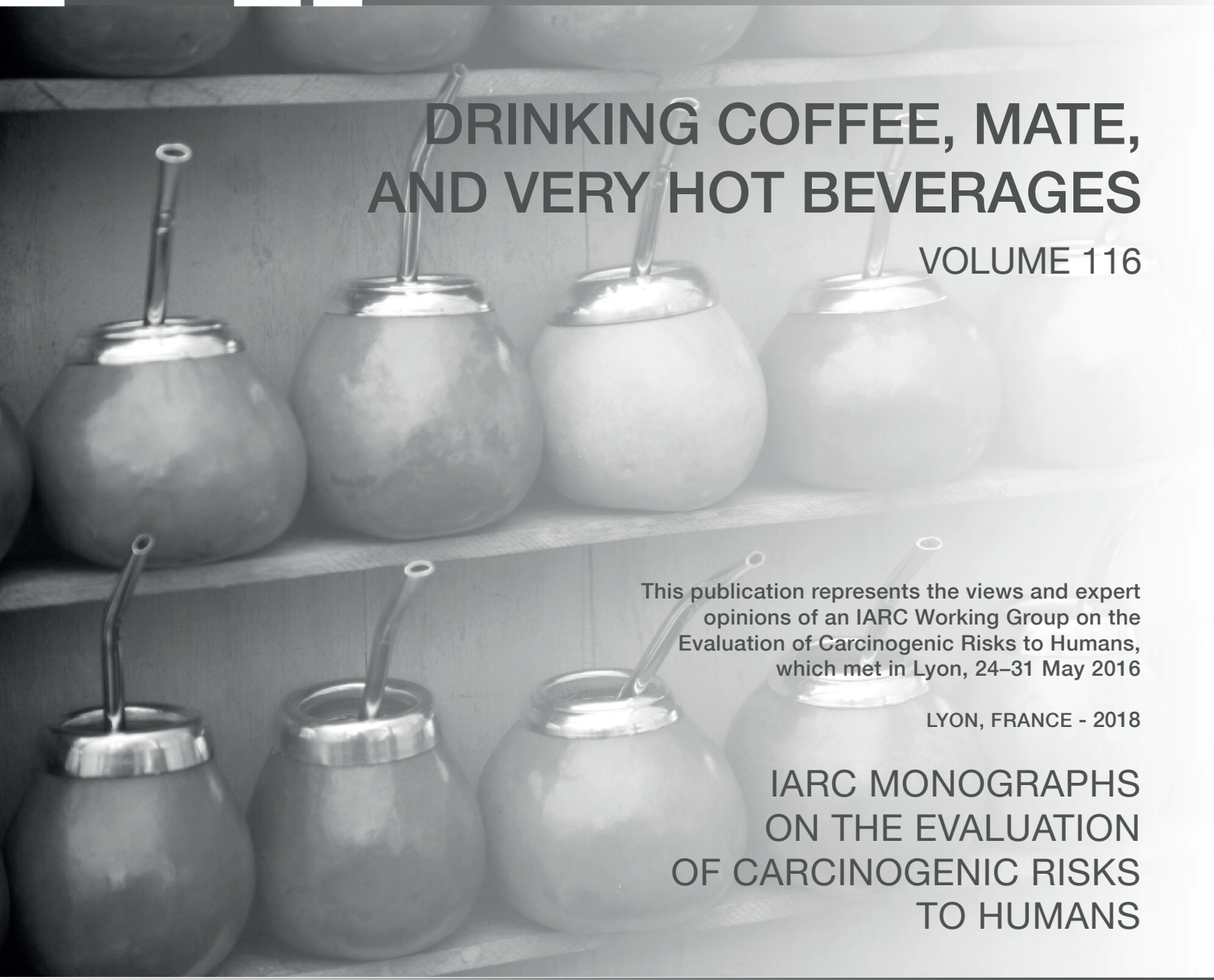
IARC MONOGRAPHS



DRINKING COFFEE, MATE, AND VERY HOT BEVERAGES

VOLUME 116

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

4. MECHANISTIC AND OTHER RELEVANT DATA

4.1 Toxicokinetic data

4.1.1 Humans

(a) Absorption

(i) Caffeine

Table 4.1 (web only; available at: http://publications.iarc.fr/566) summarizes pharmacokinetics parameters maximum plasma concentration (C_{max}), time to peak concentration (T_{max}), and the area under the curve (AUC) of caffeine from studies in humans.

Several studies in humans have shown rapid and dose-dependent absorption of caffeine in subjects administered coffee. Coffee consumption significantly increased caffeine in plasma in a single-blind, three-stage clinical trial of 11 men and 36 women, all regular coffee consumers (4.0 ± 1.7 cups/day) who had abstained from coffee consumption for 1 month (Kempf et al., 2010). [Two smokers were included in the analysis. There were no data on caffeine content in coffee used in the study.] Caffeine was rapidly absorbed, reaching C_{max} 1.2 h after consumption, in a study of healthy non-smokers (7 men, 5 women) who ingested a single dose of 70 mg caffeine as a green/roasted coffee blend dissolved in water (Martínez-López et al., 2014). [The 70 mg dose was selected to avoid possible saturation processes and nonlinear kinetics reported with higher caffeine doses.] A caffeine mean peak level of 9.7 ± 1.2 µg/mL and time to peak of 42 ± 5 minutes was reported in subjects administered coffee (400 mg caffeine) in a randomized, double-blind, single-dose, placebo-controlled, study of caffeine pharmacokinetics in 8 men and 5 women characterized as regular coffee and cola consumers (1 smoker) (Liguori et al., 1997).

Studies in humans given caffeine added to decaffeinated instant coffee (Gelal et al., 2003) or caffeine as a capsule or gum (Kaplan et al., 1997; Kamimori et al., 2002; Skinner et al., 2014) reported rapid, dose-dependent absorption.

An in vitro study using human skin membrane [of less relevance to pharmacokinetics of caffeine from coffee] demonstrated absorption of caffeine (100 µg/m^2) with time to maximum rate of 1.2 ± 0.2 hour to 5.2 ± 1.2 hour (van de Sandt et al., 2004).

(ii) Phenolic acids

Table 4.2 (web only; available at: http://publications.iarc.fr/566) is a summary of pharmacokinetics parameters C_{max}, T_{max}, and AUC of phenolic acids from studies in humans.

Hydroxycinnamic acids are rapidly absorbed after coffee consumption. Peak absorption of caffeic acid (CA) was reached 1 hour after giving 200 mL of brewed coffee to 10 healthy men who were non-smoking moderate coffee drinkers (2–4 cups/day of coffee (Nardini et al., 2002). 5-Caffeoylquinic acid (5-CQA) was the major hydroxycinnamic acid present in plasma, contributing 40.7% of AUC in 6 non-smoking healthy volunteers (2 men, 4 women) given 190 mL of decaffeinated brewed coffee (Monteiro et al., 2007). Two plasma concentration peaks

were observed in all subjects for all hydroxycinnamic acids. [Biphasic concentration peaks could be attributed to either enterohepatic circulation or to colonic metabolism.] Stalmach et al. (2009) identified 12 different compounds related to chlorogenic acid (CGA) in 11 non-smoking subjects (8 men, 3 women) who followed a polyphenol-free diet for 48 hour before administration of 200 mL of instant coffee. The T_{max} of up to 1 hour was indicative of small intestine absorption.

In the study of Kempf et al. (2010) reported above, significant increases were seen in coffee-derived compounds including CA, ferulic acid (FA), and isoferulic acid (iFA) after daily consumption. In two reports (Renouf et al., 2010a, b), CA, FA, and iFA reached C_{max} approximately 1 hour after administration of 4 g of instant coffee in 9 healthy non-smoking coffee consumers (4 men, 5 women). [Plasma was sampled up to 12 hours after coffee consumption; data on certain late-appearing phenolic acids was therefore lacking.]

In a similar randomized, crossover study of 10 healthy non-smoking coffee consumers (4 men, 6 women) (Renouf et al., 2014), phenolic acids appeared rapidly in the plasma, but the overall level of hydroxycinnamic acids remained low (AUC < 10 μM min, C_{max} < 100 nM). The hydroxycinnamic acid AUC values increased during dose escalation. [The exclusion criterion for smoking was > 5 cigarettes/day.]

In a study of 9 healthy volunteers (4 men, 5 women) who consumed a single dose of 400 mL instant coffee, dimethoxycinnamic acid was found in plasma exclusively as a free aglycone, with a C_{max} of 496 ± 110 nM reached 30 minutes after dosing (Farrell et al., 2012). [Smoking status of the subjects was not assessed.]

Several studies investigated absorption of hydroxycinnamic acids after coffee administration in individuals with an ileostomy (Stalmach et al., 2010; Erk et al., 2012, 2014b). In 3 men and 2 women, 71 ± 7% of hydroxycinnamic acids ingested as a single 200 mL dose of instant coffee drink was recovered in the form of parent compound and its metabolites in ileostomy effluent (Stalmach et al., 2010). In two studies, 5 women with ileostomies were given a single dose of decaffeinated coffee containing either hydroxycinnamic acids (4525 μmol, 2219 μmol, or 1053 μmol) (Erk et al., 2012) or CQAs (746 μmol) (Erk et al., 2014b). For hydroxycinnamic acids, 68.8 ± 9.0% and 77.4 ± 4.3% of the high and low ingested dose, respectively, were recovered in the ileal fluid [suggesting that one third of the ingested amount is absorbed in the small intestine]. For CQAs, the recovery rate was 76.2%.

In a further study of 10 non-smoking healthy volunteers (5 men, 5 women) given 170 mg of hydroxycinnamic acids via decaffeinated green coffee extract in a capsule in plasma (Farah et al., 2008), apparent bioavailability of chlorogenic acids varied considerably over the range 7.8–72.1% (mean: 33 ± 23%) [no data on regular coffee consumption were provided.]

In a study using instant coffee in vitro (Farrell et al., 2011), rapid and time-dependent membrane permeation of dimethoxycinnamic acid was seen in Caco-2 cells. Paracellular diffusion was the main transport mechanisms of hydroxycinnamic acids, and the monocarboxylic acid transporter was a mediator of CA disposition (Konishi & Kobayashi, 2004).

(iii) Other compounds

After a single dose (350 mL) of filtered coffee given to healthy non-smoking regular coffee consumers who had abstained from caffeine for 10 days, a higher maximum concentration of trigonelline was reached later in women (n = 6, C_{max} = 6547 nmol/L, T_{max} = 3.17 hours) as compared with men (n = 7, C_{max} = 5479 nmol/L, T_{max} = 2.29 hours) (Lang et al., 2010). No difference was observed for *N*-methylpyridinium.

De Roos et al. (1998) reported dose-dependent absorption of diterpenes in 9 healthy volunteers (4 men, 5 women) with an ileostomy after coffee

consumption. [No data on smoking and regular coffee consumption were available.]

(b) Distribution

A high volume of distribution was reported in a study of healthy non-smoking regular coffee drinkers (7 men and 6 women) who ingested a single 350 mL dose of coffee after a 10-day washout period (Lang et al., 2010). The volume of distribution (i.e., the theoretic-al volume that would be necessary to contain the total amount of an administered dose) was 123 L and 148 L for trigonelline and 211 L and 214 L for *N*-methylpyridinium, for women and men, respectively.

(c) Metabolism

(i) Caffeine

A general schematic of caffeine metabolism is presented in Fig. 4.1.

In the study of Martínez-López et al. (2014) described in Section 4.1.1 (a) (i) above, paraxanthine (PX) was the major metabolite followed by 1-methyluric acid (1-MU) and 1-methylxanthine (1-MX). All detected metabolites were present in plasma from the first sampling time (30 minutes after coffee consumption). [Data on regular coffee consumption were not provided.]

In 9 (7 men, 2 women) healthy non-smoking regular coffee drinkers (≥ 4 cups per day) administered caffeine (0, 4.2, or 12 mg/kg per day in decaffeinated coffee in three randomized treatment blocks of 5 days each), the higher caffeine dose resulted in plasma AUC values for all evaluated metabolites that were at least 3.3-fold higher (Denaro et al., 1990). The metabolism of caffeine under long-term dosing conditions decreased in a dose-dependent manner, leading to the accumulation of methylxanthines.

Additional studies on the modulating effect of coffee on metabolizing enzymes can be found in Section 4.1.3 of this monograph.

(ii) Phenolic acids

A general schematic of chlorogenic acids metabolism is presented in Fig. 4.2.

