Coffee consumption rapidly reduces background DNA strand breaks in healthy humans: results of a short term repeated uptake intervention study

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Abstract

Scope: Intervention studies provide evidence that long-term coffee consumption correlates with reduced DNA background damage in healthy volunteers. We here report on short-term kinetics of this effect, showing a rapid onset after normal coffee intake.

Methods and results: In a short-term human intervention study, we determined the effects of coffee intake on DNA integrity during 8 hours. Healthy male subjects ingested coffee in 200 ml aliquots every second hour up to a total volume of 800 ml. Blood samples were taken at baseline, immediately before the first coffee intake and subsequently every two hours, prior to the respective coffee intake. DNA integrity was assayed by the comet assay. The results show a significant (p<0.05) reduction of background DNA strand breaks already 2 h after the first coffee intake. Continued coffee intake was associated with further decrements in background DNA damage within the 8h intervention (p<0.01 and p<0.001, respectively). Mean tail intensities (TI%) decreased from 0.33 TI% (baseline, 0 h) to 0.22 TI% (within 8 h coffee consumption).
Conclusion: Repeated coffee consumption was associated with reduced background DNA strand breakage, clearly measurable as early as two hours after first intake resulting in a cumulative overall reduction by about one third of the baseline value.

Graphical abstract:

The long-term consumption of coffee is associated with improved DNA integrity in healthy volunteers. To test whether these effects could be shown after acute and repeated coffee consumption within a day, a short-term study was performed. The results demonstrate that the intake of coffee within hours significantly reduced background DNA strand breaks, measured by comet assay.

1. Introduction
Numerous epidemiological studies indicate potential health effects associated with coffee consumption and with reduced risk of certain chronic and degenerative diseases, such as diabetes, Parkinson’s disease, cardiovascular disorders and some types of cancer [1]. Coffee contains a multitude of compounds such as chlorogenic acids (e.g. caffeoylquinic acids, CQAs) as well as constituents generated during the roasting, including Maillard reaction products such as N-methylpyridinium or melanoidins. The latter have been reported to account for about 25% of dry matter [2]. Such constituents have been discussed to act as radical scavengers and / or to induce expression of antioxidant/electrophile response element (ARE/ERE) dependent enzymes by activation of Nrf2-signaling pathways [3-7]. Several intervention studies indicate that consumption of coffee protects against DNA-damage in humans [8-12]. We have recently reported that four weeks consumption of coffee rich in chlorogenic acid and roast products markedly reduced DNA strand breaks (background and total) in white blood cells (WBC) and increased glutathione level in plasma of healthy subjects [8]. Other research groups likewise observed in humans a decrease of total DNA strand breaks in isolated lymphocytes after coffee intake [10, 12]. In a randomized controlled study with healthy subjects, we reported a significant reduction of background DNA strand breaks in white blood cells after four weeks coffee consumption [9]. All these effects were observed after continuous coffee uptake during several days or weeks. However, acute, short-term (one day) effects of coffee on DNA integrity have not been reported yet. Such information is expected to produce new insights into potential mechanism of protection against DNA damage; rapid onset would speak for direct radical scavenging by coffee constituents whereas longer term responses may rather be attributed to other mechanism, such as those mediated by activation of Nrf2 signaling.
We here describe the results of an eight-hour short-term intervention study with 13 healthy male volunteers.

