Tumour formation in multiple intestinal neoplasia (Apc\textsuperscript{Min/+}) mice fed with filtered or unfiltered coffee

Seija I. Oikarinen\textsuperscript{1}, Iris Erlund\textsuperscript{2} and Marja Mutanen\textsuperscript{1}

\textsuperscript{1}Department of Applied Chemistry and Microbiology, Division of Nutrition, University of Helsinki, Helsinki, Finland; \textsuperscript{2}Department of Health and Functional Capacity, Biomarker Laboratory, National Public Health Institute, Helsinki, Finland

Abstract

**Background:** The aetiology of colorectal cancer has strong dietary links, and there may be an association between coffee and colorectal cancer risk.

**Objective:** To study the effects of filtered (low levels of kahweol/cafestol) and unfiltered (high levels of kahweol/cafestol) coffee on tumour formation in multiple intestinal neoplasia (Apc\textsuperscript{Min/+}) mice.

**Design:** Apc\textsuperscript{Min/+} mice (n=11 per group) were fed for 9 weeks with 10% w/w of these two types of coffee. Coffee was served as a dietary ingredient mixed with a semi-synthetic AIN-93G-based diet. Plasma levels of caffeine and paraxanthine were used as compliance markers. At the end of the feeding period intestinal tumour number and size were determined. The levels of β-catenin and cyclin D1, two cell-signalling proteins important to the progression of neoplasia, were also analysed in the tumour tissue.

**Results:** Plasma caffeine and paraxanthine concentrations were 3.2±1.4 and 1.7±0.4 μmol l\textsuperscript{-1} in the filtered coffee group and 3.6±2.3 and 1.6±0.6 μmol l\textsuperscript{-1} in the unfiltered coffee group. The level of plasma xanthines was below detection in the control group. The total number of tumours was equal between the dietary groups: 29 for the control, 30 (p=0.767) for the filtered coffee and 29 (p=0.430) for the unfiltered coffee groups. The levels of β-catenin and cyclin D1 in the nuclear fraction of the tumour tissue were also the same between the groups.

**Conclusions:** Filtered or unfiltered coffee (10% w/w) does not exert antitumorigenic activity in Apc\textsuperscript{Min/+} mice or change β-catenin and cyclin D1 signalling in the adenoma tissues. The results suggest that coffee does not change neoplasia progression in this animal model.

**Keywords:** Apc\textsuperscript{Min/+} mice; caffeine; coffee; colon cancer; paraxanthine

Received: 6 Jun. 2007; Revised: 22 Aug. 2007; Accepted: 23 Aug. 2007

**Introduction**

Colorectal cancer (CRC) is the third most common cancer worldwide (1). It occurs mainly sporadically and environmental factors, including diet, are strongly implicated in its aetiology (2). Diets high in vegetables, fruits, and perhaps fibre, or low in fat and red meat, have been suggested to be associated with a decreased risk of CRC. The association between coffee and CRC risk is a matter of controversy. The pooled results of 12 case-control studies showed a significant 28% reduction in CRC risk for a high versus low category of coffee consumption. However, a review of case-control studies showed inconsistent results between the coffee consumption and the risk of colon cancer (4). Cohort studies have not shown any reduction in CRC risk associated with coffee (3-6).

Animal studies on the effects of coffee constituents, such as caffeine, kahweol and cafestol, on colon tumorigenesis, have also shown conflicting results. Administration of caffeine at 0.1% level in drinking water enhanced heterocyclic amine-induced aberrant crypt number (7) and DNA adduct formation in the rat colon (8). Caffeine at a dose of 0.044% in drinking fluid had no inhibitory effect on control studies, reported a significant pooled relative risk of 0.76 (95% CI 0.66–0.89) for a high versus low category of coffee consumption. However, a review of case-control studies showed inconsistent results between the coffee consumption and the risk of colon cancer (4). Cohort studies have not shown any reduction in CRC risk associated with coffee (3-6).
tumour formation in the small intestine or colon of Apc\textsuperscript{Min/+} mice (9). In contrast, coffee oil and its major constituents, kahweol and cafestol, decreased the adenocarcinoma frequency in the colon of 1,2-dimethylhydrazine-treated rats, and inhibited the formation of mutagen-DNA adducts in the colonic mucosa (10, 11). Data obtained from animal studies and human interventions indicate that some putative chemopreventive mechanisms of coffee and its components could be related to the induction of several phase II xenobiotic metabolizing enzymes such as glutathione S-transferase and N-acetyltransferase, and protection against DNA damage caused by mutagens (11–16).