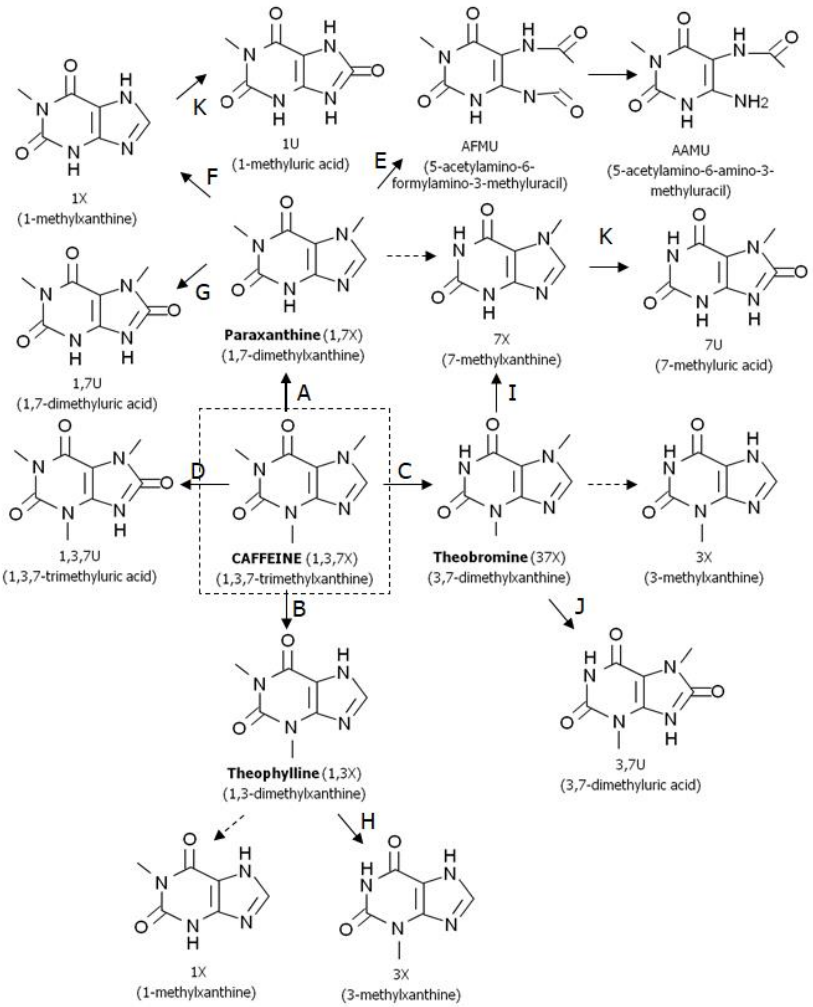
In the study of Farah et al. (2008) described in Section 4.1.1 (a) (ii) above, the hydroxycinnamic acids metabolites CA, FA, and iFA and *p*-coumaric acids contributed about 20.3% of the total phenolics detected in plasma. On the other hand, sinapic, gallic, *p*-hydroxybenzoic, and dihydrocaffeic (DHCA) acids were the major phenolic compounds found in urine (approximately 82%). [Plasma was not sampled 8 hours after dosing, when concentrations of hydroxycinnamic acids in some of the subjects were still high. No data on regular coffee consumption were available.]

In the study of Erk et al. (2012) described in Section 4.1.1 (a) (ii) above, sulfation was the dominant form of conjugation and significant inter-individual variation in metabolism of hydroxycinnamic acids was observed. [Only women were included in the study. Most of the observed inter-individual differences came from a single outlier.]

In two studies conducted by Renouf et al. (Renouf et al., 2010a, b), DHCA and dihydroferulic acid (DHFA) reached maximum plasma concentration (approximately 200 nM and 550 nM, respectively) 10 hours after ingestion. [Plasma was sampled up to 12 hours after the coffee consumption; the complete kinetics of certain late-appearing phenolic acids was therefore lacking.]

Fumeaux et al. (2010) identified and characterized several hydroxycinnamic acids for the first time in the plasma and urine of 11 healthy volunteers given a single dose of hydroxycinnamic acids of 412 µmol consumed as instant coffee. Four were identified in plasma (CA and DHCA 3′-sulfate, and FA and DHFA 4′-sulfate), and ten in urine (CA 3′- and 4′-sulfates, DHCA 3′-O-glucuronide and 3′-sulfate, FA 4′-sulfate, iFA 3′-sulfate, DHFA 4′-O-glucuronide and

Fig. 4.1 Important metabolic pathways for caffeine and its metabolites



A, F: CYP1A2; B, D: CYP1A2, CYP2C8, CYP2C9, CYP2E1, CYP3A4; C: CYP1A2, CYP2E1, E: NAT2; G: CYP1A2, CYP2A6; H: methylxanthine N1 demethylase; I: methylxanthine N3 demethylase; J, K: xanthine oxidase
Compiled by the Working Group

4′-sulfate, FA and dihydroisoferulic acid 3′-O-glucuronides). [Sex, smoking status, and regular coffee consumption of study subjects were not reported.]

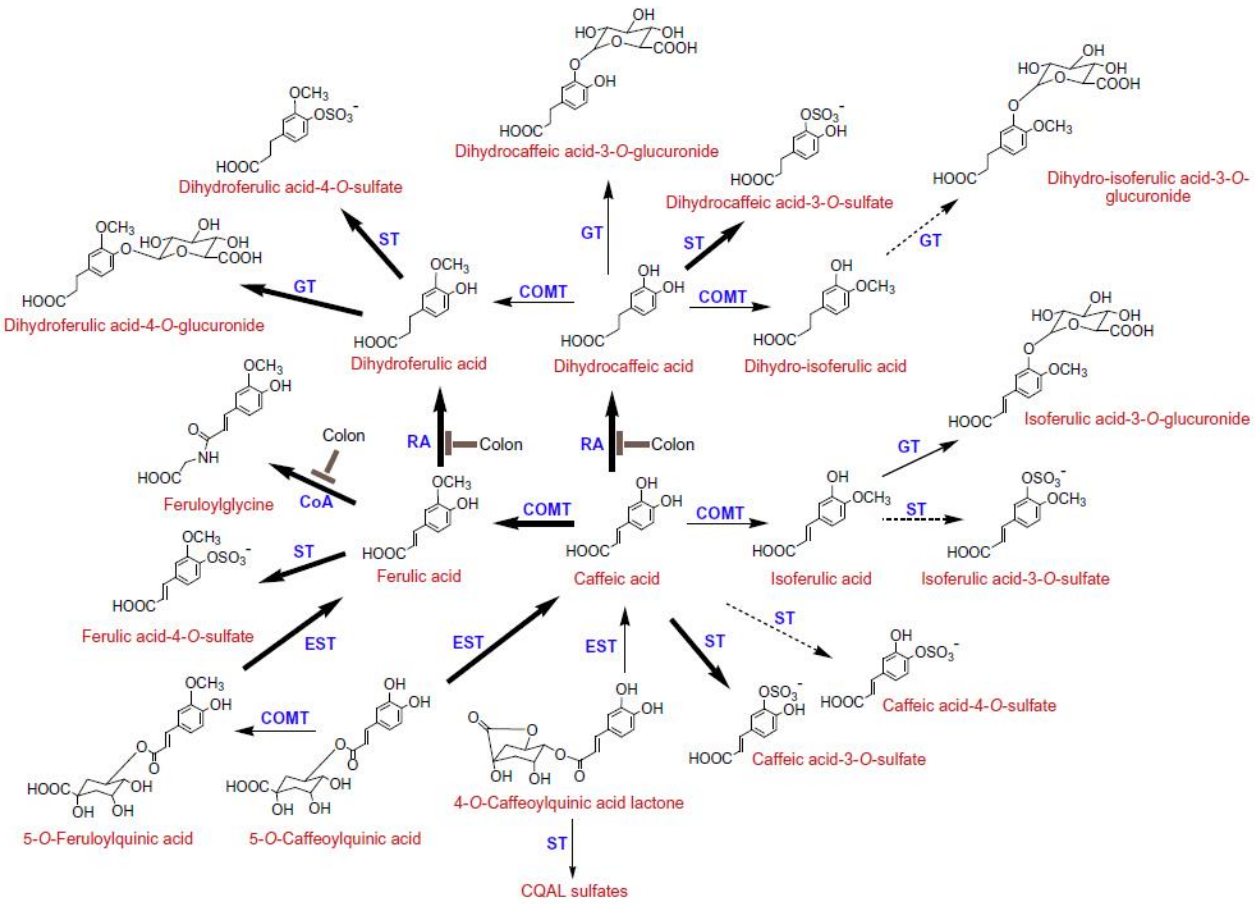
Several previously unidentified coffee metabolites were detected by Redeuil et al. (2011) in the plasma of 9 healthy non-smoking regular coffee consumers (4 men and 5 women) after the administration of a single 400 mL dose of instant coffee. A total of 22 phenolic acid derivatives and 12 CGA derivatives were detected, including 19 newly identified substances such as feruloylquinic acid lactone (FQA), sulfated and glucuronidated forms of FQA lactone, and sulfated forms of coumaric acid.

(d) Elimination

(i) Caffeine

Martínez-López et al. (2014) detected 11 caffeine metabolites in urine after a single dose of green/roasted coffee, with 1-methyluric acid as the major compound representing 67.7%

Fig. 4.2 Metabolism of chlorogenic acids after the ingestion of coffee in humans



5-O-CQA and 5-O-FQA are illustrated structures, but their respective 3- and 4-isomers would be metabolized in a similar manner, and likewise for 4- and 3-O-CQAL. COMT, catechol-O-methyltransferase; ET, esterase; RA, reductase; GT, UDP-glucuronyltransferase; ST, sulfuryl-O-transferase; Co-A, co-enzyme A. Bold arrows indicate major routes, dotted arrows minor pathways. Steps blocked in subjects with an ileostomy and hence occurring in the colon are indicated.
From Stalmach et al. (2010), with permission from Elsevier

of the total urinary metabolites. Unmetabolized caffeine represented about 2.7% of the total amount of urinary metabolites, with a urinary T_{max} of 6.00 ± 2.71 hours. [Data on regular coffee consumption were not reported.]

In the study of Denaro et al. (1990) reported in Section 4.1.1 (c) (i) above, elimination of caffeine was dose-dependent; a higher caffeine dose was associated with a progressive decrease in caffeine clearance (0.118 ± 0.049 L/h/kg, 0.069 ± 0.018 L/h/kg, and 0.054 0.019 L/h/kg for placebo, low caffeine dose, and high caffeine dose, respectively) and a consequent increase in the half-life of caffeine (4.0 ± 1.4 hours, 6.1 ± 1.6 hours, and 8.7 ± 2.3 hours for placebo, low caffeine dose, and high caffeine dose, respectively).

Förster et al. (2005) showed increased urinary levels of free pentosidine (from 3.9 ± 1.2 µg/day to 10.2 ± 2.9 µg/day) in 18 healthy volunteers

(7 men, 11 women) given coffee. On the contrary, the elimination of free pyrraline was not affected by coffee consumption. [No data were available on smoking and coffee consumption habits.]

Other studies in which caffeine was administered as a capsule or gum demonstrated urinary elimination of caffeine and its metabolites (Kaplan et al., 1997; Kamimori et al., 2002; Gelal et al., 2003; Skinner et al., 2014).

(ii) Phenolic acids

In the study of Farah et al. (2008) described in Section 4.1.1 (a) (ii) above, the only intact hydroxycinnamic acids identified in urine were 5-CQA and 4-CQA. DHCA, sinapic, gallic, and *p*-hydroxybenzoic acids were the major (85%) phenolic compounds.

In 5 non-smokers (men) who consumed 4 g of instant coffee powder dissolved in water, significant urinary elimination of FA, iFA, DHFA, and vanillic acid was observed (Rechner et al., 2001).

In the study of Monteiro et al. (2007) described in Section 4.1.1 (a) (ii) above, the only intact CGA identified in urine was 5-CQA. Gallic and dihydrocaffeic acid represented the most abundant phenolic acids in urine, comprising about 56% of the total urinary concentration of all detected compounds. [No data on regular coffee consumption were provided.]

After ingestion of 200 mL of instant coffee by 11 non-smokers (8 men, 3 women), the major urinary CGA-related compound was DHCA-3-*O*-sulfate (Stalmach et al., 2009). In the study described above by the same group (Stalmach et al., 2010) in 5 ileostomy volunteers (3 men, 2 women), sulfated FA, CA, and DHCA and glucuronidated iFA were the main compounds in the 24-h ileostomy effluent after a single dose of instant coffee.

In 5 non-smoking volunteers (2 men, 3 women) given instant coffee in water or milk, the main coffee compounds identified in urine were hippuric, 3,4-dihydroxyphenylacetic, dihydrocaffeic, vanillic, and gallic acids (Duarte & Farah, 2011).

