

Dark roast coffee is more effective than light roast coffee in reducing body weight, and in restoring red blood cell vitamin E and glutathione concentrations in healthy volunteers

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Recent results from prospective cohort studies have shown that moderate coffee consumption is associated with a reduced risk for diabetes mellitus type II or Alzheimer's disease. Since reactive oxygen species (ROS) are believed to be involved in the pathogenesis of these diseases, antioxidants in coffee might contribute to this risk reduction. We aimed at elucidating whether a dark roast coffee beverage (CB) rich in *N*-methylpyridinium ions (NMP: 785 µmol/L) and low in chlorogenic acids (CGA: 523 µmol/L) has stronger antioxidant effects on human erythrocytes than a CB prepared from a light roast with opposite proportions (CGA: 4538 µmol/L; NMP: 56 µmol/L). Following a 2-wk wash out period, 500 mL of the respective CB was administered to 30 subjects daily for 4-wk. Blood and spot urine samples were collected at the beginning and at the end of each intervention. Intake of the dark roast CB most effectively improved the antioxidant status of erythrocytes: superoxide dismutase and glutathione peroxidase activity decreased by 5.8 and 15%, respectively, whereas tocopherol and total glutathione concentrations increased by 41 and 14%, respectively. Furthermore, administration of the NMP-rich CB led to a significant body weight reduction in pre-obese subjects, whereas the CGA-rich CB did not.

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Recent results from prospective cohort studies have shown that moderate coffee consumption is associated with a reduced risk for diabetes mellitus type II, several types of

cancer or Parkinson's disease [1–3]. Since reactive oxygen species (ROS) are believed to be involved in the pathogenesis of these diseases, antioxidants in coffee might contribute to this risk reduction [3–7]. We aimed at elucidating whether a dark roast coffee beverage (CB) rich in *N*-methylpyridinium (NMP) and low in chlorogenic acids (CGA) has stronger antioxidant effects on human erythrocytes than a CB prepared from a light roast with opposite proportions. This hypothesis was based on the results from animal studies and cell-culture experiments that indicate a pivotal role of CGA [8, 9] and NMP [10] in the antioxidant activity of coffee brews. In addition, NMP has been identified as a strong inducer of ARE (antioxidant responsive element)-

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Abbreviations: AOC, antioxidant capacity; CAT, catalase; CB, coffee beverage; CGA, chlorogenic acids; GPX, glutathione peroxidase; MDA, malondialdehyde; NMP, *N*-methylpyridinium; ROS, reactive oxygen species; SOD, superoxide dismutase

dependent phase II gene expression *in vitro*, indicative for an elevation of antioxidative cell defence [11, 12].

The effect of the roasting degree on the antioxidative potential of coffee *in vitro* has been studied by Nicoli et al. [13] who reported a higher antioxidant activity for a medium roast coffee compared with a light or dark roast. Moreira et al. [14] ascribed a reduced antioxidant activity of dark versus light roast coffee *in vitro* to its lower content of CGA. However, these results have been obtained from *in vitro* experiments which might not display *in vivo* conditions correctly since CGA are metabolized to structurally different metabolites after dietary intake [15].

In this randomized, longitudinally designed 12-wk human intervention trial, healthy volunteers ($n = 30$) were asked to follow a dietary regime low in polyphenols and were subjected to a controlled, 4-wk intake of 500 mL of a light roast coffee which was preceded and followed by a 2-wk wash out phase. After the second wash out phase, subjects were asked to drink the same amount of a dark roast coffee prepared from the same bean variety for another 4-wk (see Supporting Information, Fig. 1). CBs were prepared using a common coffee drip filter machine (TCM, Germany) with 30 g of roast coffee powder and 600 mL of bi-distilled water. The CBs used for the intervention were derived from the same batch of green coffee beans (Arabica Brazil). For the first intervention period, light roast conditions were applied (260°C , 2 min), resulting in a CB with high concentrations of CGA (4.5 mmol/L: CGA-CB) and low NMP concentrations (0.06 mmol/L). For the second intervention period, dark roast conditions were applied (260°C , 5 min) that resulted in a CB with high concentrations of NMP (0.079 mmol/L: NMP-CB) and low CGA contents (0.05 mmol/L) (see Supporting Information, Table I). For compliance control, trigonelline and NMP were quantified in spot urine samples from all volunteers (see Supporting Information, Materials and Methods). During the period of CGA-CB intervention, large amounts of trigonelline were excreted, whereas NMP concentrations were negligible. In comparison, trigonelline levels in urine samples collected during NMP-CB intervention decreased while NMP levels increased, indicating a significant uptake of NMP from the dark roast coffee brew (see Supporting Information, Fig. II).