Colon carcinogenesis can be studied either by inducing aberrant crypt foci or tumour formation with chemical carcinogens or by using mouse models with defects in the Apc gene. One such mouse model for human familial adenomatous polyposis (FAP) is multiple intestinal neoplasia (Apc\textsuperscript{Min/+}) mouse (17). Apc\textsuperscript{Min/+} mice have a point mutation in the Apc allele, which in turn causes truncated Apc protein formation. Mutations in the APC/Apc gene lead to the accumulation of hypophosphorylated β-catenin protein in the cytosol and later in the nucleus, where β-catenin together with transcription factors (18) constitutively activate the expression of target genes such as C-myc and cyclin-D1 (19, 20). Mutations in the APC/Apc gene predispose people to CRC, whereas in Apc\textsuperscript{Min/+} mice, owing to their short lifespan, most of the tumours are benign adenomas. This experiment studied the possible chemopreventive effect of filtered or unfiltered coffee (10% w/w) on tumour formation in Apc\textsuperscript{Min/+} mice. Both filtered and unfiltered coffees contain caffeine, but the concentration of kahweol and cafestol depends on brewing methods (21). Unfiltered coffee, which represents the traditional type of coffee consumed particularly in Scandinavia, contains cafestol and kahweol, whereas filtered coffee mostly lacks these compounds.

Materials and methods

Animal experiment
C57BL/6J–Apc\textsuperscript{Min/+} mice were bred at the Laboratory Animal Centre of the University of Helsinki, Finland. Mice positive for the Apc\textsuperscript{Min/+} genotype, as determined by polymerase chain reaction (PCR) amplification of weaning tail biopsy DNA (22), were assigned randomly to the experimental diets at a mean age of 34 ± 2 days (5 weeks) with 11 mice per group. Each diet group contained seven males and four females. Animals were housed in plastic cages in a temperature- and humidity-controlled animal facility, with a 12 h light/dark cycle. They had free access to the experimental semi-synthetic diets and tap water for the feeding period of 9 weeks. Fresh diets were provided four times per week. Body weights were recorded weekly. The experimental protocol was approved by the Laboratory Animal Ethics Committee of the University of Helsinki.

Diets
All the experimental diets were semi-synthetic AIN-93G-based (23) high-fat diets. The fat concentration (20 g per 100 g) and fat composition of the experimental diets were designed to approximate those in a typical Western-type diet; the diet provided intakes of saturated, monounsaturated and polyunsaturated fatty acids in an approximate ratio of 3:2:1 (24). Three diets were included: a control diet, a filtered coffee diet (10% w/w) and an unfiltered coffee diet (10% w/w). All diets were similar with respect to protein (20%), fat (40%) and carbohydrates (40%) on an energy basis (kJ). The filtered coffee was prepared in a regular procedure by adding 14 g of coffee per 1.25 dl (1 cup) of water and using a paper filter. The unfiltered coffee was prepared by boiling ground coffee beans with water (14 g of coffee per 1.25 dl). After cooling, the unfiltered coffee was decanted. The coffee diets were prepared by adding to the control diet 10% (w/w) of filtered or unfiltered coffee beverage. Coffee was served as a dietary ingredient and not a drink because it was found that coffee drink separated into layers in a drinking bottle. Food consumption per mouse was estimated to be 2.4 g per day. Therefore, the estimated amount of coffee consumed per body weight of the animals was approximately equivalent to 0.6 litres per day for humans. The composition of the experimental diets is shown in Table 1.