(iii) Other compounds

In 13 healthy non-smokers (7 men, 6 women) given a single 350 mL oral dose of coffee, the plasma half-life ($t_{1/2}$) of trigonelline and *N*-methylpyridinium was 4.65 hours versus 5.5 hours and 2.35 hours versus 2.15 hours in men compared with women, respectively (Lang et al., 2010). Differences between the sexes were also observed in terms of the extent of elimination, the 8-hour urinary excretion being slightly less in women than in men.

Habitual coffee consumption did not alter the concentration of two trigonelline metabolites, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-5-carboxamide, in urine samples of healthy volunteers 4 hours after consumption of a cup of coffee (Wong et al., 2002).

In 9 ileostomists (4 men, 5 women) consuming French-press coffee, only free kahweol and cafestol were found in 14-hour ileostomy effluent (De Roos et al., 1998). Both diterpenes were present in 24-hour urine in either glucuronidated or sulfated form.

4.1.2 Experimental systems

(a) Absorption

(i) In vivo

In Wistar rats given coffee or coffee and milk for 3 weeks, the absorption of CQA was found to be weak and not disrupted by the addition of milk, regardless of the fat content (Dupas et al., 2006). [Only skimmed and semi-skimmed milk was used in the study.]

Several studies evaluated absorption in rats treated with phenolic acids. Almost all ingested CGA (98.6%) remained intact in the small intestine 6 hours after administration in Wistar rats, suggesting poor absorption from the gastrointestinal tract (Azuma et al., 2000). In rats given

CGA, intact CGA was detected in urine samples, indicating that it was absorbed in its native form (Gonthier et al., 2003). In rats given CA, the major compounds in both urine and plasma were CGA metabolites of microbial origin (*m*-coumaric acid and derivatives of phenylpropionic, benzoic, and hippuric acids), accounting for 57.4% (mol/mol) of the CGA intake. In Sprague-Dawley rats, CA was rapidly absorbed with a peak plasma concentration (C_{max}) of 7870 ± 2480 ng/mL achieved 0.33 ± 0.13 hours after the oral administration of a 20 mg/kg dose (Wang et al., 2015).

In C57BL/6J mice treated with a single dose of cafestol (1.5 mg dose of [^{3}H]-labelled compound), cafestol was efficiently absorbed into the portal vein as the parent compound, a glucuronide, and an unidentified metabolite (Cruchten et al., 2010).

(ii) In vitro and ex vivo

In an ex vivo experiment with pig jejunal mucosa, hydroxycinnamic acids (at concentrations achievable in the gut lumen, 0.02–3.5 mM) were absorbed by passive diffusion in the jejunum with active efflux transport, mediated by MDR1 and MDR2 (Erk et al., 2014a). Using an in vitro Dunkin-Hartley guinea-pig stomach cell model, FQA and diCQA (dicaffeoylquinic acid) permeated across the gastric barrier as intact compounds with a relative permeability coefficient (P_{app}) of approximately 0.2 cm/s and 2–10 cm/s, respectively (Farrell et al., 2011).

The net absorption of CGA and CA accounted for 8% and 19.5% of their respective perfused flux using an in situ intestinal perfusion model derived from rat (ileum/jejunum) (Lafay et al., 2006). In a model of digestion model in vitro, the most abundant compound detected after digestion of coffee was caffeine (94%), followed by 5-CQA, 4-CQA, and 3-CQA (87.9–92.0%) (Cha et al., 2012).

(b) Distribution

In C57BL/6J mice given a single dose of cafestol (1.5 mg dose of ^{3}H-labelled compound), almost all radioactivity was found in small intestines and liver; trace amounts were detected in kidneys and none in other organs (van Cruchten et al., 2010).

(c) Metabolism

(i) In vivo

In Wistar rats given hydroxycinnamic acids, CA, or quinic acid (250 µmol/day) in the diet for 8 days, the major compounds in both urine and plasma were CGA metabolites of microbial origin (*m*-coumaric acid and derivatives of phenylpropionic, benzoic, and hippuric acids), accounting for 57.4% (mol/mol) of the CGA intake (Gonthier et al., 2003).

In mice given cafestol via the portal vein, epoxy-glutathione, glutathione, and glucuronide conjugates were identified (van Cruchten et al., 2010). With ^{3}H-labelled cafestol intravenously injected to mice (van Cruchten et al., 2010), the most abundant cafestol metabolites in bile (41%) was the glucuronide conjugate. The same metabolite was also detected in portal blood 18 minutes after administration.

(ii) In vitro

In a study of the metabolism of caffeine (100 mM) in vitro using rat P450s and liver microsomes, CYP1A2 was the most important enzyme overall (Kot & Daniel, 2008a). The main oxidation pathway (70%) was 8-hydroxylation, with CYP1A2 and CYP3A2 catalysing 72% and 15% of the reaction, respectively.

Hydrolysis of CGA was shown to take place in the gut mucosa, using an in situ intestinal perfusion model derived from rat (ileum/jejunum) (Lafay et al., 2006).

CA was shown to be methylated by catechol-*O*-methyltransferase in gastric cells, with iFA as the major metabolite, using a Dunkin-Hartley guinea-pig stomach cell model (Farrell et al., 2011).

(d) Elimination

When given as a single dose to Sprague-Dawley rats, CA was rapidly eliminated with $t_{1/2}$ values of about 1 hour after intravenous (1 mg/kg) or oral (20 mg/kg) administration (Wang et al., 2015).

In C57BL/6J mice, 20% of the administered radiolabelled cafestol dose was detected in bile 5 hours after intravenous administration (van Cruchten et al., 2010). Within 48 hours after oral administration, all radiolabel was eliminated.

4.1.3 Modulation of metabolic enzymes

(a) Humans

(i) In vivo

Several studies investigated the effect of coffee consumption on cytochrome P4501A2 (CYP1A2) activity. An increase of almost 2-fold (6.26 vs 3.94, $P = 0.01$) in CYP1A2 activity was seen in regular coffee consumers (1–10 cups/day) compared with non-consumers (< 1 cup/day) in a case–control study involving 43 adenocarcinoma patients and 47 controls matched by sex, age, and ethnicity (Le Marchand et al., 1997). In a study of 100 Serbian and 149 Swedish healthy volunteers, daily consumption of at least 3 cups of coffee was associated with significantly increased caffeine metabolism and CYP1A2 enzyme activity (Djordjevic et al., 2008). Additional genotyping of subjects for *CYP1A2* revealed that a significant association between heavy coffee consumption and high CYP1A2 enzyme activity exists only in carriers of –163 A/A genotype, suggesting that the –163A allele (rs762551) is a recessive factor necessary for the *CYP1A2* induction (Djordjevic et al., 2010). The effect of the single nucleotide polymorphism (SNP) –163C > A on *CYP1A2* inducibility persisted after adjusting for smoking and oral contraceptive use in women ($P \leq 0.022$). In a similar study with 194 Swedish and 150 Korean healthy volunteers, Ghotbi et al. (2007) reported a significantly lower rate of caffeine metabolism in Koreans as compared with Swedes ($P < 0.0001$). Increased caffeine metabolism was detected in cigarette smokers and carriers of –163C > A *CYP1A2* polymorphism ($P \leq 0.0007$), while sex-specific effects were not observed.

The effect of coffee on phase II metabolizing enzymes was also reported. In 10 healthy volunteers who consumed 1 L/day of filtered or unfiltered coffee over a period of 5 days, a significant increase in glutathione *S*-transferase (GST) enzymatic activity and immunoassays for GSTA and GSTP isozymes revealed that the induction can be assigned exclusively to the latter (Steinkellner et al., 2005). The same inductive effect was observed with both filtered and unfiltered coffee preparations [suggesting that coffee diterpenes kahweol and cafestol, known to be removed from coffee by paper filtration, are not responsible for the GST induction]. In contrast, colorectal GST activity was not affected by coffee consumption in 64 healthy regular coffee consumers drinking 1 L/day of unfiltered coffee for two intervention periods of 2 weeks (Grubben et al., 2000).

(ii) In vitro

In an assay in vitro using cultured lymphocytes from 239 healthy Japanese volunteers, regular coffee consumption increased the expression of aryl hydrocarbon hydroxylase (AHH) (Kiyohara & Hirohata, 1997).

In human colon carcinoma Caco-2 cells, coffee inhibited sulfotransferase (SULT) activity in a dose-dependent manner, an inhibitory effect that could not be attributed to caffeine (Okamura et al., 2005). Neither coffee nor caffeine affected glucuronidation, that is, UDP-glucuronosyl transferase (UGT) activity. Exposure of Caco-2 cells to 5% coffee resulted in an 81.4% decrease in SULT activity (Saruwatari et al., 2008). Likewise, Isshiki et al. (2013) also reported a 60% and 25% reduction of the expression of *SULT1E1* gene and SULT activity, respectively, in Caco-2 cells treated with 2.5% coffee for 24 hours.

Filtered coffee, decaffeinated coffee, and instant coffee induced UGT1A expression in HepG2 and Caco-2 cells (Kalthoff et al., 2010), indicating that the observed upregulation is independent of caffeine, kahweol, or cafestol content.

Kahweol and cafestol slightly increased overall GST activity and significantly increased the level of GST-mu protein in transformed liver epithelial cell lines (THLE) (Cavin et al., 2001). Similarly, kahweol and cafestol decreased sulfotransferase SULT1A1 by 38%, while GST and UGT activity increased by 1.4- and 1.2-fold, respectively, in human HepG2 cells (Majer et al., 2005).

In human lymphoblastoid cell lines (LCLs), caffeine caused a significant downregulation in *CYP1A1* levels (by 1.29-fold), but had no effect on *CYP1A2* (Amin et al., 2012). Likewise, caffeine did not alter the expression of the *CYP1A2* in primary human hepatocytes (Vaynshteyn & Jeong, 2012).

(b) Experimental systems

(i) In vivo

In wildtype mice, coffee (3% and 6%) increased hepatic levels of GSTA1 (5-fold and 6-fold, respectively), GSTA4 (3-fold and 4-fold, respectively), and CYP1A2 (3-fold in the 6% coffee group), while GSTA3 and UGT1A6 were unaffected (Higgins et al., 2008). On the contrary, in Nrf2 null mice, both the normal constitutive expression of enzymes and the alteration in their level and activity in the liver was diminished; only the UGT1A6 level was increased by 4-fold in *nrf2*$^{-/-}$ mice fed 6% coffee. In the small intestine of the wildtype mice, induction followed the same Nrf2-dependent pattern.

In Fischer rats fed a coffee-containing diet (0%, 1%, or 5% w/w) for 2 weeks, there was a strong, concentration-dependent induction of CYP1A2 (by up to 16-fold) (Turesky et al., 2003). In addition, coffee (5%) (but not caffeine) increased rGSTA1 and rGSTA3 (by 1.4- and 2.6-fold, respectively), and UGT (2-fold). Similarly, Abraham et al. (1998) showed that coffee caused a modest increase in GST activity in Swiss albino mice.

Coffee significantly increased enzyme expression in different organs of humanized UGT transgenic mice, ranging from 10-fold for liver UGT1A1 to 11-fold and 14-fold for stomach UGT1A1 and UGT1A6, respectively (Kalthoff et al., 2010). Several studies (Huber et al., 2003, 2004, 2008) have demonstrated that coffee given to rats for 10–20 days induced hepatic GST and UGT activities (up to 30% and approximately 2-fold, respectively), as well as hepatic CYP1A1, CYP1A2, CYP2B1, and CYP2B2 (ranging from 2-fold for CYP2B2 to 6-fold for CYP1A2).

In male Fischer 344 rats, caffeine (0.04%) significantly increased the CYP1A2 protein level by 3.8-fold (Chen et al., 1996). Similarly, caffeine (20 mg/kg) given to Swiss albino mice for 8 weeks increased the level of CYP1A2 in the brain (Singh et al., 2009). Kahweol/cafestol (47% kahweol, 47% cafestol, 5% isomeric derivatives) increased GST and UGT activity in rat liver and kidney (Huber et al., 2002).

(ii) In vitro

In rat primary hepatocytes, caffeine (50 µM for 72 hours) resulted in an increase of 9-fold in *Cyp1a2* expression (Vaynshteyn & Jeong, 2012). A mixture of kahweol and cafestol (52.5:47.5) for 48 hours inhibited CYP3A2 and activated GST in a dose-dependent manner in primary rat hepatocytes (Cavin et al., 2001).

CA significantly inhibited both human (Uwai et al., 2011) and rat (Uwai et al., 2013) organic anion transporters (OATs) expressed in *Xenopus laevis* oocytes. CGA significantly inhibited only hOAT3, while quinic acid was without effect on the transporters.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

(a) Humans

The results of investigations on the effect of coffee drinking by exposed humans and in human cells in vitro are listed in Table 4.3 and Table 4.4, respectively.

