}p < 0.01$ (one-sided, paired *t*-test).

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Table 1. Body weight, body mass index (BMI) and body composition of 33 probands at the beginning of the study (start) and at the end of each study phase

Body composition	Time point of measurement			
	Start	1st wash-out	Coffee phase	2nd wash-out
Body weight (kg)	85.19 ± 13.25	84.85 ± 12.95	84.24 ± 13.3**	84.50 ± 13.69
BMI (kg/m^2)	25.6 ± 3.7	25.5 ± 3.7	25.3 ± 3.8*	25.3 ± 3.9
Body fat (kg)	20.42 ± 8.19	20.0 ± 8.54	19.36 ± 8.56*	19.49 ± 8.74
Fat-free mass (kg)	64.78 ± 6.73	64.82 ± 6.47	64.87 ± 6.76	64.93 ± 6.94
Water (L)	47.42 ± 4.9	47.44 ± 4.8	47.49 ± 4.9	47.5 ± 4.9

Data are means and SD from 33 probands; significantly different from preceding measurement: *$p<0.05$, **$p<0.01$ (one-sided paired *t*-test).

almost unchanged in our study, the coffee mediated loss in body weight can largely be attributed to body fat reduction.

Nutrient intake In the coffee phase, significant reduction in energy and nutrient intake was observed as compared to the first wash-out phase. In the second wash-out phase, a significant re-increase of kcal and carbohydrate intake occurred without, however, reaching the values of the first wash-out. Two weeks after study termination, ingestion of kcal/nutrient was not found increased, compared to the second wash-out, suggesting a prolonged effect of the study coffee (Supporting Information Fig. 3).

Influence of independent predictors The results show a significant influence of the study phases on all dependent variables, underlining the antioxidant and body-weight-reducing effectiveness of the study coffee. Individual probands significantly affected all parameters except for total DNA damage; body weight, body fat and tGSH levels were significantly influenced by BMI. No influence of age on the variables was found (Supporting Information Table 3).

Taken together, consumption of coffee brew particularly rich in both green coffee bean constituents and roast products resulted in markedly reduced DNA damage ± FPG, concomitant with significant elevation of GSH level and GSR activity in probands. The effects on GSH level were more pronounced in normal weight (BMI < 25), compared to overweight participants. The GSH status was similarly elevated during coffee intervention; therefore, the rise of GSH level is supposed to result from both elevated GSH synthesis and GSSG reduction by GSR. These findings partially can be ascribed to the presence of phenolic coffee constituents such as CQAs, known to exert marked radical scavenging activity and elevation of antioxidant defense [18]. In human trials, consumption of lightly roasted coffee rich in chlorogenic acids resulted in decreased oxidative damage of DNA and other macromolecules and elevated antioxidant defense [2, 13]. The particularly high effectiveness observed for the coffee used in the present study suggests that roast products (e.g. NMP) substantially contribute to its in vivo antioxidant activity. NMP has been described to induce GST activity in vitro and in rats [17]. Moreover, a markedly enhanced transcription and nuclear translocation of Nrf2 has been observed in HT-29 cells after NMP incubation [19] (Supporting Information, Further considerations).

In addition to its antioxidant activity, the study coffee significantly diminished body weight and body fat. The concomitant reduction of energy and nutrient intake observed in the coffee intervention phase suggests a potential influence of coffee constituents, particularly of CQAs, on the regulation of hunger and satiety. Other potential mechanisms, such as modulation of glucose absorption and carbohydrate metabolism and/or caffeine-mediated induction of thermogenesis may also have contributed.

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The authors have declared no conflict of interest.

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