Intestinal tumour scoring and tissue samples
At the age of 14 weeks, the mice were killed by carbon dioxide asphyxiation. Blood was collected from the caudal vena cava into heparinized tubes and centrifuged at 6000 \times g for 1 min, after which plasma was stored at −70°C. The small intestine, caecum and colon were removed, opened along the longitudinal axis and rinsed with ice-cold saline.
The small intestine was divided into five sections of equal length. The caecum and colon were kept together. The small intestine and colon and caecum were then spread flat on a microscope slide. The number, diameter and location of intestinal tumours were determined with an inverse light microscope with a magnification of 67 by two observers blind to the dietary treatment. The minimum detection limit of the adenoma diameter was 0.3 mm. Adenoma tissue was excised, and the normal-appearing mucosa tissue was scraped. Tissues were snap-frozen in liquid nitrogen and stored at −70°C for further analysis.

Plasma xanthine analysis

Plasma concentrations of caffeine, paraxanthine, theophylline and theobromine were determined to verify coffee intake. The analyses were performed by a new method based on liquid–liquid extraction and high-performance liquid chromatography (HPLC). Fifty microlitres of plasma was incubated with 5 μl of β-glucuronidase/sulfatase solution, 10 mM of ascorbic acid and 85 mM of sodium acetate for 18 h to cleave potential glucuronide and sulfate conjugates. Fifty microlitres of sodium acetate buffer (0.7 M, pH 2) and 1 ml of ethyl acetate were added, and xanthines were extracted into the upper phase during thorough mixing. The procedure was repeated three times, whereafter the upper phases were combined and dried down under nitrogen. The dried sample was dissolved into the HPLC mobile phase. Xanthines were then separated by HPLC. The equipment consisted of an Agilent 1100 Series components including quaternary pump, autosampler, degasser and variable-wavelength ultraviolet (UV) detector (Palo Alto, CA, USA). Chromatographic separations were achieved using an Agilent Eclipse XDP-C18 (5 μM, 4.6 x 150 mm) (Palo Alto, CA, USA). The mobile phase consisted of 15% methanol in 10 mM sodium acetate buffer (adjusted to pH 5 with orthophosphoric acid). The flow rate was set at 1.5 ml min⁻¹ and the wavelength at 274 nm. The recovery levels of the four xanthines were >90%. Intra-assay and interassay precision was below 4% and 9%.

Western blot analysis

The adenoma tissues of the small intestine were fractionated into nuclear, cytosolic and membranous fractions as described by Pajari et al. (24) for Western blot analysis. Normalized amounts of the nuclear proteins (5–10 μg), and constant amounts of rat brain or TMK (a human gastric cancer cell) homogenate (controls for interassay variation) were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and each sample was run twice, the duplicates being loaded on a different gel. The proteins were blotted onto a nitrocellulose membrane (Hybond ECL; Amersham Pharmacia Biotech, UK) at 100 V for 1 h, and blots were blocked with 5% non-fat milk powder in Tris-buffered saline containing 0.1% Tween (TBS-Tween) overnight at +4°C. Antibodies against β-catenin (sc-7199) and a horseradish peroxidase (HRP)-conjugated secondary antibody (sc-2030) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cyclin D1 was from NeoMarkers (Lab Vision, Fremont, CA, USA). The blots were first probed with the primary antibodies for 2 h at room temperature, washed three times with TBS-Tween and incubated with the HRP-conjugated secondary antibody for 2 h. Signals were visualized by using the ECL reagents and film (Amersham Corporation, Arlington Heights, IL, USA) according to the manufacturer’s instruction. Specificities of signals were verified either with incubations without appropriate primary antibody or by using a blocking peptide provided by a supplier (Lab Vision, Fremont, CA, USA). The specificity of the β-catenin bands was ensured earlier by using two other commercially available antibodies (24). Blots were scanned and analysed using a GS-800 Calibrating

---

Table 1. Composition of experimental diets (g kg⁻¹ diet)¹

| Ingredient                  | Control | Filtered coffee | Unfiltered coffee |
|-----------------------------|---------|-----------------|------------------|
| Casein                      | 236.2   | 212.6           | 212.6            |
| Dextrose                    | 479     | 431.1           | 431.1            |
| Butter                      | 148.9   | 134.0           | 134.0            |
| Sunflower oil               | 13.3    | 12.0            | 12.0             |
| Rapeseed oil                | 62.2    | 56.0            | 56.0             |
| Mineral mix AIN-93-MX        | 41.6    | 37.4            | 37.4             |
| Vitamin mix AIN-93-VX        | 11.8    | 10.6            | 10.6             |
| L-cystine                   | 3.6     | 3.2             | 3.2              |
| Choline chloride            | 3.6     | 3.2             | 3.2              |
| Tert-butylhydroquinone       | 0.014   | 0.014           | 0.014            |
| Coffee¹                     | 100     | 100             | 100              |