(i) Exposed humans

See Table 4.3.

DNA damage

A protective effect on DNA damage in lymphocytes was found in studies conducted with coffee containing increased amounts of chlorogenic acids (green coffee bean extract) and *N*-methylpyridinium (Bakuradze et al., 2011, 2014, 2015, 2016).

While several other studies found no protective effect on DNA damage in unexposed lymphocytes, it was demonstrated that lymphocytes isolated from coffee consumers exhibited reduced DNA damage after in vitro exposure to DNA-damaging agents (Steinkellner et al., 2005; Bichler et al., 2007). In contrast to the protective effects seen in peripheral lymphocytes, non-smoking, coffee-consuming men had an approximately 20% higher percentage tail DNA under neutral, but not alkaline, conditions compared with men who consumed no caffeine (Schmid et al., 2007).

Oxidative and other DNA damage end-points reported in studies of oxidative stress markers are discussed in Section 4.2.2.

Cytogenetic effects

One study reported a significant increase in lymphocyte chromosomal aberrations with coffee intake, independent of smoking status or folate levels (Chen et al., 1989). In sperm cells, a statistically significant positive association was found between drinking coffee daily and the lack of chromosome X or Y. In addition, coffee drinking 1–6 times per week was associated with an additional chromosome 18 (Jurewicz et al., 2014).

In splenectomized individuals, consumption of caffeinated (but not decaffeinated) coffee was associated with an approximately 2-fold higher frequency of micronuclei (MN) in reticulocytes and erythrocytes (Smith et al., 1990). In an Italian lifestyle study described by Barale et al. (1998), no increase of MN formation was found in coffee drinkers compared with non-drinkers.

Several studies that reported on the relationship between coffee consumption and sister-chromatid exchange (SCE) in lymphocytes focused on a variety of lifestyle factors rather than primarily on the effects of coffee. [The Working Group noted shortcomings regarding the study design.] Reidy et al. (1988) reported a positive linear relationship between SCE) and coffee consumption that was similar for male smokers (n = 30) and non-smokers (n = 30). [The Working Group noted a lack of details on coffee consumption.] Similarly, coffee intake was associated with a significant increase in SCE in a study of women of the Republic of Korea (Shim et al., 1989). However, a follow-up report from the same group found no effect of coffee consumption on SCE in male smokers (Shim et al., 1995). A borderline increase in SCE frequencies with coffee drinking was reported by Barale et al. (1998), and no difference in spontaneous SCE between coffee drinkers and non-drinkers was reported in another study in Italy (Sbrana et al., 1995). Finally, reporting on a cross-sectional study of twins, Hirsch et al. (1992) found that individuals who consumed at least 5 cups/day of coffee had half the number of SCE/cell (after adjusting for smoking) compared with those who drank < 5 cups/day.

Gene mutations

Several studies from one research group (Porta et al., 1999, 2009; Morales et al., 2007) reported an association between coffee consumption and

Table 4.3 Genetic and related effects of drinking coffee in exposed humans

Cell type	End-point	Test system	Description of exposure and controls	Response	Comments	Reference
Lymphocytes	DNA damage	Comet assay	33 male non-smoking subjects consumed 750 mL/day of coffee for 4 wk	(PE) ($P < 0.001$)		Bakuradze et al. (2011)
Lymphocytes	DNA damage	Comet assay	84 non-smoking subjects consumed 750 mL/day of coffee for 4 wk	(PE) ($P < 0.001$)		Bakuradze et al. (2014)
Lymphocytes	DNA damage	Comet assay	84 male non-smoking subjects; 42 consumed 750 mL/day of coffee and 42 controls consumed water only for 4 wk	(PE) ($P = 0.0002$)		Bakuradze et al. (2015)
Lymphocytes	DNA damage	Comet assay	13 male non-smoking subjects; sampling every 2 h before and after coffee drinking; 200 mL every 2 h (total 800 mL)	(PE) $P < 0.001$		Bakuradze et al. (2016)
Lymphocytes	DNA damage	Comet assay	10 healthy subjects (3 men, 7 women) consumed 1 L/day of unfiltered coffee for 5 days	– (PE)	Reduction in DNA damage induced by BPDE ($P = 0.0001$)	Steinkellner et al. (2005)
Lymphocytes	DNA damage	Comet assay	8 healthy men and women; 600 mL/day coffee (400 mL paper- and 200 mL metal-filtered) for 5 days	– SC (PE)	Reduction in DNA damage induced by H_2O_2 or Trp-P-2 ($P < 0.05$)	Bichler et al. (2007)
Sperm	DNA damage	Comet assay	80 healthy male non-smokers: 58 coffee drinkers vs 22 non-drinkers	+ coffee drinkers vs non-drinkers ($P = 0.005$)	+ for neutral, but not alkaline, assay	Schmid et al. (2007)
Lymphocytes	Chromosomal damage	Chromosomal aberration	25 subjects who consumed > 4 cups/day of coffee vs 34 subjects < 4 cups/day of coffee	+ ($P < 0.019$)		Chen et al. (1989)
Sperm	Chromosomal damage	Chromosomal aberration, aneuploidy (FISH)	212 healthy men	+		Jurewicz et al. (2014)
Reticulocytes and erythrocytes	Chromosomal damage	Micronucleus formation	44 splenectomized subjects (26 men, 18 women); 29 drank 1–2 cups/day of coffee, 10 drank decaffeinated coffee (< 1 cup/day), 12 drank tea	+ reticulocytes ($P = 0.05$), erythrocytes ($P = 0.03$)	No effect with decaffeinated coffee	Smith et al. (1990)
Lymphocytes	Chromosomal damage	Micronucleus formation	564 female coffee drinkers vs 165 non-drinkers; 414 male coffee drinkers vs 107 non-drinkers	–		Barale et al. (1998)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	30 smoking and 30 non-smoking men	+ Coffee intake vs abstinence ($P = 0.0006$)	Linear increase with cups of coffee intake ($P < 0.01$)	Reidy et al. (1988)

Table 4.3 (continued)

Cell type	End-point	Test system	Description of exposure and controls	Response	Comments	Reference
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	11 coffee-drinking (1–2 cups/day) women vs 41 women non-drinkers	+ ($P < 0.01$)	Few coffee consumers	Shim et al. (1989)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	14 male smokers who drank coffee (> 2–3 cups/day for 6 mo) vs 14 male non-drinking smokers	–	Few coffee consumers	Shim et al. (1995)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	564 coffee-drinking women vs 165 non-drinkers; 414 coffee drinker men vs 107 non-drinkers	–		Barale et al. (1998)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	86 coffee drinkers versus 22 non-drinkers	–		Sbrana et al. (1995)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	In a study of twins, 29 coffee drinkers who consumed ≥ 5 cups/day vs 195 consuming < 5 cups/day	+ ($P < 0.001$)	Linear increase with cups of coffee ($P < 0.001$)	Hirsch et al. (1992)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	86 coffee drinkers versus 22 non-drinkers	–		Sbrana et al. (1995)
Tumour	K-*RAS* mutation	DNA analysis, PCR	121 patients with pancreatic cancer (70 men and 51 women)	+ ($P = 0.018$)	Increase with cups of coffee consumed, but not duration	Porta et al. (1999)
Tumour	K-*RAS* mutation	DNA analysis, PCR	107 pancreatic cancer patients with (83 cases) or without (24 cases) K-*RAS* mutation	+ ($P = 0.026$)	Increase with cups of coffee ($P = 0.038$)	Morales et al. (2007)
Tumour	K-*RAS* mutation	DNA analysis, PCR	103 pancreatic ductal adenocarcinoma patients	+ ($P < 0.015$)	Increase with cups of coffee consumed	Porta et al. (2009)

+, positive; –, negative; BPDE, (±)-anti-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; FISH, fluorescence in situ hybridization; h, hour; PCR, polymerase chain reaction; PE, protective effect; SC, standard conditions; Trp-P-2, amine 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole-acetate; wk, week(s); vs, versus

Table 4.4 Genetic and related effects of coffee in human cells in vitro

Tissue, cell line	Coffee type and preparation	End-point	Test	Results		Concentration (LEC or HIC)	Comments	Reference
				Without activation	With metabolic activation			
HT29 and HepG2 cells	Green coffee extract	DNA damage	DNA strand break, comet assay	– (PE)	NT	6 µg/mL	Reduction in H_2O_2-induced DNA damage	Glei et al. (2006)
Peripheral lymphocytes	Metal filtered coffee, French press method	DNA damage	DNA strand break, comet assay	+ (PE)	NT	50 µL/mL	Coffee increased DNA damage and reduced H_2O_2-induced DNA damage	Bichler et al. (2007)
HeLa cells	Spent coffee grounds	DNA damage	DNA strand break, comet assay	– (PE)	NT	333 µg/mL	Reduction in H_2O_2-induced DNA damage	Bravo et al. (2013)
p53R cells (colorectal cell line expressing *TP53* reporter gene)	Brewed coffees (regular and decaffeinated)	DNA damage	p53 activation assay	+	NT	1:20		Hossain et al. (2013)
Transformed liver epithelial cell lines expressing CYP 1A2, 3A4, and 2B6	Coffee diterpenes: cafestol and kahweol	DNA damage	DNA adduct	(PE)	NT	1 µg/mL	Reduction in AFB1-DNA adducts formation	Cavin et al. (2001)
Primary hepatocytes	Coffee (caffeinated and decaffeinated)	DNA damage	DNA adducts	(PE)	NT	200 µg/mL	Reduction in AFB1-DNA adduct formation	Cavin et al. (2008)
Lymphocytes	Instant coffee (caffeinated and decaffeinated)	Chromosomal damage	Chromosomal aberration	+	+	2.5 mg/mL	Lower in the presence of S9	Aeschbacher et al. (1985)
Peripheral lymphocytes	Brewed coffee	Chromosomal damage	Sister-chromatid exchange	+	NT	0.2 mg/mL		Tucker et al. (1989)
Liver HepG2 cell line	Coffee diterpenes: cafestol and kahweol (C+K)	Chromosomal damage	MN formation	– (PE)	NT	0.3 µg/mL	Inhibited MN induced by PhIP or NDMA	Majer et al. (2005)

+, positive; – negative; AFB1, aflatoxin B_1; C+K, cafestol and kahweol; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; MN, micronucleus; NDMA, *N*-nitrosodimethlyamine; NT, not tested; PE, protective effect; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

K-*RAS* mutations in ductal adenocarcinoma of the pancreas. Mutations in K-*RAS* on codon 12 were found in tumours from 94 of 121 patients (77.7%), and were more common among regular coffee drinkers than non-regular coffee drinkers (82.0% vs 55.6%, $P = 0.018$, $n = 107$, adjusted for smoking and alcohol drinking) (Porta et al., 1999). Similar results were obtained in two follow-up studies that also adjusted for other lifestyle factors and exposures to organochlorine chemicals (Morales et al., 2007; Porta et al., 2009).

Mutagenicity of urine

Aeschbacher & Chappuis (1981) found no evidence of mutagenicity in Salmonella strains TA98 and TA100 of polar and non-polar fractions with urine samples from 6 coffee drinkers and 6 non-drinkers. However, chromosomal damage in Chinese hamster ovary (CHO) cells was induced by fractions prepared from urine of coffee drinkers (Dunn & Curtis, 1985).

(ii) Human cells in vitro

See Table 4.4.

Coffee increased DNA damage by comet assay in one study (Bichler et al. 2007) and reduced the DNA damage induced by H_2O_2 in several studies using different cell types (Bichler et al. 2007; Bravo et al., 2013), as did a green coffee extract (Glei et al., 2006). Coffee increased TP53 activation, via a stably transfected luciferase reporter, in a human colorectal cell line (Hossain et al., 2013). Although reportedly confirmed in comet and histone γH2AX phosphorylation experiments, the latter results were not shown. [The Working Group noted that this study is difficult to interpret.]

Coffee protected against aflatoxin-induced DNA adducts in transformed human liver epithelial cells (Cavin et al., 2001), as did two diterpenes (cafestol and kahweol) in human primary hepatocytes (Cavin et al., 2008). Similar protection by the diterpenes was seen against MN induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and *N*-nitrosodimethylamine (NDMA) (Majer et al., 2005).

In human lymphocytes, coffee induced chromosomal aberrations in the absence of a metabolic activation system (S9), but S9 reduced the clastogenic properties (Aeschbacher et al., 1985). Tucker et al. (1989) reported a significant increase in SCE with brewed coffee, an effect reduced by bisulfite addition. [The Working Group noted that this suggested that bicarbonyls (which are complexed by bisulfite) may have accounted for this effect.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.5.