2. Materials and Methods

This study was approved by the local ethic committee of Rhineland-Palatine, Mainz, Germany, no. 837.218.12 (8319-F). Male volunteers (n = 14, ages 20-50, BMI 19-25), who fulfilled the inclusion criteria (healthy non-smokers, no intake of pharmaceutical drugs or food supplements during the study period) were recruited. After informed written consent, volunteers were subjected to medical examination including standard clinical blood biochemistry. They showed the following anthropometric characteristics: average age of 23 ± 2.4 years, BMI of 23.8 ± 1.6 kg/m², body fat content of 14.4 ± 5.1 kg (Bioelectrical impedance analysis, BIA 101, SMT medical GmbH, Wuerzburg, Germany). One individual dropped out for private reasons. The remaining 13 volunteers completed the eight hour intervention study. This was performed with four to five volunteers per three consecutive study days, following a design as shown (see Figure 1). Volunteers were kept for the intervention day in a clinical facility under tightly controlled environmental conditions. They received identical quantities of the same food and beverage (water) throughout the intervention day. Three days before (wash-out period) and during the study, volunteers were instructed to avoid coffee (except study coffee) and caffeine-containing products as well as dietary supplements and foods rich in polyphenols. The volunteers entered the facility in fasted state and venous blood samples were taken. Thereafter the volunteers consumed the first 200 mL aliquot of black coffee (with/without sugar, no milk), brewed in a commercially
available standard coffee pad machine (2 x coffee pads á 7.5 g / 200 mL). The coffee blend was prepared from a special roasted and blended Arabica (Coffea arabica) coffee, rich in both, green and roast bean coffee constituents (see Table 1) and was packed into coffee pads (Tchibo GmbH, Hamburg). Every brew was freshly prepared immediately before each individual consumption. After 2, 4, 6, and 8 h this procedure was repeated (total coffee consumption 800 mL/volunteer). Blood samples were drawn from the participants immediately prior to the next coffee intake every two hours after each coffee intake. During the study the volunteers consumed a light meal (bread roll with cheese) and a light dinner (tortellini carbonara), respectively. Water consumption was allowed *ad libitum*.

The ground coffee was analytically characterized using HPLC-DAD and HPLC-MS/MS methods as previously described [13]. In brief, caffeine and CQAs (sum of 5-O-, 4-O- and 3-O-CQA) were quantified by HPLC-DAD (272 and 324 nm, respectively) with external calibration. NMP and trigonelline were quantified by stable isotope dilution analysis with d3-trigonelline and d3-N-methylpyridinium after sample clean-up via solid phase extraction (SPE) on cationic exchange material [14].

Venous blood samples were collected in EDTA tubes and worked up as described for the comet assay. DNA strand breaks were determined as reported before by the comet assay, a well established method of alkaline single cell gel electrophoresis [8]. DNA migration was calculated as mean tail intensity (TI%: DNA in the comet tail in percent of total DNA).

Results from 13 volunteers are reported as means and SD. The Shapiro-Wilk test was used for analysis of normal distribution. Differences of parameters between baseline and each coffee intake were analysed by analysis of variance (ANOVA). Differences without normal distribution were analysed by ANOVA based on Blom- rank transformation [15].
3. Results and discussion

To characterize the ground study coffee, concentrations of selected compounds are presented in Table 1. The roast product N-methylpyridinium, NMP (1.1 mg/g), was present at a level associated with dark roast, whereas the total amount of green bean coffee constituents, as exemplified by CQAs (10.4 mg/g, sum of 3-, 4- and 5-caffeoylquinic acids) was in the range expected for medium roasted coffee. Normally, dark roasts contain lower concentrations of these compounds than light roasts [16, 17]. The caffeine level (16.7 mg/g) was in the upper range of commercial ground coffee [14]. The trigonelline content (3.9 mg/g) likewise was in the range of medium roast coffee. Trigonelline degrades during the roast process into a spectrum of thermal degradation products, including NMP and further pyridine derivatives and, nicotinic acid. More than 90% of the compounds mentioned are known to be extracted into the coffee brew when brewing is performed at a water to coffee ratio (v/w) >16, as used here [18]. During this eight hour intervention study volunteers consumed four portions of 200 mL coffee brew every two hours (see Figure 1) resulting in a total uptake of 800 ml during the intervention day. As exemplified in Figure 2, a significant (p < 0.05) reduction of background DNA strand breaks became observable already two hours after the first coffee (200 ml) intake, compared to the baseline. The mean tail intensities (TI%) decreased from 0.33 TI% (baseline) to 0.28 TI% (after 200 ml coffee). Further consumption of coffee brew every two hours up to a total of 800 mL revealed a significant further incremental reduction of DNA damage (p < 0.01 and p < 0.001, respectively) in relation to the baseline. The respective TI % values decreased from 0.33 TI% (baseline) to 0.25 TI% (after 400 ml coffee), 0.23 TI% (after 600 ml coffee) and 0.22 TI% (after 800 ml coffee).
These results indicate an additive protective effect on DNA integrity by repeated coffee intake, summing up to about 30%.