¹Casein was obtained from Kainuun Osuusmeijeri (Sotkamo, Finland), dextrose from Six Oy (Helsinki, Finland), mineral and vitamin mix from Harlan Teklad (Madison, WI, USA), L-cystine, choline chloride and tertiary butylhydroquinone from Yliopiston Apteekki (Helsinki, Finland). Butter, sunflower oil, rapeseed oil and ground coffee beans for filtered and unfiltered coffee were from a local market.

²See Materials and methods.
Densitometer and the Quantity One program (BioRad Laboratories, Hercules, CA, USA). Results in duplicate were expressed as sample band intensity (optical density of the band multiplied by the band area) divided by control band intensity.

Statistical analysis
The Mann–Whitney non-parametric test was used to compare the two coffee groups with the control group. Tumour incidence in the colon was analysed by Fisher’s exact test. The SPSS statistical program, version 12 (SPSS Inc., Chicago, IL, USA), was used for all statistical analysis. Differences were considered significant at $p < 0.05$.

Results

Weight gain of the $Apc^{Min/-}$ mice
The mice grew well during the feeding period and the weight gain and the final body weights of the $Apc^{Min/-}$ mice did not differ between the groups (Fig. 1). At a few time points, coffee-fed mice had slightly lower body weights than controls. The male mice in the filtered coffee group had lower body weights at the age of 11 weeks ($p < 0.05$), and the females in the unfiltered coffee group had lower body weights at the ages of 7, 8, 9 and 10 weeks ($p < 0.05$) compared with the control animals. The authors suggest that these small differences in body weights at certain time-points are not likely to be biologically significant.

Adenoma results
The male and female $Apc^{Min/-}$ mice did not differ significantly in the number or diameter of adenomas and therefore the data from male and female mice were pooled. The range of adenoma number and adenoma distribution between the small intestine and the colon here were similar to those found in other studies (25).

The tumour number or size in the small intestine and the colon did not differ between dietary groups (Table 2). The total tumour number was 29 for the control group, 30 for the filtered coffee group and 29 for the unfiltered coffee group.

$\beta$-Catenin and cyclin D1 analysis
The subcellular localization, particularly nuclear $\beta$-catenin and cyclin D1, is considered to be an important determinant of their function. The effects of coffee on $\beta$-catenin and cyclin D1 in tumours were therefore analysed by a semi-quantitative Western blot method. As observed in a previous study (24), immunoblots of $\beta$-catenin gave one band at 98 kDa in rat brain homogenate (control), and one band (or two bands) at 92 kDa (full-length $\beta$-catenin) in the adenoma tissue of $Apc^{Min/-}$ mice (Fig. 2A). The $\beta$-catenin results are expressed as the intensity of the full-length band, which could be detected in almost all samples. Immunoblots of cyclin D1 gave one band at 35 kDa in the TMK (a human gastric cancer cell) homogenate (control), and two bands at 35 and 36 kDa, which probably represent full-length cyclin D1 with different levels of phosphorylation. The cyclin D1 results are expressed as the intensity of these two bands. The levels of nuclear $\beta$-catenin (Fig. 2B) and cyclin D1 (Fig. 2C) in the adenoma tissue did not differ between the dietary groups.