Several in vivo studies tested coffee in combination with genotoxic agents. Turesky et al. (2003) found evidence for coffee-associated reduction of PhIP-induced DNA adducts in the liver of rats. Ferk et al. (2014) found a significant reduction of DNA damage induced by aflatoxin B_1 in the liver of rats with paper- and metal-filtered coffee brews, whereas a decaffeinated coffee brew had a lesser effect.

A significant dose-dependent increase in 8-OHdG levels, as well as an increase in the concentrations of CGA in the urine, was found in Wistar rats given freeze-dried coffee (Sakamoto et al., 2003). Salomone et al. (2014) showed that coffee reduced the hepatic levels of 8-OHdG and other markers of oxidative stress in rats fed a high-fat diet. A study in ICR mouse by Morii et al. (2009) reported no effect of instant coffee consumption (0.1% w/v) on DNA oxidation, on the activity of superoxide dismutase (SOD), or on 8-OHdG repair-associated gene expression (*Ogg1*).

A protective effect of coffee on the induction of MN in mouse bone marrow was reported by Abraham and co-workers. A significant inhibition of MN formation by coffee was observed after co-treatment with dimethylbenz[*a*]anthracene,

Table 4.5 Genetic and related effects of coffee in non-human mammalian cells in vivo

Species, strain, sex	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regiment	Comments	Reference
Rat, Fischer-344, male	Liver, colon, pancreas	DNA damage	DNA adducts	(PE) against PhIP	1% lyophilized coffee	Diet, 14 day, sampling 24 h after PhIP treatment	$P < 0.01$; PhIP detected (0.75 mg/kg bw); DNA adducts in liver, colon, pancreas; liver adducts decreased by 50%; 1% coffee protects against PhIP in liver, 5% in pancreas	Turesky et al. (2003)
Rat, Him-OFA, male	Liver	DNA damage	DNA strand breaks, comet assay	(PE) against AFB1	Metal-filtered coffee: 9.65 g/day; paper-filtered coffee or decaffeinated coffee: 19.3 g/day	Orally, 8 day, sampling 4 h after AFB1 (2 mg/kg bw)		Ferk et al. (2014)
Rat, Wistar, male	Urine	Oxidized DNA damage	8-OHdG	+	0.62% (125 mg/day) freeze-dried coffee	Orally in diet, 130 d		Sakamoto et al. (2003)
Rat, Wistar, male	Liver	Oxidized DNA damage	8-OHdG	(PE), high-fat diet	1.5 mL/animal Paper-filtered decaffeinated coffee	Orally as solution, 12 wk		Salomone et al. (2014)
Mouse, ICR, male	Liver	Oxidized DNA damage	8-OHdG	–	0.1% w/v instant coffee	Orally as solution, up to 8 mo		Morii et al. (2009)
Mouse, Swiss, male/female	Bone marrow	Chromosomal damage	MN formation	– (PE) against MMC, CP, PCZ but not adriamycin	500 mg/kg bw coffee/instant coffee	Gavage, 1×, sampled after 25–28 h		Abraham (1989)
Mouse, Swiss, female	Fetal liver, blood, maternal bone marrow	Chromosomal damage	MN formation	(PE) against CP, NEU and MMC	350 mg/kg bw during gestation (15–16 days)	Gavage; 1×, sampled after 22 or 28 h	Protective effect in embryos and dams	Abraham (1995)
Mouse, Swiss albino, male	Bone marrow	Chromosomal damage	MN formation	– (PE) against urethane	125 mg/kg bw filtered coffee	Gavage, 1×, sampled after 24 or 48 h	Coffee increased GST	Abraham et al. (1998)

Table 4.5 (continued)

Species, strain, sex	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regiment	Comments	Reference
Mouse, Swiss, male	Bone marrow	Chromosomal damage	MN formation	(PE) against DMBA, AFB1, B[*a*]P, UR, CP	250 mg/kg bw instant coffee	Gavage, 2× (2 h, 20 h before i.p. carcinogen treatment), sampled 24 h or 48 h after last dose		Abraham (1991)
Mouse, Swiss, male	Bone marrow	Chromosomal damage	MN formation	– (PE) against DMBA, B[*a*]P, UR, CP, MMC	140 mg/kg bw decaffeinated, caffeinated instant coffee	Gavage, 1×/10 d, sampled 24 h or 48 h after dose	Same result with 2 g/100 mL oral	Abraham & Singh (1999)
Mouse, MS/Ae	Bone marrow	Chromosomal damage	MN formation	– (PE) against MU + $NaNO_2$	150–1000 mg/kg bw instant coffee	Orally, 1×, sampled 24 h after dose	No PE against MNU	Aeschbacher & Jaccaud (1990)
Mouse, Swiss, OF-1, male	Bone marrow	Chromosomal damage	MN formation	–	3000 mg/kg bw instant coffee	Gavage, 1×/5 days, sampled 6 h after dose		Aeschbacher et al. (1984)
Chinese hamster, male	Bone marrow	Chromosomal damage	Sister-chromatid exchange	–	2500 mg/kg bw instant coffee	Gavage, 1×, sampled 25–26 h after dose		Aeschbacher et al. (1984)

+, positive; –, negative; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AFB1, aflatoxin B_1; B[*a*]P, benzo[*a*]pyrene; bw, body weight; CP, cyclophosphamide; DMBA, dimethylbenz[α]anthracene; GST, glutathione *S*-transferase; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneally; LED, lowest effective dose; MMC, mitomycin C; MN, micronucleus; mo, month(s); MNU, *N*-methylnitrosourea; MU, methyl urea; $NaNO_2$, sodium nitrite; NEU, *N*-nitroso-*N*-ethylurea; PCZ, procarbazine; PE, protective effect; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; UR, urethane; wk, week(s)

aflatoxin B_1, benzo[*a*]pyrene, cyclophosphamide, mitomycin, procarbazine, and urethane, but not adriamycin (Abraham, 1989, 1991; Abraham et al., 1998). Oral administration of coffee to pregnant mice before administration of cyclophosphamide, *N*-nitroso-*N*-ethyl urea, or mitomycin C reduced the formation of MN in the fetal liver and blood and in maternal bone marrow (Abraham, 1995). Coffee was also protective against urethane-mediated reduction in the activity of the detoxifying enzyme glutathione *S*-transferase (Abraham et al., 1998). In a comparative study of caffeinated and decaffeinated brews, both displayed similar protective effects against chemically-induced MN (Abraham & Singh, 1999). Notably, several of these studies included coffee-only control groups; no evidence for induction of MN by coffee itself was detected.

Coffee administered in a dose equivalent to the consumption of 5 cups of coffee had a protective effect on nitrosourea-induced MN in bone marrow cells in mice (Aeschbacher & Jaccaud, 1990). In addition, no prevention of MN induced by exogenous *N*-methylnitrosourea was found, suggesting that the protective effect of coffee may be through prevention of endogenous nitrosation (Aeschbacher & Jaccaud, 1990). The same group reported that instant coffee had no effect on MN and SCE in mice or in Chinese hamsters (Aeschbacher et al., 1984).

(ii) Non-human mammalian cells in vitro

See Table 4.6.

Overall, experiments with coffee or its constituents in mammalian cells fall into two categories: the first group concerns the effect of coffee per se on damage of the genetic material, and the second group deals with the protective effects towards chemical carcinogen-associated damage.

In a CHO cell line (AUXB1), coffee induced SCE; this was reduced by bisulfite addition but not by catalase and peroxidase (Tucker et al., 1989). In CHO-K1 cells, SCE frequencies were increased with caffeinated or decaffeinated coffee (brewed and instant), although decaffeinated coffee was less potent and only positive in the absence of S9 (Santa-Maria et al., 2001). No increase was seen with green coffee prepared from unroasted beans (with and without S9).

In Chinese hamster lung (CHL) cells, a mutagenic effect of instant coffee was suppressed by sodium bisulfite, a scavenger of carbonyls (Nakasato et al., 1984).

Protection by coffee against PhIP as measured by the single-cell gel electrophoresis assay was seen in the Chinese hamster fibroblast V79 cell line expressing CYP1A2 and sulfotransferase SULT1C1 (Edenharder et al., 2002).

Caffeine-containing instant coffee protected against DNA damage and MN induced by different genotoxic chemicals, such as *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), mitomycin C, methyl methanesulfonate, and γ-radiation in mouse lymphoma cells (Abraham et al. (2004). No difference in the protective properties of caffeinated and decaffeinated brews against MNNG was detected (Abraham & Stopper, 2004). Coffee itself was devoid of genotoxic activity, and no reduction of MN was detected when cells were exposed to the mutagen before coffee.

(iii) Non-mammalian experimental systems

See Table 4.7.

No clear effects were found in germ cell assays in *Drosophila melanogaster*, but moderate activities were detected regarding the induction of mosaic spots in the wings in repair-proficient and also repair-deficient cells (Graf & Würgler, 1986). Coffee had a protective effect when administered in combination with a variety of genotoxins such as urethane, cyclophosphamide, mitomycin C, and diethylnitrosamine (Abraham, 1994; Abraham & Graf, 1996).

The majority of studies with *Salmonella typhimurium* and other bacterial tester strains were published before 1990 and were reviewed by the

Table 4.6 Genetic and related effects of coffee in non-human mammalian cells in vitro

Species	Cell model	End-point	Test system	Results		Concentration (LEC or HIC)	Reference
				Without metabolic activation	With metabolic activation		
Chinese hamster	CHO (AUXBI)	Chromosomal damage	Sister-chromatid exchange	+	NT	0.1–1.2 mg/mL; brewed coffee	Tucker et al. (1989)
Chinese hamster	CHO-K1	Chromosomal damage	Sister-chromatid exchange	+	+	10 mg/mL; blend or instant coffee	Santa-Maria et al. (2001)
Chinese hamster	CHO-K1	Chromosomal damage	Sister-chromatid exchange	–	–	10 mg/mL; roasted, green coffee	Santa-Maria et al. (2001)
Chinese hamster	CHO-K1	Chromosomal damage	Sister-chromatid exchange	+	–	10 mg/mL; blend or instant decaffeinated coffee	Santa-Maria et al. (2001)
Chinese hamster	Lung fibroblasts V79-rCYP1A2-rSULT1C1	DNA damage	Comet assay	– (PE) against PhIP	NT	2% v/v; coffee (not specified)	Edenharder et al. (2002)
Mouse	Lymphoma L5178Y	DNA damage	Comet assay	– (PE) against MNNG and MMS	NT	125 µg/mL; caffeinated instant coffee	Abraham & Stopper (2004); Abraham et al. (2004)
Mouse	Lymphoma L5178Y	Gene mutation	*Tk*$^{\pm}$ locus	– (PE) against MNNG	NT	125 µg/mL; caffeinated instant coffee	Abraham et al. (2004)
Mouse	Lymphoma L5178Y	Chromosomal aberration	MN formation	–	NT	250 µg/mL; caffeinated instant coffee	Abraham & Stopper (2004)
Mouse	Lymphoma L5178Y	Chromosomal aberration	MN formation	–	NT	125 µg/mL; caffeinated instant coffee or filtered and unfiltered instant coffee 60 µg/mL boiled coffee	Abraham et al. (2004); Abraham & Stopper (2004)
Mouse	Lymphoma L5178Y	Chromosomal aberration	MN formation	– (PE) against MNNG	NT	60–250 µg/mL; caffeinated, decaffeinated, filtered, unfiltered instant coffee, and boiled coffee	Abraham & Stopper (2004)
Mouse	Lymphoma L5178Y	Chromosomal aberration	MN formation	– (PE) against MNNG; MMS; MMC; γ- radiation.	NT	125 µg/mL; caffeinated instant coffee	Abraham et al. (2004)

+, positive; –, negative; HIC, highest ineffective concentration; LEC, lowest effective concentration; MMC, mitomycin C; MMS, methyl methanesulfonate; MN, micronucleus; MNNG, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; NT, not tested; PE, protective effect; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

Working Group in a previous *IARC Monograph* on coffee (IARC, 1991).

The first description of an investigation of the effects of coffee on *Salmonella typhimurium* strains was published by Nagao et al. (1979). Regular, instant, and decaffeinated instant coffee were mutagenic in strain TA100 but not TA98, and only without metabolic activation. Similar results were reported by Aeschbacher & Würzner (1980), with positive results in TA100 but not in other tester strains (TA98, TA1535, TA1537, TA1538). Subsequent host-mediated assays in which bacterial indicator cells were injected into host animals (mice received instant coffee at 6 /kg bw) and subsequently recovered from the liver yielded consistently negative results.