During each wash out and intervention period, subjects were not allowed to drink any additional coffee, were asked to avoid vitamin supplements and foods rich in polyphenols, and were told not to change their habitual life style (e.g. physical activity or sleep pattern). Nutrient intake was calculated on the basis of seven-day dietary records analysed using the computer-based nutrient calculation program PRODI[®] (see Supporting Information, Materials and Methods).

As a result, the intake of the antioxidant vitamins ascorbic acid and tocopherol did not change over the total time of intervention (see Supporting Information, Table II). Therefore, we hypothesize that any changes in the selected parameters of the antioxidative defence observed in this study were induced by the respective coffee administered.

Total energy intake was significantly lower during NMP-CB intervention when compared with the first wash out period and to the intervention with CGA-CB (see Supporting Information, Table II). The subject's initial mean body weight was 73.1 ± 3.8 kg and decreased after intervention with NMP-CB compared with the preceding wash out period by 0.6 ± 0.4 kg ($p < 0.05$; see Supporting Information, Table III). For the pre-obese subjects subset, a mean weight loss of 2.5 ± 1.0 kg ($p < 0.05$) was noted, whereas normal weight subjects did not lose any weight after NMP-CB intervention. Administration of CGA-CB resulted in a slight, but statistically significant increase of 0.2 ± 0.5 kg ($p < 0.05$; see Supporting Information, Table III). The loss of body weight in pre-obese subjects after administration of the dark roast NMP-CB is an interesting finding aside from our primary hypothesis. Prospective epidemiological data [16] and results from a 12-wk human intervention trial [17] support coffee-associated weight loss. However, no data are available on this effect of dark roast coffee brews, necessitating the verification of the here presented results in the future intervention trials.

The primary hypothesis of this study was that the intake of a dark roast CB rich in NMP (NMP-CB) is more effective than a light roast CB rich in chlorogenic acids (CGA-CB) in restoring parameters of the antioxidant defence system in erythrocytes from healthy volunteers. The 4-wk intake of the CGA-CB coffee resulted in increased erythrocyte activities of superoxide dismutase (SOD), glutathione-peroxidase (GPX) and catalase (CAT) and by 12, 25 and 22%, respectively (Fig. 1). An elevated SOD activity can be caused by an increased formation of superoxide radical anions, whereas elevated GPX and CAT activity indicate elevated levels of peroxides. Owing to the strict polyphenol-poor dietary regimen the participants had to follow during the course of the study, an increased formation of intracellular ROS and subsequent induction of antioxidant enzyme activities might be one of the underlying mechanisms. However, this hypothesis needs further testing, in particular since no increase in malondialdehyde (MDA), as a marker of lipid peroxidation, and no decrease in tocopherol or tGSH as major antioxidants in erythrocytes was observed (Fig. 2), indicating that the enzymatic activities were sufficient to prevent the erythrocytes from major lipid peroxidation. In the plasma, however, where these enzyme activities are generally very low, a decrease in MDA levels was accompanied with a decrease in the total antioxidant capacity (AOC) (see Supporting Information, Fig. III). These results clearly indicate that the 4-wk intake of the CGA-CB induced enzymes of the antioxidant defence system during a polyphenol-poor dietary regimen. This effect was not seen up to 180 min after a bolus dose of the CGA-CB coffee, neither at the beginning nor the end of the 4-wk CGA-CB intervention (data not shown).

Bolus administration of the dark roasted NMP-rich CB significantly decreased SOD and CAT activity during the first 180 min after coffee consumption (Fig. 3A and B). These effects were more pronounced at the end than at the beginning of the 4-wk NMP-CB intervention, indicating a

constitutive effect that is likely to be caused by lower amounts of ROS. This hypothesis is supported by an increase in tGSH concentrations in erythrocytes and the total AOC in the plasma (Fig. 3C and D), and by results from the 4-wk NMP-CB intervention, which resulted in a 5.8% and a 16% decrease in the SOD and GPX activity in erythrocytes (Fig. 1). Although the CAT activity increased by

15% ($p = 0.051$), an augmented lipid peroxidation after the 4-wk intervention is very unlikely since the concentrations of MDA were not changed and those of tGSH and tocopherol even increased upon administration of NMP-CB by 14 and 41%, respectively (Fig. 2). Therefore, it is unlikely that the decrease in SOD and GPX enzyme activity was caused by ROS-induced damage to these enzyme proteins, as