Plasma xanthine analysis
Caffeine and its metabolites paraxanthine, theobromine and theophylline were analysed from mouse plasma samples. Paraxanthine was clearly the dominant metabolite, and theophylline as well as theobromine was present in minute amounts only. Therefore, caffeine and paraxanthine were used as markers of compliance. Plasma caffeine and paraxanthine concentrations were $3.2 \pm 1.4$ and $1.7 \pm 0.4 \mu mol l^{-1}$ in the filtered coffee group and $3.6 \pm 2.3$ and $1.6 \pm 0.6 \mu mol l^{-1}$ in the unfiltered coffee group. Xanthine concentrations in the control group were below the limit of quantification.
Discussion

To the authors’ knowledge, this is the first study investigating the effects of filtered or unfiltered coffee on adenoma formation in the ApcMin/+ mouse. The main difference between these two types of coffee is the levels of kahweol and cafestol, which are abundantly present only in unfiltered coffee. Kahweol and cafestol have some bioactivity in the human body, since unfiltered coffee has been shown to raise serum cholesterol in man (26). In the present study, filtered and unfiltered coffee was evaluated for possible antitumorigenic activities. Consumption of a coffee-free diet, or filtered or unfiltered coffee (10% w/w) for 9 weeks had a similar effect on tumour formation in ApcMin/+ mice. Supplementation of the diet with coffee increased significantly the levels of plasma caffeine and paraxanthine in the plasma, indicating good compliance with coffee supplementation. The results show that filtered or unfiltered coffee, their constituents (caffeine, kahweol and cafestol) and the caffeine metabolite paraxanthine had no chemoprotective effect in the ApcMin/+ mice. The caffeine result is in line with another study with ApcMin/+ mice, which showed that administration of caffeine at a dose of 0.044% in drinking fluid had no inhibitory effect on tumour formation in this animal model (9).

Even though the adenoma result did not differ between the groups, the authors wanted to determine whether coffee changes the subcellular localization of β-catenin and its target protein cyclin D1, since the improper regulation of cellular β-catenin pools is one of the driving forces in Apc-induced colonic neoplasia (18, 27). Dietary components have been shown to affect β-catenin localization in both the normal-appearing mucosa and the tumour tissue, thus diminishing the progression of neoplasia in Apc-induced mice (9, 28, 29). In the present study no differences in the nuclear β-catenin and cyclin D1 levels were found between dietary groups, and therefore it may be concluded that coffee consumption does not affect cancer progression in the adenoma tissue. However, these results do not exclude the possibility that coffee consumption has a protective effect against other type of cancers in which the aetiology of the disease if different.

Table 2. Tumour number, and size in the small intestine and the colon of ApcMin/+ mice fed with a control diet or diets supplemented with either filtered or unfiltered coffee for 9 weeks

| Diet               | Control Median | Control Range | Filtered coffee Median | Filtered coffee Range | Unfiltered coffee Median | Unfiltered coffee Range |
|--------------------|----------------|---------------|------------------------|-----------------------|--------------------------|-------------------------|
| n                 | 11             |               | 11                     |                       | 11                       |                         |
| Small intestine    |                |               |                        |                       |                          |                         |
| Tumours per mouse | 29             | 23–202        | 29                     | 15–127                | 29                       | 21–66                   |
|                   |                |               | p = 0.693              |                       | p = 0.430                |                         |
| Tumours per mousea| 29             | 23–65         | 29                     | 15–62                 | 29                       | 21–66                   |
|                   |                |               | p = 0.677              |                       | p = 0.646                |                         |
| Tumour size (mm)  | 1.1            | 1.0–1.4       | 1.2                    | 0.7–1.4               | 1.1                      | 0.9–1.4                 |
|                   |                |               | p = 0.768              |                       | p = 0.768                |                         |
| Colon             |                |               |                        |                       |                          |                         |
| Incidence         | 4/11           |               | 7/11                   |                       | 3/11                     |                         |
| Tumours per mouse | 0              | 0–2           | 1                      | 0–4                   | 0                        | 0–2                     |
|                   |                |               | p = 0.207              |                       | p = 0.811                |                         |
| Tumour size (mm)  | 3.0            | 1.7–4.1       | 2.9                    | 2.0–4.0               | 2.4                      | 2.3–3.3                 |
|                   |                |               | p = 0.850              |                       | p = 0.593                |                         |
| Total             |                |               |                        |                       |                          |                         |
| Tumours per mouse | 29             | 25–202        | 30                     | 15–131                | 29                       | 21–68                   |
|                   |                |               | p = 0.767              |                       | p = 0.430                |                         |

Results are expressed as median and range (minimum–maximum). The Mann–Whitney non-parametric test was used to compare the filtered and unfiltered coffee groups with the control group. Differences were considered significant at p < 0.05.

aThe adenoma data from male (n = 7) and female (n = 4) mice were pooled.

bExclusion of two extreme values (202 and 127) from the control and filtered coffee groups, respectively, did not change the statistical significance between the groups.
from CRC. The results of three Japanese cohort studies showed that coffee consumption decreased the risk of liver cancer, although the exact mechanism of action remained unclear (30, 31).