Many subsequent studies attempted to discern the components accounting for mutagenicity in the Ames assay, determining that the addition of glutathione reduced mutagenicity (Kosugi et al., 1983; Friederich et al., 1985). The first evidence that methylglyoxal accounts for the bacterial mutagenicity of coffee beverages was provided by the studies of Kasai et al. (1982) and Fujita et al. (1985a). Later studies confirmed this assumption; that is, it was shown that the addition of glyoxalase reduced the mutagenicity of methylglyoxal and also the mutagenic activity of coffee brews by up to 80% (Friederich et al., 1985). [The Working Group noted that the compounds accounting for the induction of bacterial mutagenesis may be inactivated under in vivo conditions in humans.]

Apart from methylglyoxal, other dicarbonyls (in particular glyoxal and ethylglyoxal) are also present in coffee brews (Nagao et al., 1986). These compounds are less mutagenic in TA100 than methylglyoxal itself and are present in lower quantities; nevertheless, they may contribute to a certain extent to the overall effects of coffee.

A systematic comparison of the effects of a broad variety of components indicated that the effects (in TA100 and TA102) were mainly caused by dicarbonyls and not by other constituents such as furans, heterocycles, and sulfur-containing compounds (Aeschbacher et al., 1989). Another coffee constituent that may be involved in the bacterial mutagenesis is trigonelline (Wu et al., 1997). In contrast to coffee, however, trigonelline compounds were highly active in TA98 and its derivative strains (YG1024) in the presence of S9 mix. One investigation (Johansson et al., 1995) of instant coffee found some evidence of mutagenicity in TA98 with S9, which may be due to trigonelline reaction products. The mutagenic activity of instant coffee was seen in TA98, YG1024, and YG1029 with S9 (the latter strains overexpress *N*-acetyltransferase, which catalyses the activation of heterocyclic aromatic amines) (Johansson et al., 1995).

The mutagenic activity of instant coffee in strain TA100 increased significantly after nitrosation, and involved compounds such as chlorogenic acid, cathechol, and caffeic acid (Duarte et al., 2000). However, whereas coffee and coffee components inhibit the nitrosation of methylurea under in vitro conditions, the reduced formation of *N*-nitroso compounds was observed in vivo (Stich et al., 1982, 1984).

Evidence for the genotoxic properties of coffee was also found in several other bacterial test systems, for example in assays for phage induction with *Escherichia coli* (Suwa et al., 1982; Kosugi et al., 1983) and in experiments with *Escherichia coli* WP2 *uvrA* and *Escherichia coli* WP2 *uvrA*/pKM101 (Kosugi et al., 1983). Based on a comparison of coffee components using the L-arabinose resistance assay, methylglyoxal, glyoxal, caffeic acid, and caffeine contributed little, if at all, to the bacterial mutagenicity of coffee, whereas hydrogen peroxide content could explain 40–60% of the genotoxic activity of the brews (Dorado et al., 1987). These findings are in contrast to results obtained with Ames tester strains, which are more responsive to methylglyoxal (Ariza et al., 1988). The assumption that the peroxide accounts for the effects of coffee in the L-arabinose resistance test was further

Table 4.7 Genetic and related effects of coffee in non-mammalian experimental systems

Experimental system Species, strain	End-point	Test[a]	Results		Type of coffee	Concentration (LEC or HIC)	Comments	Reference
			Without activation	With metabolic activation				
Drosophila melanogaster	Germ cells mutation	Sex-linked recessive lethals	–	NA	Instant coffee	4%		Graf & Würgler (1986)
Drosophila melanogaster	Germ cells mutation	Dominant lethal sex chromosome loss	–	NA	Home-brew coffee	3%		Graf & Würgler (1986)
Drosophila melanogaster	Somatic mutation	SMART	+ + –	NA	Instant coffee Home-brew coffee Decaffeinated	4% 3% 20%	Moderate effect	Graf & Würgler (1986)
Drosophila melanogaster	Somatic mutation	SMART	– (PE) against DEN, MMC, UR, CP	NA	Instant coffee	2%		Abraham (1994); Abraham & Graf (1996)
Salmonella typhimurium TA100	Gene mutation	Reverse mutation	+ +	– –	Coffee from roasted beans Instant caffeinated and decaffeinated coffee	4.7–21 mg/plate 1 mg/plate		Nagao et al. (1979)
Salmonella typhimurium TA98	Gene mutation	Reverse mutation	–	–	Coffee from roasted beans, instant caffeinated and decaffeinated coffee	5–35 mg/plate		Nagao et al. (1979)
Salmonella typhimurium TA98, TA1535, TA1537, TA1538	Gene mutation	Reverse mutation	–	–	Brewed, instant coffee	35 mg/plate		Aeschbacher & Würzner (1980)
Salmonella typhimurium TA100	Gene mutation	Reverse mutation	+	–	Brewed, instant coffee	5–15 mg/plate		Aeschbacher & Würzner (1980)

Table 4.7 (continued)

Experimental system Species, strain	End-point	Test[a]	Results		Type of coffee	Concentration (LEC or HIC)	Comments	Reference
			Without activation	With metabolic activation				
Salmonella typhimurium TA100	Gene mutation	Reverse mutation	+ + +	NT NT NT	Brewed coffee Instant coffee Instant decaffeinated	10 mg/plate 7.5 mg/plate 5 mg/plate	Suppression of the mutagenic properties of all brews by scavenging of 1,2-dicarbonyl diacetyl and glyoxal L-ascorbic acid increased the effect of coffee	Suwa et al. (1982)
Escherichia coli K12	Prophage induction	Plaque formation	+	NT	Instant coffee, decaffeinated instant	20 mg/plate		Suwa et al. (1982)
Salmonella typhimurium TA100	Gene mutation	Reverse mutation	+	NT	Coffee from roasted beans	15 mg/plate	No effects of green coffee	Kosugi et al. (1983)
Escherichia coli K12	Prophage induction	Plaque formation	+ –	NT	Coffee from roasted beans Coffee from green beans	20–30 mg/plate 60 mg/plate	No effects of green coffee	Kosugi et al. (1983)
Escherichia coli WP2*uvr*A/pKM101	Gene mutation	Reverse mutation	+ –	NT	Coffee from roasted beans Coffee from green beans	40 mg/plate 75 mg/plate	No effects of green coffee	Kosugi et al. (1983)
Salmonella typhimurium TA100, TA102	Gene mutation	Reverse mutation	+ (TA100) + (TA102)	– (TA100) – (TA102)	Instant coffee Instant coffee	7 mg/plate 10 mg/plate	Reduction of mutagenic effect by glutathione	Friederich et al. (1985)
Salmonella typhimurium TA100	Gene mutation	Reverse mutation	+ + (TA100) + (TA102) – (TA104) – (YG1024)	NT – (TA100) + (TA102) – (TA104) – (YG1024)	Instant coffee Instant coffee Instant coffee Instant coffee Instant coffee	10 mg/plate 10 mg/plate NR NR NR	Methylglyoxal in coffee caused only a moderate effect. Reduction of the coffee effects by catalase	Fujita et al. (1985a)

Table 4.7 (continued)

Experimental system Species, strain	End-point	Test[a]	Results		Type of coffee	Concentration (LEC or HIC)	Comments	Reference
			Without activation	With metabolic activation				
Salmonella typhimurium TA100, TA102	Gene mutation	Reverse mutation	+ (TA100) + (TA102)	NT NT	Instant coffee Instant coffee	10 mg/plate 20 mg/plate		Aeschbacher et al. (1989)
Salmonella typhimurium TA98, TA100	Gene mutation	Reverse mutation	+ (TA98) – (TA100)	NT NT	Fractions of instant coffee	Fractions from 250 mg/mL		Kato et al. (1994)
Salmonella typhimurium TA98, YG1024, YG1029	Gene mutation	Reverse mutation	NT NT NT NT NT NT	+ (TA98) + (YG1024) + (YG1029) + (TA98) + (YG1024) + (YG1029)	Extracts of grain-based coffee Extracts of instant coffee	0.75 gEq/plate 0.2 gEq/plate NR 0.75 gEq/plate 0.2 gEq/plate NR	Higher sensitivity in YG1024 with S9 mix	Johansson et al. (1995)
Escherichia coli K12 (catalase proficient UC1217 and catalase deficient UC1218)	Gene mutation	*Lac* I Test	+	NT	Instant coffee	4 mg/plate (UC1218); 15 mg/plate (UC1217)	Similar spectrum of mutations (coffee vs H_2O_2)	Ruiz-Laguna & Pueyo (1999)
Salmonella typhimurium TA102, TA104	Gene mutation	Reverse mutation	+ (TA102) + (TA104)	NT NT	Paper-filtered coffees	5 mg/plate 5 mg/plate		Dorado et al. (1987)
Salmonella typhimurium (L-Arabinose resistant) BA1, BA3, BA9, BA13	Gene mutation	Forward mutation	+ (BA13) + (BA13) + (B13)	NT NT NT	Coffee beans Ground coffee Instant coffee	1 mg/plate 1 mg/plate 0.5 mg/plate	Instant coffee was more active than ground coffee	Dorado et al. (1987)
Salmonella typhimurium (L-Arabinose resistant) BA13	Gene mutation	Forward mutation	+ + +	NT NT NT	Ground coffee static Ground coffee agitated Instant coffee agitated	0.5 mg/plate 0.5 mg/plate 0.5 mg/plate	Caffeine was not mutagenic	Ariza et al. (1988)
Salmonella typhimurium (L-Arabinose resistant) BA13	Gene mutation	Forward mutation	+	–	Instant coffee	2.5 mg/plate	Only one dose tested; reduction of mutagenicity by addition of catalase	Ariza & Pueyo (1991)

Table 4.7 (continued)

Experimental system Species, strain	End-point	Test[a]	Results		Type of coffee	Concentration (LEC or HIC)	Comments	Reference
			Without activation	With metabolic activation				
Plasmid pBR322 DNA	DNA damage	DNA strand breaks	–	NT	Instant coffee	0.8 mg/assay		Kato et al. (1994)
Plasmid pBR322 DNA	DNA damage	DNA strand breaks	+	NT	Fractions of instant coffee	Fractions from 100 mg/mL		Kato et al. (1994)
Plasmid pBR322 DNA	DNA damage	DNA strand breaks	+	NT	Fractions of instant coffee	Fractions from 100 mg/mL		Hiramoto et al. (1998)

[a] Unless otherwise indicated, the experiments were plate incorporation assays

+, positive results; –, negative results; cat. def., catalase deficient; cat. pro., catalase proficient; CP, cyclophosphamide; DEN, diethylnitrosamine; gEq, gram equivalent; HIC, highest ineffective concentration; LEC, lowest effective concentration; MMC, mitomycin C; NA, none applicable; NR, not reported; NT, not tested; PE, protective effect; SMART, somatic mutation and recombinant test; UR, urethane

confirmed by experiments showing that the addition of catalase attenuates the activity of the beverage (Ariza et al., 1988; Ariza & Pueyo, 1991). Ruiz-Laguna & Pueyo (1999) compared mutation spectra induced by coffee and H_2O_2 in the *LacI* gene in catalase-deficient and -proficient *E. coli* strains. Coffee caused a similar spectrum of mutational events as H_2O_2, which was in turn different from the spontaneous spectrum.

(iv) Acellular systems

Chlorogenic acid, caffeic acid, pyrogallol, and hydroquinone cause a pH-dependent degradation of deoxyribose (Kato et al., 1994; Duarte et al., 1999). In isolated bacteriophage (PM2) DNA treated with Maillard products (isolated from coffee extracts) and a Fe^{2+} catalysed Fenton reaction, DNA single-strand breaks were detected (Wijewickreme & Kitts, 1998). Hydroxyhydroquinone was identified as the active component of coffee inducing DNA damage (Hiramoto et al., 1998).

4.2.2 Oxidative stress and antioxidant status

This section describes the effects of coffee on oxidative stress and on antioxidant status. In contrast to potentially enhancing oxidative stress, coffee also has antioxidant properties that might reduce oxidative stress. The antioxidant properties of coffee and its constituents, for example chlorogenic acids, have been demonstrated using various assays including ferric ion-reducing antioxidant power (FRAP), total peroxyl radical-trapping antioxidant parameter (TRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and oxygen radical antioxidant capacity (ORAC) (reviewed in Liang & Kitts, 2014). In cell- and animal-based studies, coffee is also able to induce mRNA and protein expression of antioxidant enzymes via the Nrf2/ARE (antioxidant response element) pathway, thus enhancing endogenous defence mechanisms.

(a) Exposed humans

See Table 4.8.