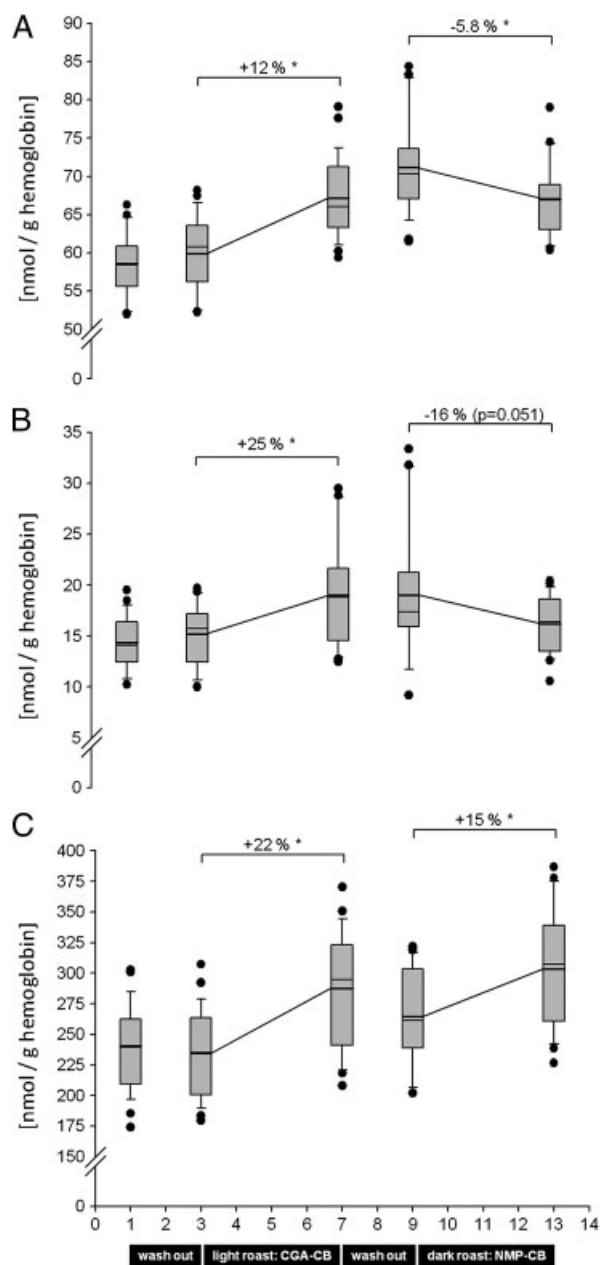


Figure 1. Catalytic activities of superoxide dismutase (A), glutathione peroxidase (B) and catalase (C) in erythrocytes from healthy volunteers during three months of intervention (start of the intervention at week 1, end of the intervention at week 13) with a light roast (CGA-CB) and a dark roast (NMP-CB) coffee. Box plots show group median and mean (connected) values (* $p \leq 0.05$).