Previously published intervention studies described longer term effects of coffee consumption on DNA integrity. Although using slightly different experimental conditions, a significant reduction of background and total DNA strand breaks in white blood cells was invariably reported [8, 9, 12]. Background DNA strand breaks may be conceived to result from endogenous exposure to DNA damaging agents from internal or external sources and/or may reflect incomplete DNA repair. In addition to background DNA strand breaks, total DNA damage includes DNA damage as provoked by reactive oxygen species (ROS) and other electrophilic reactants, causing lesions detectable by processing with specific enzymes of the (bacterial) DNA repair machinery, such as formamidopyrimidine glycosylase (FPG) [19].

In the present study, we observed a decrease in background DNA damage already within two hours after coffee consumption. Such an early onset of DNA protection most probably reflects direct mitigating effects of certain coffee constituents. For instance, polyphenols like chlorogenic acids (e.g. CQA and their metabolites) as well as compounds formed during roasting (e.g. melanoidins) have been found to exert radical scavenging as well as metal chelating activities in vitro and in vivo [20, 21]. Bioavailability studies of coffee constituents have shown that chlorogenic acids after absorption in the small intestine rapidly appear in the circulatory system. Within 60 min after ingestion of coffee, free chlorogenic, caffeic, ferulic or isoflavanic acids, as well as their sulfate and glucuronide conjugates have been detected [22-25]. Furthermore, metabolites conceived to be generated primarily by the gut microbiome, including dihydroferulic and dihydrocaffeic acids and corresponding conjugates occur later in plasma (t_{max} = 6 - 10 hours) [23, 24]. The latter metabolites are more slowly
excreted and are present in plasma samples up to 20 h after ingestion [24]. In contrast, the caffeic acid derivatives (chlorogenic acids, caffeic and ferulic and isoferulic acids) were found to reach baseline after 6 hours. Depending on the ingested dose, up to 25% of chlorogenic acids were recovered in the urine [24, 26].

Based on these kinetic data, it can be concluded that plasma concentrations of chlorogenic acids and their metabolites attained two hours after the first coffee intake will suffice to contribute to the rapid onset of the observed protective effects on DNA background damage. On the premise that residual plasma concentrations of relevant coffee constituents are still present from the foregoing intake at time points of subsequent coffee intakes, incrementally enhanced DNA protective effects may be expected. Indeed, stepwise decrements in DNA background damage became apparent, in parallel with the cumulative coffee intake during the eight hours intervention. It thus can be inferred that the fractionated coffee uptake results in a steady built-up of plasma levels of chlorogenic acids and their metabolites, most probably also of melanoidins and their potential metabolites [27, 28]. Plasma kinetics of trigonelline and N-methylpyridinium (the latter generated from trigonelline during roasting) are within a similar range. For example, maximal plasma concentrations were reached at about ~ 1-3 h after coffee intake, and an elimination half-life of about 2-5 h was reported [29]. Of note, because these well water soluble compounds are excreted primarily in the urine (about 60-70 % within 8h) they have been utilized to verify compliance in coffee intervention studies [29].

