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Impact of paper filtered coffee on oxidative DNA-damage: Results of a clinical trial

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ABSTRACT

Coffee is among the most frequently consumed beverages worldwide and epidemiological studies indicate that its consumption is inversely related to the incidence of diseases in which reactive oxygen species (ROS) are involved (liver cirrhosis, certain forms of cancer and neurodegenerative disorders). It has been postulated that antioxidant properties of coffee may account for this phenomenon. To find out if consumption of paper filtered coffee which is the most widely consumed form in Central Europe and the US protects humans against oxidative DNA-damage, a controlled intervention trial with a cross-over design was conducted in which the participants ($n = 38$) consumed 800 ml coffee or water daily over 5 days. DNA-damage was measured in peripheral lymphocytes in single cell gel electrophoresis assays. The extent of DNA-migration attributable to formation of oxidised purines (formamidopyrimidine glycosylase sensitive sites) was decreased after coffee intake by 12.3% ($p = 0.006$). Biochemical parameters of the redox status (malondialdehyde, 3-nitrotyrosine and the total antioxidant levels in plasma, glutathione concentrations in blood, intracellular ROS levels and the activities of superoxide dismutase and glutathione peroxidase in lymphocytes) were not markedly altered at the end of the trial, also the urinary 8-isoprostaglandine F2α concentrations were not affected. Overall, the results indicate that coffee consumption prevents endogenous formation of oxidative DNA-damage in human, this observation may be causally related to beneficial health effects of coffee seen in earlier studies.

1. Introduction

Coffee is one of the most widely consumed beverages worldwide. Epidemiological studies found that its consumption is associated with beneficial health effects, i.e. reduction of the risks of certain forms of cancer [1,2], diabetes type 2 [3] and neurodegenerative disorders [4]. It is possible that these effects are due to inactivation of reactive oxygen species (ROS) which play a role in the etiology of these diseases.

It was shown in a number of *in vitro* studies that coffee contains constituents such as chlorogenic acids, Maillard products and caffeine which are able to inactivate ROS by direct scavenging [5–8]. Furthermore, specific components such as N-methylpyridinium, which is formed from trigonelline during roasting and the diterpenoids kahweol and cafestol are able to induce the activities of antioxidant enzymes [9–13]. However, only few studies with laboratory rodents and humans have been published in which the antioxidant properties of coffee were investigated and the results are controversial (for review see Ref. [5]).

Recently, Bichler et al. [14] reported on prevention of oxidative DNA-damage in lymphocytes in a small intervention trial ($n = 8$). Furthermore, they found also an increase in the activity of glutathione peroxidase (GPx) and Cu–Zn superoxide dismutase (SOD). In an Italian intervention study [15], increased plasma levels of the antioxidant glutathione (GSH) were found after consumption of espresso, while no evidence for alterations of several markers of lipid peroxidation and of antioxidant enzymes was obtained in a large Finnish intervention trial [16] and Giovannucci and coworkers [17] reported even an increase in DNA-migration due to

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formamidopyrimidine glycosylase (FPG) sensitive sites in lymphocytes in a questionnaire based study. One of the reasons for these inconsistent results may be that different types of coffee were tested in these trials. It is assumed that the concentrations of individual bioactive components in coffee depend strongly on the mode of preparation, for example, diterpenoids are found in much higher concentrations in metal filtered and Turkish style brews than in paper filtered coffee [18].

The primary aim of the present study was to investigate the impact of paper filtered coffee (the most widely consumed form in Central Europe and the US) on DNA-damage in a controlled intervention trial. It is assumed that DNA stability plays a key role in the etiology of a large number of diseases including cancer [1,19,20]. As suggested by Moller and Loft [20], a cross-over design was used to avoid seasonal effects. DNA-damage was monitored in peripheral lymphocytes in single cell gel electrophoresis (SCGE or comet) assays which are based on the measurement of DNA-migration in an electric field. This technique is increasingly used in dietary human intervention trials (for review see Ref. [21]). The experiments were conducted under alkaline standard conditions, which detect single and double strand breaks and apurinic sites [22], with lesion specific enzymes FPG and endonuclease III (Endo III) that reflect formation of oxidatively damaged bases [23] and after H_2O_2 treatment of the cells which enables to monitor alterations of the sensitivity of the cells towards ROS induced DNA-damage [24].

In addition, a number of biochemical parameters of the redox status were monitored in the indicator cells and in body fluids to obtain a more comprehensive picture of the impact of coffee consumption on the redox status in humans. Intracellular ROS levels and the activities of the enzymes SOD, GPx were determined in the lymphocytes. These latter measurements provide information on indirect effects that involve the activation of transcription factors [25]. Furthermore, additional biochemical parameters of the redox status, namely the total antioxidant capacity (TAC), maldondialdehyde (MDA), oxidized low density lipoprotein (oxoLDL), GSH and the 3-nitrothyrosine (3-NT) levels were determined in plasma or blood, the concentrations of 8-isoprostaglandin F2α (8-iso PGF2α) was monitored in urine. The measurements reflects also direct scavenging effects and were carried out in body fluids for reasons of comparison with earlier intervention trials and *in vitro* studies with coffee and its components and other dietary factors [5,16].