(i) Cross-sectional studies

Several cross-sectional studies investigated the effects of coffee consumption on oxidative DNA damage. Coffee drinking (0 to > 4 cups/day) was inversely associated with DNA damage as measured by 8-hydroxydeoxyguanosine (8-OHdG) (van Zeeland et al., 1999; Hori et al., 2014). Hori et al. (2014) adjusted for smoking status. In the latter study, the association was attenuated in women after adjusting for ferritin. [Coffee is known to inhibit iron absorption and therefore might decrease iron-induced oxidative damage.] In another study, coffee and tea consumption significantly decreased DNA damage as measured by 8-oxodeoxyguanosine (8-OxodG), another marker for DNA damage (Lodovici et al., 2005). However, the effects of coffee and tea were not separately studied. Coffee drinking was associated with decreased derivatives of reactive oxygen metabolites (d-ROM), a measure of lipid peroxidation, in men only in a large cross-sectional study of 9877 Japanese subjects (Ishizaka et al., 2013). The highest quartile of coffee consumption (≥ 5 cups/day) had a significantly lower d-ROM than the lowest quartile. d-ROM was increased in male current smokers compared with male never-smokers. Antioxidant status was not affected by coffee in either men or women, but was decreased in male smokers compared with male never-smokers (Ishizaka et al., 2013).

(ii) Randomized controlled trials

Several randomized controlled trials (RCTs) studied the effects of coffee drinking on various markers of DNA damage and lipid peroxidation. Consumption of filtered coffee (800 mL/day) for 5 days significantly decreased DNA damage as measured by the comet assay (Mišík et al., 2010). Another study using 800 mL of instant coffee enriched with CGA did not find significant effects

with this assay (Hoelzl et al., 2010). Mišík et al. (2010) also measured a range of oxidative stress markers, such as nitrotyrosine (3-NT), oxidized low-density lipoprotein (oxLDL), thiobarbituric acid-reactive substances (TBARS), 8-epi-prostaglandin F2α (PGF2α), and reactive oxygen species (ROS), but none of these changed significantly. In the study of Hoelzl et al. (2010), plasma 3-NT and urinary PGF2α decreased significantly. Coffee significantly decreased 8-OHdG in a crossover trial comparing coffee drinking (4 cups/day) and abstinence in 37 patients with chronic hepatitis (Cardin et al., 2013). However, advanced protein oxidation products (AOPP) did not change. Coffee with reduced hydroxyhydroquinone (HHQ), a roasting product of coffee beans, decreased lipid peroxidation (F2-isoprostanes) (Ochiai et al., 2009). In contrast, roasting did not appear to affect PGF2α and oxLDL as there were no differences between light- and medium-roast coffee (each 480 mL) (Corrêa et al., 2012).

Markers of antioxidant status were studied in several randomized controlled trials with coffee. A significant increase in glutathione (GSH) was reported by Ochiai et al. (2009), which is in line with the simultaneous decrease in lipid peroxidation mentioned in the paragraph above. In another study, a range of markers of the antioxidant status did not change, such as total antioxidant capacity (TAC), total glutathione (tGSH), and the activities of the antioxidant enzymes SOD and glutathione peroxisidase (GPx) (Mišík et al., 2010). This is consistent with the lack of effect on oxidative stress markers (see paragraph above), although DNA damage decreased significantly. Light- and medium-roast coffee both increased markers of antioxidant status, including SOD, GPx, and catalase (CAT), total antioxidant status (TAS), and oxygen radical absorbance capacity (ORAC) (Corrêa et al., 2012). In a crossover trial of 64 healthy subjects, coffee (1 L/day) did not change the activity of GST in the mucosa, but increased GSH in mucosa and plasma (Grubben et al., 2000).

(iii) Interventions (≥ 7 days)

No effects on lipid peroxidation and antioxidant enzymes were seen in a study comparing the consumption of 0, 3, or 6 cups of filtered coffee (Mursu et al., 2005). Yukawa et al. (2004) found that coffee drinking (150 mL daily for 7 days) reduced lipid peroxidation in plasma in 11 participants; the lag time of LDL oxidation increased substantially, whereas TBARS decreased.

The effects of light- and dark-roast coffee (500 mL daily for 4 weeks) on antioxidant enzymes and antioxidants in erythrocytes were studied by Kotyczka et al. (2011). Dark-roast coffee decreased SOD and GPx activity, but increased CAT activity and tGSH and tocopherol. Light-roast coffee increased SOD, GPx, and CAT activity, but did not change tGSH and tocopherol. Light- and dark-roast coffee (500 mL) did not significantly increase the expression of transcription factor Nrf2 and the antioxidant enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) regulated by Nrf2 (Boettler et al., 2011). However, a later study (Boettler et al., 2012) reported that the consumption of 750 mL/day of coffee for 4 weeks increased expression of Nrf2 in peripheral blood lymphocytes in male volunteers (n = 18). Similar findings were reported by Volz et al. (2012) in a pilot intervention study. Daily consumption of 750 mL of coffee for 4 weeks by healthy male volunteers (n = 29) increased NRF2 transcription in peripheral blood lymphocytes.

(iv) Acute interventions

In several studies, the concentration of H_2O_2 in urine increased 3–10-fold 1–2 hours after consumption of coffee (Long & Halliwell, 2000; Hiramoto et al., 2002; Ziobro & Bartosz, 2003; Halliwell et al., 2004). [This may suggest that H_2O_2 is absorbed from coffee, enters the circulation, and may reach tissues.] In subjects who drank green tea and instant coffee containing the same concentrations of H_2O_2, Halliwell et al. (2004) found that, in contrast to coffee, none of the subjects showed a rise in urinary H_2O_2

Table 4.8 Effects of drinking coffee on oxidative stress markers in exposed humans

Tissue	Cell type	End-points	Test	Description of exposure[a] and controls	Response[b]/ significance	Comments	Reference
Cross-sectional studies							
Blood	Lymphocytes	DNA damage	8-OxodG	Cross-sectional; 87 men (18–60 yr): 30 smokers, 29 non-smokers, 28 secondary smokers	Coffee and tea consumption decreased 8-OxodG [$P < 0.05$]; 8-OxodG higher in smokers than non-smokers [$P < 0.0001$]	Tea and coffee not separated	Lodovici et al. (2005)
Blood	Leukocytes	DNA damage	8-OHdG	Cross-sectional; 102 (51 M, 51 F) healthy Italians (24–45 yr); 0 to > 4 cups/day	Coffee and smoking inversely associated with 8-OHdG		van Zeeland et al. (1999)
Urine	–	DNA damage	8-OHdG	Cross-sectional, 507 (298 M, 209 F) healthy (21–67 yr); < 1, 1, 2–3, ≥ 4 cups/day)	Coffee inversely associated with 8-OHdG in women [P-trend < 0.05] but adjustment for ferritin attenuated the association		Hori et al. (2014)
Plasma	–	Redox status	d-ROM, BAP	Cross-sectional, 9877 (7633, 2627 F, 5006 M) Japanese subjects (mean, 59 ± 10 yr); quartiles of coffee intake (0, 1–2, 3–4, ≥ 5 cups/day)	Decrease in d-ROM [$P < 0.001$ for trend] with coffee intake in men only; in male current smokers vs never smokers, d-ROM increased [$P < 0.001$] while BAP decreased [$P < 0.001$]		Ishizaka et al. (2013)
Randomized controlled trials							
Blood	Leukocytes	DNA damage	8-OHdG, AOPP	RCT crossover, 37 (29 M, 8 F) patients with chronic hepatitis C (58 ± 11 yr); 4 cups/day unfiltered coffee, abstinence; 30 days	Coffee vs no coffee decreased 8-OHdG [$P < 0.05$] but AOPP was not changed	No placebo	Cardin et al. (2013)
Blood, plasma, urine	Lymphocytes	DNA damage, redox status	Comet assay, various ROS measures	RCT crossover, 38 (14 M, 24 F) healthy non-smokers (28 ± 8 yr); filtered coffee (800 mL); 5 days, washout 5 wk	Coffee vs water decreased DNA damage (+FPG) [$P < 0.05$]; no significant change: 3-NT, oxLDL, TBARS, PGF2α, ROS, TAC, tGSH, SOD, GPx	No placebo	Mišík et al. (2010)

Table 4.8 (continued)

Tissue	Cell type	End-points	Test	Description of exposure[a] and controls	Response[b]/ significance	Comments	Reference
Blood, plasma, urine	Lymphocytes	DNA damage, lipid peroxidation, protein nitrosation	Comet assay, PGF2α, 3-NT	RCT crossover, 36 (13 M, 16 F) healthy non-smoking subjects (27 yr); 800 mL unfiltered coffee, 800 mL water; 5 days, washout 5 wk	Coffee vs water decreased plasma 3-NT ($P < 0.02$) and urinary PGF2α ($P < 0.02$); no change in DNA damage (+FPG)	No placebo	Hoelzl et al. (2010)
Plasma, urine	–	Lipid peroxidation, antioxidants	F2-isoprostanes, tGSH	RCT, placebo, double-blind, parallel; 9 on coffee (184 mL), 12 on placebo (184 mL); 8 wk	Coffee decreased isoprostanes [$P < 0.05$] and increased tGSH [$P < 0.05$]	Coffee with reduced HHQ	Ochiai et al. (2009)
Plasma	Erythrocytes	Redox status	TAS, ORAC, oxLDL, PGF2α, activity SOD, GPx, CAT	RCT crossover; 20 (6 M, 14 F) healthy non-smoking (20–65 yr); 480 mL paper-filtered coffee light roast for 4 wk, 480 mL paper-filtered coffee medium roast for 4 wk, no washout in between	Coffee increased TAS [$P < 0.01$], ORAC [$P < 0.01$], SOD [$P < 0.01$], GPx [$P < 0.01$], and CAT [$P < 0.01$]; PGF2α and oxLDL were not changed	No placebo	Corrêa et al. (2012)
Colorectal tissue, plasma	Mucosa	Glutathione status	GST activity, GSH	RCT crossover, 64 (31 M, 33 F) healthy subjects (43 ± 11 yr); 1 L/day unfiltered coffee, no coffee; 2 wk, washout 8 wk	Coffee vs no coffee GSH content but not GST activity increased in mucosa ($P = 0.01$) and plasma ($P = 0.003$)	No placebo	Grubben et al. (2000)
Interventions (≥ 7 days)							
Plasma, serum	–	Lipid peroxidation, antioxidant enzymes	F2-isoprostanes, hydroxy fatty acids, LDL-conjugated dienes, activity GPx and PON	Intervention, parallel, 43 healthy non-smoking men (26 ± 6 yr); 0, 3, or 6 cups filtered coffee, 3 wk; acute intervention (in 35 of the subjects) 0, 1, or 2 cups filtered coffee	No change in lipid peroxidation or antioxidant enzyme activity	Subjects not randomized across three treatment groups	Mursu et al. (2005)
Plasma	–	Lipid peroxidation	Lag time LDL oxidation, TBARS	11 healthy male students (20–31 yr); wash-in (water, 7 days); coffee (150 mL, 7 days); washout (water, 7 days)	Coffee increased LDL oxidation lag time [$P < 0.001$] and decreased TBARS [$P < 0.005$]; both returned to baseline after washout		Yukawa et al. (2004)

Table 4.8 (continued)

Tissue	Cell type	End-points	Test	Description of exposure[a] and controls	Response[b]/ significance	Comments	Reference
Blood	Erythrocytes	Antioxidant enzymes, antioxidants	SOD, GPx, CAT activity; erythrocyte GSH, tocopherol, MDA	30 healthy subjects; 2 wk washout, 4 wk 500 mL light-roast filtered coffee daily, 2 wk washout, 4 wk dark-roast filtered coffee daily	Light roast increased SOD, GPx, and CAT [all $P < 0.05$]; no change in tGSH, Toc, and MDA Dark roast decreased SOD and GPx activity, and increased CAT, tGSH (total GSH), and Toc (tocopherol) concentrations [all $P < 0.05$]; no change: MDA	Tocepherol not defined	Kotyczka et al. (2011)
Blood	Peripheral blood lymphocytes	mRNA, NQO1, and Nrf2	RT–PCR	27 healthy non-smoking subjects (26 ± 1 yr); 2 wk washout, 4 wk 500 mL light-roast filtered coffee, 2 wk washout, 4 wk dark-roast filtered coffee	No change in Nrf2, NQO1		Boettler et al. (2011)
Acute interventions							
Urine	–	Oxidative stress	H_2O_2	4 subjects (26–49 yr); 1 cup instant coffee; 0, 50, 100 min	Increased urinary H_2O_2		Long & Halliwell (2000)
Urine	–	Oxidative stress	H_2O_2	10 (2 F, 8 M) healthy subjects (20–70 yr); 187 mL canned coffee; 1–4 h	Increased urinary H_2O_2		Hiramoto et al. (2002)
Urine	–	Oxidative stress, antioxidants	H_2O_2	8 healthy subjects; 200 mL instant coffee; 0, 60 min	Increased urinary H_2O_2; no change in antioxidants		Ziobro & Bartosz (2003)
Urine	–	Oxidative stress	H_2O_2	9 subjects; 200 mL instant coffee; 0, 1, 2, 3, 4 h	Increased urinary H_2O_2		Halliwell et al. (2004)
Plasma	–	Lipid peroxidation	Lag time LDL oxidation	10 (5 F, 5 M) healthy (24–35 yr); 200 mL filtered coffee; 0, 30, 60 min	Coffee increased LDL oxidation lag time [$P < 0.05$]	No control	Natella et al. (2007)
Plasma	–	Antioxidants	TRAP, SH groups, crocin test, ascorbic acid	Acute intervention, 10 healthy non-smoking (age NR; 200 mL coffee; 0, 1, 2 h	Coffee increased uric acid [$P < 0.005$] and TRAP [$P < 0.05$] but not ascorbic acid or total SH	No control	Natella et al. (2002)