Taken together, a comprehensive spectrum of coffee constituents and their metabolites can be inferred to be responsible for the observed rapid onset of mitigating effects on DNA background damage.
Beyond these immediate DNA protective responses also long term effects have been observed in previous human intervention studies [8, 9, 12]. It has been found that chlorogenic acids, but also roast-associated constituents such as NMP, as well as certain Maillard reaction products ingested in coffee can up-regulate several physiological defense systems. These constituents have been identified as activators of the Nrf2/ARE pathway, activating nuclear Nrf2 translocation as well as gene- and protein expression of different phase II enzymes, such as glutathione transferase and reductase, glucuronyl transferase, heme oxygenase, superoxide dismutase, catalase and some others, in vitro and in vivo [4, 5]. Of note, roasted, but not green coffee extract has been reported to up-regulate nuclear translocation of Nrf2 in macrophages [2]. Likewise, only a heated, but not an unheated Maillard reaction mixture was found to exert a strong Nrf2 activating effect [2]. Thus, long-term activation of Nrf2-mediated defense may be inferred to result from green bean and roast-associated coffee constituents in combination. In addition to radical scavenging and induction of detoxification systems, DNA repair may also be influenced by coffee constituents. However, as yet there is only limited and inconclusive information concerning effects of coffee on DNA repair modulation. No effects of instant coffee consumption was reported on DNA damage and DNA repair in mouse liver [30]. At variance, in rats, Turkish coffee and its diterpene components kahweol and cafestol were reported to increase the expression of DNA repair protein O\(^6\)-methylguanine-DNA methyltransferase (MGMT), in parallel with some phase II enzymes [31].

4. Concluding remarks

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We conclude that regular daily consumption of coffee is associated with mitigating activity against background DNA damage. This DNA protective activity has a very rapid onset, observed already two hours after the first intake. It is further increasing with continuing coffee intake throughout the here described one-day intervention. Former studies revealed sustained DNA protective effects during longer term regular coffee consumption (4 weeks). Thus, in line with these earlier findings, this study clearly shows a very early onset of DNA protective efficacy, augmented during further coffee intake during the day and becoming sustained during long-term coffee ingestion.
We are grateful for the contribution of the participants in the study. The authors thank Sylvia Schmidt for preparing blood samples and performing the comet measurements. We thank Dirk Galan and Axel Stachon for their support during the study. The authors thank Tchibo GmbH, especially G. Bytof, I. Lantz and H. Stiebitz for providing the study coffee, packed into pads and the coffee pad machines.

Potential conflict of interest statement: G. Eisenbrand is scientific advisor with Tchibo GmbH and with the Institute for Scientific Information on Coffee, La Tour de Peilz, Switzerland (ISIC).

All other authors have no interests declared.
5. References


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[29] Lang, R., Wahl, A., Skurk, T., Yagar, E. F., et al., Development of a hydrophilic liquid interaction chromatography-high-performance liquid chromatography-tandem mass spectrometry based stable...


Legends to the figures:

**Figure 1:** Design of short-term intervention study. ↑ Blood sampling
**Figure 2:** Background DNA strand breaks in WBC of 13 volunteers at start of study and two hours after each coffee consumption (total 800 ml). Data are expressed as TI % showing means and SD; significantly different DNA strand breaks: ***p<0.001, ** p<0.01, * p<0.05, Variance analysis ANOVA, influences factor “time”. BS: blood sampling.
Table 1: Concentration [mg/g of selected constituents in the ground study coffee

<table>
<thead>
<tr>
<th>compound</th>
<th>c (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Caffeine structure" /></td>
<td>16.7 ± 0.7</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Caffeoylquinic acids" /></td>
<td>10.4 ± 0.9</td>
</tr>
<tr>
<td>Caffeoylquinic acids&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>
N-Methylpyridinium

\[
\begin{align*}
\text{N-Methylpyridinium} & \quad 1.1 \pm 0.15 \\
\text{Trigonelline} & \quad 3.9 \pm 0.32
\end{align*}
\]

Values are expressed as mean ± SD. * Sum of 3-, 4- and 5-caffeoylquinic acid