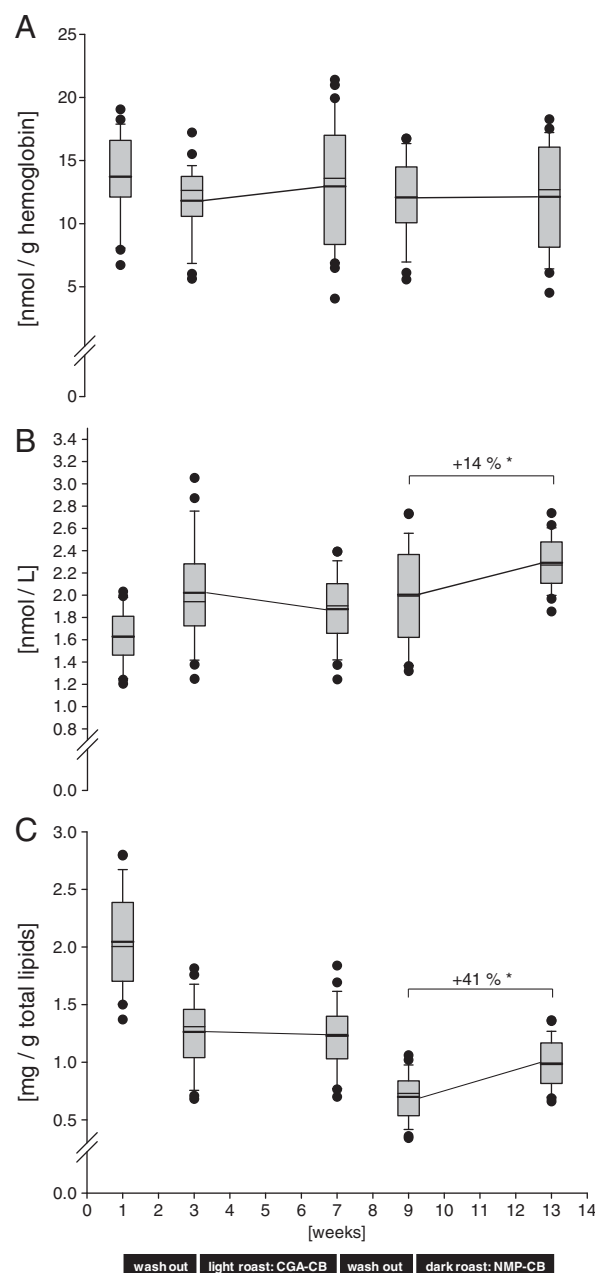


Figure 2. Concentrations of MDA (A), total glutathione (B) and tocopherol (C) in erythrocytes from healthy volunteers during three months of intervention (start of the intervention at week 1, end of the intervention at week 13) with a light roast (CGA-CB) and a dark roast (NMP-CB) coffee. Box plots show group median and mean (connected) values (* $p \leq 0.05$).

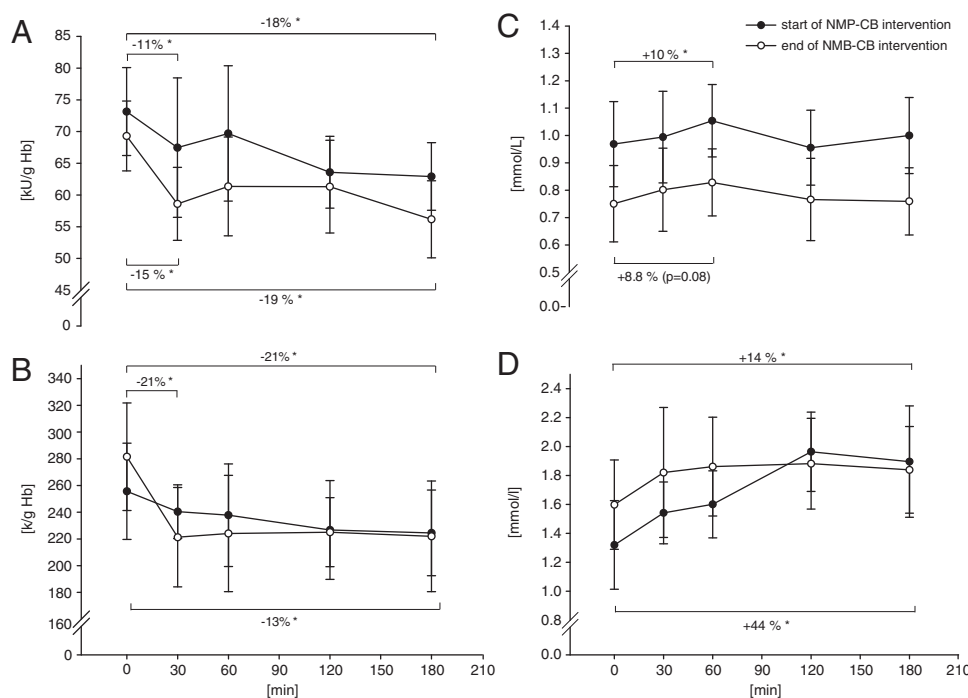


Figure 3. Erythrocyte SOD (A) and erythrocyte CAT (B), plasma total AOC (C), plasma total glutathione (D), catalytic activity in healthy volunteers after bolus administration of 500 mL NMP-CB at the beginning and end of the 4-wk NMP-CB intervention period. Line plots show group mean (connected) and standard variation values (* $p \leq 0.05$).

discussed by Hassan et al. [18]. Mursu et al. [19] could also not detect any increase in indicators of lipid peroxidation in healthy volunteers after administration of either a bolus dose or after a 3-wk intervention with up to 900 mL of coffee per day. These results further support our findings that coffee exhibits antioxidant properties in vivo. The finding that the dark roast NMP-CB has a more pronounced antioxidant effect in vivo than the light roast CGA-CB is in agreement with results reported by Esposito et al. [20], who described a sparing effect on plasma glutathione by 16% after administration of five cups of dark roasted Italian style coffee per day for five days, and Grubben et al. [21], who reported increased glutathione concentrations after a 2-wk intervention period with 1 L of dark roasted cafetière (French press) coffee per day in mucosa biopsies from 64 healthy volunteers.

In our study, the increased levels of tocopherol also support the antioxidant effect of dark roast, NMP-rich coffee in vivo. This result is in agreement with previous findings of our own group where a tocopherol restoring in vivo effect was demonstrated for NMP [11].