In the present study, caffeine and paraxanthine were present in the plasma samples of all mice receiving coffee, but not the controls. Paraxanthine appeared to be a good compliance marker and, because of the smaller variation in paraxanthine results, a better marker than plasma caffeine. These findings are in line with those of Klebanoff et al. (32), who suggested that plasma paraxanthine reflects coffee consumption more accurately than plasma caffeine in humans, owing to its more favourable pharmacokinetic properties. To the authors’ knowledge, this type of information is not available from animal studies, although the primary metabolism of caffeine, i.e. demethylation by CYP1A2, has been extensively studied in many species (33). The caffeine and paraxanthine concentrations were in the same range as those found among the Finnish population (I. Erlund, unpublished results). As the intake of coffee in the present experiment was similar to the mean daily consumption in Finnish adults, it may be concluded that caffeine bioavailability is not dramatically different in humans and in mice. Since weight gain was also similar in the three different groups, non-compliance cannot explain the results of the study.

In conclusion, this study found that filtered or unfiltered coffee (10% w/w) does not exert anti-tumorigenic activity in ApcMin+/C27 mice or change β-catenin and cyclin D1 signalling in the adenoma tissues.

Acknowledgement

The study was supported in part by a grant from the Institute for Scientific Information on Coffee (ISIC).

References

1. Stewart BW, Kleihues P, eds. World Cancer Report. Lyon: IACR Press; 2003.
2. Potter JD. Colorectal cancer: molecules and populations. J Nat Cancer Inst 1999;91:916–32.
3. Giovannucci E. Meta-analysis of coffee consumption and risk of colorectal cancer. Am J Epidemiol 1998;147:1043–52.
4. Tavani A, La Vecchia C. Coffee, decaffeinated coffee, tea and cancer of the colon and rectum: a review of epidemiological studies, 1990–2003. Cancer Causes Control 2004;15:743–57.
5. Michels KB, Willett WC, Fuchs CS, Giovannucci E. Coffee, tea, and caffeine consumption and incidence of colon and rectal cancer. J Natl Cancer Inst 2005;97:282–92.
6. Larsson SC, Bergkvist L, Giovannucci E, Wolk A. Coffee consumption and incidence of colorectal cancer in two prospective cohort studies of Swedish women and men. Am J Epidemiol 2006;163:638–44.
7. Tsuda H, Sekine K, Uehara N, Takasuka N, Moore MA, Kenno Y, et al. Heterocyclic amine mixture carcinogenesis and its enhancement by caffeine in F344 rats. Cancer Lett 1999;143:229–34.
8. Takeshita F, Ogawa K, Asamoto M, Shirai T. Mechanistic approach of contrasting modifying effects of caffeine on carcinogenesis in the rat colon and mammary gland induced with 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Cancer Lett 2003;194:25–35.
9. Ju J, Hong J, Zhou J-n, Pan Z, Bose M, Yang G-u, et al. Inhibition of intestinal tumorigenesis in ApcMin+/C27 mice
by (−)-epigallocatechin-3-gallate, the major catechin in green tea. Cancer Res 2005;65:10623–31.

10. Gershbein LL. Action of dietary trypsin, pressed coffee oil, silymarin and iron salt on 1,2-dimethylhydrazine tumorigenesis by gavage. Anticancer Res 1994;14:1113–6.

11. Huber WW, McDaniel LP, Kaderlik KR, Teitel CH, Lang NP, Kadlubar FF. Chemoprotection against the formation of colon DNA adducts from the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat. Mutat Res 1997;376:115–22.

12. Cavin C, Holzhauer D, Constable A, Huber WW, Schilter B. Cafestol and kahweol, two coffee specific deterpenes with anticarcinogenic activity. Food Chem Toxicol 2002;40:1155–63.