Table 1
Characteristics of the study group[a].

Parameter[b]		Overall ($n = 38$)
Age (y)		27.6 ± 8.0
Weight (kg)		67.2 ± 1.1
Body mass index		22.3 ± 2.8
Height (m)		1.7 ± 0.1
Pulse	(a)	75.1 ± 12.9
	(b)	74.8 ± 13.9
Blood pressure (systolic, mm Hg)	(a)	125.4 ± 14.8
	(b)	124.9 ± 15.9
Blood pressure (diastolic, mm Hg)	(a)	77.9 ± 6.6
	(b)	79.1 ± 8.2

[a] Numbers indicate mean ± SD.
[b] (a) before coffee consumption/(b) after coffee consumption.

2. Materials and methods

2.1. Study population

Only individuals who fulfilled the inclusion criteria (healthy non-smokers, no intake of pharmaceutical drugs, no intake of food supplements 4 weeks prior and during the study, no participation in another clinical trial, no pregnancy, compliance with the protocol, no blood withdrawal 3 weeks before the study) were included. At the start and at the end of the trial blood pressure and pulse frequencies were monitored. Two participants were excluded from the final data analysis because of illness. In total, samples from 38 participants (14 males and 24 females) were analysed. The characteristics of the participants are listed in Table 1.

The study was approved by the Ethical Commission of the Medical University of Vienna and informed consent was obtained from all participants.

2.2. Design

Fig. 1 gives a schematic overview of the design of the intervention trial. Nine days before the beginning of the intervention phase the participants were asked to control their consumption of fruit juices and other dietary components which may have an impact on the outcome of the study and to avoid physical exercise, since it is known that it may affect DNA-migration (for details see Ref. [26]). The compliance of the participants was controlled by oral questioning. The individuals were allocated into 2 groups (18 coffee/water and 20 water/coffee).

The individuals in the coffee/water group drank 800 ml coffee/day over 5 consecutive days. The brew and the water were consumed without a fixed daily schedule. After a washout phase (5 weeks) and a restriction phase (1 week) they consumed 800 ml water/day instead of coffee. The participants in the water/coffee group followed the study protocol in reversed order. All individuals were asked to restrict their coffee consumption to 1 cup per day 3 weeks before the start of the study and during the washout phase.

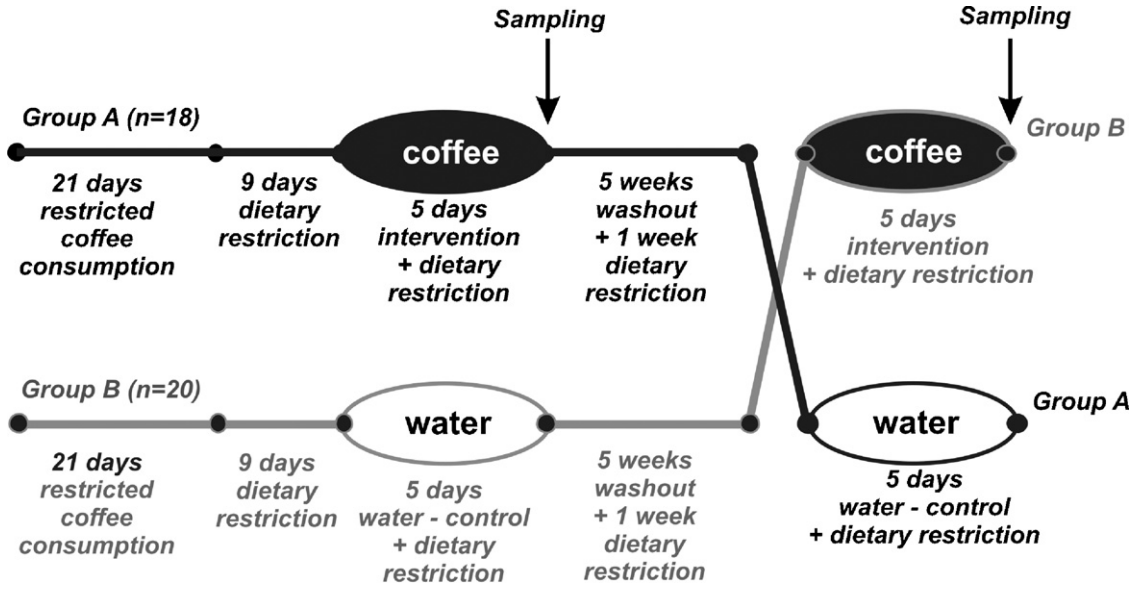

Fig. 1. Design of the intervention trial.