Overall, our data indicate the light roasted CGA-rich CB being a weaker modulator of the antioxidative defence system compared with the dark roasted NMP-rich coffee. Moreover, the NMP-CB intervention reduced the body weight in pre-obese subjects, indicating a significant potential for body weight control by dark roast coffee brews containing high amounts of NMP. Future studies have to prove whether these effects can be attributed to NMP or to other coffee constituents.

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References

- [1] Greenberg, J. A., Boozer, C. N., Geliebter, A., Coffee, diabetes, and weight control. *Am. J. Clin. Nutr.* 2006, **84**, 682–693.
- [2] Tavani, A., La Vecchia, C., Coffee and cancer: a review of epidemiological studies, 1990–1999. *Eur. J. Cancer* 2000, **9**, 241–256.
- [3] Dawson, T. M., Dawson, V. L., Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 2003, **302**, 819–822.
- [4] Jenner, P., Oxidative stress in Parkinson's disease. *Ann. Neurol.* 2003, **53**, 26–36.
- [5] Mena, S., Ortega, A., Estrela, J. M., Oxidative stress in environmental-induced carcinogenesis. *Mutat. Res.* 2009, **674**, 36–44.
- [6] Ranheim, T., Halvorsen, B., Coffee consumption and human health – beneficial or detrimental? – mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. *Mol. Nutr. Food Res.* 2005, **49**, 274–284.

- [7] Natella, F., Nardindi, M., Gianetti, I., Dattilo, C. et al., Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food Chem.* 2002, 50, 6211–6216.
- [8] Daglia, M., Racchi, M., Papetti, A., Lanni, C. et al., In vitro and ex vivo antihydroxyl radical activity of green and roasted coffee. *J. Agric. Food Chem.* 2004, 24, 1700–1704.
- [9] Onishi, M., Morishita, H., Iwahasi, H., Toda, S. et al., Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. *Phytochemistry* 1994, 36, 579–583.
- [10] Somoza, V., Lindenmeier, M., Wenzel, E., Frank, O. et al., Activity-guided identification of a chemopreventive compound in coffee beverage using in vitro and in vivo techniques. *J. Agric. Food Chem.* 2003, 51, 6861–6869.
- [11] Boettler, U., Sommerfeld, K., Volz, N., Pahlke, G. et al., Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression. *J. Nutr. Biochem.* 2011, 22, 426–440.
- [12] Boettler, U., Volz, N., Pahlke, G., Teller, N. et al., Coffees rich in chlorogenic acid or *N*-methylpyridinium as inducers of chemopreventive phase II enzymes via the Nrf2/ARE pathway in vitro and in vivo. *Mol. Nutr. Food Res.* 2011, 55, 798–802.
- [13] Nicoli, M. C., Manzocco, L., Lerici, C. R., Antioxidant properties of coffee brews in relation to the roasting degree. *LWT-Food Science and Technol.* 1997, 30, 292–297.
- [14] Moreira, D. P., Monteiro, M. C., Ribeiro-Alves, M., Donangelo, C. M. et al., Contribution of chlorogenic acids to the iron-reducing activity of coffee beverages. *J. Agric. Food Chem.* 2005, 53, 1399–1402.
- [15] Stalmach, A., Steiling, H., Williamson, G., Crozier, A., Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Arch. Biochem. Biophys.* 2010, 501, 98–105.
- [16] Greenberg, J. A., Boozer, C. N., Geliebter, A., Coffee, diabetes, and weight control. *Am. J. Clin. Nutr.* 2006, 84, 682–693.
- [17] Thom, E., The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. *J. Int. Med. Res.* 2007, 35, 900–908.
- [18] Hassan, H. A., Yousef, M. I., Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. *Food Chem. Toxicol.* 2009, 47, 2332–2337.
- [19] Mursu, J., Voutilainen, S., Nurmi, T., Alfthan, G. et al., The effects of coffee consumption on lipid peroxidation and plasma total homocysteine concentrations: a clinical trial. *Free Radic. Biol. Med.* 2005, 38, 527–534.
- [20] Esposito, F., Morisco, F., Verde, V., Ritieni, A. et al., Oxidative conjugation of chlorogenic acid with glutathione: Structural characterization of addition products and a new nitrite-promoted pathway. *Bioorg. Med. Chem.* 2003, 11, 4797–4805.
- [21] Grubben, M. J., Van Den Braak, C. C., Broekhuizen, R., De Jong, R. et al., The effect of unfiltered coffee on potential biomarkers for colonic cancer risk in healthy volunteers: a randomized trial. *Aliment. Pharmacol. Ther.* 2000, 14, 1181–1190.