13. Huber WW, Teitel CH, Coles BF, King RS, Wiese FW, Kaderlik KR, et al. Potential chemopreventive effects of coffee components kahweol and cafestol palmitates via modulation of hepatic N-acetyltransferase and glutathione S-transferase activities. Environ Mol Mutagen 2004;44:265–76.

14. Grubben MJ, van den Braak CC, Broekhuizen R, de Jong R, van Rijn J, de Ruiter E, et al. The effect of unfiltered coffee on potential biomarkers for colonic cancer risk in healthy volunteers: a randomized trial. Aliment Pharmacol Ther 2000;14:118–90.

15. Esposito F, Morisco V, Verde V, Ritiene A, Alezio A, Caporaso N, Fogliano V. Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects. Aliment Pharmacol Ther 2003;17:595–601.

16. Steinkellner H, Hoelzl C, Uhl M, Cavin C, Haidinger G, Gsur A, et al. Coffee consumption induces GSTP in plasma and protects lymphocytes against (+)-anti-bezo[ap]yrene-7,8-dihydrodiol-9,10-epoxide induced DNA damage: results of controlled human intervention trials. Mutat Res 2005;591:264–75.

17. Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 1990;247:322.

18. Korinek V, Barker N, Morin PJ, Vaarbaard J, Wiese FW, Kaderlik KR, et al. Identification of the major modifier locus affecting multiple intestinal neoplasia in the mouse. Cell 1993;75:631–9.

19. Urgert R, van der Weg G, Kosmeijer-Schuil TG, van der Bovenkamp P, Hovenier R, Katan MB. Levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee brews. J Agric Food Chem 1995;43:2167–72.

20. Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, Luongo C, et al. Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. Cell 1993;75:631–9.

21. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.

22. Pourjavan A-M, Pourkian A, Kosma V-M, Rafter J, Mutanen M. Promotion of intestinal tumour formation by inulin is associated with an accumulation of cytosolic β-catenin in Min mice. Int J Cancer 2003;106:653–60.

23. Carpentier DE, Pierre F. Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in Min mice and choice of model system. Cancer Epidemiol Biomarkers Prev 2003;12:391–400.

24. Weusten-Van der Wouw MP, Katan MB, Vianon R, Huggett AC, Liardon R, Lund-Larsen PG, et al. Identity of the cholesterol-raising factor from boiled coffee and its effect on liver function enzymes. J Lipid Res 1994;35:721–33.

25. Henderson BR. Nuclear-cytoplasmic shuttling of APC regulates β-catenin subcellular localization and turnover. Nature Cell Biol 2000;2:653–60.

26. Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, et al. Plant phenolics decrease intestinal tumours in an animal model of familial adenomatous polyposis. Carcinogenesis 2000;21:921–7.

27. Schmelz EM, Roberts PC, Kustin EM, Lemonnier LA, Sullards MC, Dillehay DL, Merrill AH Jr. Induction of intracellular β-catenin localization and intestinal tumorigenesis in vivo and in vitro by sphingolipids. Cancer Res 2001;61:6723–9.

28. Inoue M, Yoshimi I, Sobue T, Tsugane S. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. J Natl Cancer Inst 2005;97:293–300.

29. Shimazu T, Tsubono Y, Kuriyama S, Ohmori K, Koizumi Y, Nishino Y, et al. Coffee consumption and the risk of primary liver cancer: pooled analysis of two prospective studies in Japan. Int J Cancer 2005;116:150–4.

30. Klebanoff MA, Levine RJ, Dersimonian R, Clemens JD, Wilkins DG. Serum caffeine and paraxanthine as markers for reported caffeine intake in pregnancy. Ann Epidemiol 1998;8:107–11.

31. Walton K, Dorne JL, Renwick AG. Uncertainty factors for chemical risk assessment: interspecies differences in the in vivo pharmacokinetics and metabolism of human CYP1A2 substrates. Food Chem Toxicol 2001;39:667–80.

Seija Oikarinen, PhD
Department of Applied Chemistry and Microbiology
Division of Nutrition
PO Box 66
FI-00014 University of Helsinki
Helsinki, Finland Tel: +358 9 19158974
Fax: +358 9 19158269
E-mail: seija.oikarinen@helsinki.fi