Table 4.8 (continued)

Tissue	Cell type	End-points	Test	Description of exposure[a] and controls	Response[b]/ significance	Comments	Reference
Plasma/ serum	–	Antioxidants	FRAP, TRAP, ascorbic acid, tocopherols (α, γ), albumin, bilirubin, uric acid	Acute intervention, randomized crossover, 10 (7 F, 3 M) healthy subjects (22–57 yr); 200 mL instant coffee, 200 mL water; 0, 90 min; 7 days washout	Coffee increased FRAP and TRAP [both $P < 0.05$] but did not change ascorbic acid, α-tocopherol, or γ-tocopherol		Moura-Nunes et al. (2009)

[a] Unless otherwise specified, the term coffee is used to mean brewed, caffeinated coffee

[b] +, positive; –, negative; differences: coffee vs control

3-NT, 3-nitrotyrosine; 8-OHdG, 8-hydroxydeoxyguanosine; 8-OxodG, 8-oxodeoxyguanosine; AOPP, advanced oxidation protein products; BAP, biological antioxidant potential; CAT, catalase; d-ROM, derivatives of reactive oxygen metabolites; F, female; FPG, formamidopyrimidine-DNA *N*-glycosylase; FRAP, ferric-reducing antioxidant parameter; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione *S*-transferase; h, hour; HHQ, hydroxyhydroquinone; LDL, low-density lipoprotein; M, male; MDA, malondialdehyde; min, minute; mo, month(s); NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear factor-erythroid-2-related factor; ORAC, oxygen radical absorbance capacity; oxLDL, oxidized LDL; PGF2α, 8-epi-prostaglandin F2α; PON, paraoxonase; RCT, randomized controlled trial; ROS, reactive oxygen species; RT–PCR, real time polymerase chain reaction; SH, sulfhydryl; SOD, superoxide dismutase; TAC, total antioxidant capacity; TAS, total antioxidant status; TBARS, thiobarbituric acid-reactive substances; tGSH, total glutathione; Toc, tocopherol; TRAP, total radical-trapping antioxidant parameter; vs, versus; wk, week(s); yr, year(s)

after green tea. [The Working Group took note of Halliwell's hypothesis that H_2O_2 of coffee is not excreted into urine, but very likely originates from the hydroxyhydroquinone present in coffee, which is subsequently oxidized in urine to produce H_2O_2.]

The lag time of LDL oxidation increased by 1 hour after consumption of 200 mL of coffee (Natella et al., 2007). Regarding measures of antioxidant status, uric acid and TRAP in plasma increased, whereas ascorbic acid and total sulfhydryl groups did not change 2 hours after coffee consumption (Natella et al., 2002). The antioxidant capacity (TRAP and FRAP) of plasma also increased 90 minutes after coffee consumption, but individual antioxidants including ascorbic acid and tocopherols did not change significantly (Moura-Nunes et al., 2009).

(b) Human cells in vitro

Colon-derived HT-29 and CaCo-2 cells exposed to coffee and coffee extracts showed protection against induced ROS (Bakuradze et al., 2010). Light-roasted coffee induced electrophile response element (EpRE)-dependent antioxidant enzymes γ-glutamylcysteine ligase (γ-GCL), NQO1, and GSR (Bakuradze et al., 2010). Roasted coffee extracts increased the expression of GPx in CaCo-2 cells by more than 10-fold (Yazheng & Kitts, 2012). Roasted coffee induced other antioxidant enzymes such as sulfiredoxin, thioredoxin reductase, and peroxiredoxin.

Exposure of hepatocytes (HepG2) to an unfiltered dark-roast coffee extract induced EpRE by more than 10-fold, but the filtered extract had a slightly lesser effect (Paur et al., 2010). [The Working Group noted that coffee components first have to be absorbed in the gastrointestinal tract, and are very likely metabolized upon absorption before they reach lymphocytes and hepatocytes. Some coffee components, for example phenolics, will be extensively metabolized during their passage through the gastrointestinal tract and upon their subsequent absorption.]

Treatment of human hepatoma (HepG2), colon carcinoma (Caco-2), and oesophagus carcinoma (KYSE70) cells with regular and decaffeinated coffee for 24 hours significantly increased expression of NRF2 (Kalthoff et al., 2010). Similar findings were reported in several other studies (Paur et al., 2010; Boettler et al., 2011; Volz et al., 2012; Sauer et al., 2013). In particular, Paur et al. (2010) demonstrated that treatment of hepatoma HepG2 cells with dark-roast coffee extract for 17 hours significantly increased expression of NRF2.

A coffee extract enriched by *N*-methylpyridinium and CGAs, each known as a potent activator of the Nrf2/ARE pathway, increased nuclear Nrf2 translocation and enhanced the transcription of ARE-dependent genes NAD(P)H:quinone oxidoreductase (*NQO1*) and *GSTA1* in HT29 human colon carcinoma cells (Volz et al., 2012).

(c) Non-human mammals in vivo

(i) Rat

See Table 4.9 (web only; available at: http://publications.iarc.fr/566).

Biomarkers of DNA damage (8-OHdG) and lipid peroxidation (F2-isoprostanes) in rat urine after long-term exposure (up to 130 days) of a coffee dose equivalent to 9 and 20 cups/day were determined (Sakamoto et al., 2003). Only 8-OHdG increased, and the increase was dependent upon dose. In another subchronic study, Morakinyo et al. (2013) reported no significant effects on TBARS.

In several experiments in rats, the effects of coffee were studied after induction of oxidative stress using a variety of stressors: a high-fat diet (Vitaglione et al., 2010; Salomone et al., 2014); exercise (Viana et al., 2012); carbon tetrachloride (CCl_4) (Ozercan et al., 2006; Poyrazoglu et al., 2008); and dimethylnitrosamine (DMN) (Shin et al. 2010). For instance, a high-fat diet increased F2-isoprostanes and 8-OHdG, both of

which were suppressed by coffee (Salomone et al., 2014). Exercise increased carbonyls, a measure of protein oxidation, and TBARS (Viana et al., 2012). Coffee partly normalized the effects of exercise on carbonyls and TBARS, but decaffeinated coffee had no effect. Carbon tetrachloride ($CCl_{4)}$ increased TBARS in plasma and liver, and unfiltered coffee was able to partly suppress the effect of CCl_4 on lipid peroxidation (Poyrazoglu et al., 2008). Coffee normalized the DMN-induced effects on TBARS (Shin et al. 2010).

Regarding antioxidant status, Morakinyo et al. (2013) found no effects of coffee on tGSH and SOD after 12 weeks of coffee. In an acute study, Vicente et al. (2011) showed that the activity of GPx, SOD, and CAT in liver increased significantly after only 1 hour, and returned to basal levels > 4 hours later. ORAC did not change. Decaffeinated coffee increased GSH and glutathione disulfide (GSSG) (Vitaglione et al., 2010). Coffee normalized the DMN-induced reduction of tGSH and SOD (Shin et al., 2010). In male Wistar rats, 2.0 mL/day of regular coffee for 28 days increased the expression of Nrf2 in the liver by 2.3-fold Vicente et al. (2014).

(ii) Mouse

See Table 4.9 (web only; available at: http://publications.iarc.fr/566).

No significant changes in 8-OHdG levels were observed in the livers of coffee-fed mice (Morii et al., 2009).

Activation of the EpRE by coffee was studied in transgenic EpRE/luciferase mice after induction by lipopolysaccharide (LPS). Coffee increased whole-body luminescence, especially that of the liver (Paur et al., 2010). A related experiment studied Nrf2 transcription by comparing the effects of coffee in *nrf2*$^{+/+}$ and *nrf2*$^{-/-}$ mice (Higgins et al., 2008). In nrf2$^{+/+}$ mice, coffee significantly increased the mRNA and protein expression of GST and NQO1. Moreover, patterns of GST and NQO1 expression in the liver, colon, and small intestine were different (Higgins et al., 2008).

Coffee did not significantly impact the expression of a range of antioxidant enzymes in the liver (Morii et al., 2009). In another study, both regular (caffeinated) and decaffeinated coffee significantly increased the content of sulfhydryls and the activity of GST in the liver. However, a dose–response relation could not be demonstrated (Abraham & Singh, 1999).

4.2.3 Chronic inflammation and immunosuppression

(a) Chronic inflammation

(i) Exposed humans

Cross-sectional studies

See Table 4.10 (web only; available at: http://publications.iarc.fr/566).

C-reactive protein (CRP) as a single biomarker of inflammation has been studied in cross-sectional studies of coffee consumption, ranging from large studies of thousands of subjects (Maki et al., 2010; Pham et al., 2011) to studies involving about 100 subjects (Kotani et al., 2010). In a healthy Japanese population of 10 325 subjects, the men (4407) in the highest quintiles of coffee consumption (> 7 cups/day) had 20% lower levels of high-sensitivity CRP (hsCRP) compared with men in the lowest quintile (0 cups/day) (Maki et al., 2010; Pham et al., 2011). In 7574 healthy men and women of the Republic of Korea, there was no difference in serum CRP levels between the highest and the lowest quartile of coffee intake (Lee et al., 2014). In a multiple regression model, Rebello et al. (2011) found that coffee drinking had no effect on hsCRP levels in 4139 healthy Asian men and women. Arsenault et al. (2009) found lower hsCRP values in the highest quartile of coffee intake in 344 healthy women. In 114 healthy Japanese, coffee drinkers had lower hsCRP values than non-drinkers of coffee (Kotani et al., 2010).

In a European population of 3042 healthy men and women (M/F: 50/50), levels of

inflammatory biomarkers (C-reactive protein, CRP; interleukin-6, IL-6; tumour necrosis factor alpha, TNF-α; and serum amyloid-A, SAA) were higher in the highest quartile of coffee intake compared with the lowest quartile for both men and women (Zampelas et al., 2004). Leukocyte counts were also higher in the highest quartile.

In a cross-sectional study of 1393 women of the US Nurses' Health Study I cohort, caffeinated and decaffeinated coffee consumption was inversely related to a range of inflammatory biomarkers (Lopez-Garcia et al., 2006). In drinkers of caffeinated coffee, CRP and E-selectin levels were lower in women with type 2 diabetes, but not in healthy women. For decaffeinated coffee, both CRP and E-selectin levels were lower in non-diabetics, whereas no difference was observed in women with diabetes (Lopez-Garcia et al., 2006).

IL-6 and plasminogen-activator inhibitor type 1 (PAI-1) were increased among 30 drinkers of high quantities of coffee (> 4 cups/day) compared with 30 drinkers of low quantities of coffee (< 1 cup/day) in a study of hypertensive smokers (Tsioufis et al., 2006).

A large number (77) of inflammatory and immune biomarkers were measured in 1728 older non-Hispanic white US subjects (age, 55–74 years). After correction for multiple comparisons and the exclusion of markers with < 25% detectability, only the soluble tumour necrosis factor receptor II (sTNFRII) was found to be significantly lower in drinkers of high quantities of coffee (> 2.5 cups/day) (Loftfield et al., 2015).

Prospective studies

See Table 4.10 (web only; available at: http://publications.iarc.fr/566).

In 2040 subjects from the prospective Nurses' Health Study, coffee drinking (highest quartile of intake ≥ 4 cups/day) was inversely associated with CRP and TNFα receptor-2 levels (Williams et al., 2008).

A prospective nested case–control study on coffee drinking and the primary form of liver cancer, hepatocellular carcinoma, included 125 cases of hepatocellular carcinoma and 250 controls (Aleksandrova et al., 2015). The multivariable-adjusted relative risk (RR) for subjects drinking ≥ 4 cups/day compared with < 2 cups/day was 0.25 (95% CI, 0.11–0.62) (*P* for trend = 0.006). Additionally, coffee drinking was inversely associated with IL-6, and that IL-6 attenuated the association of coffee with hepatocellular carcinoma.

Randomized controlled clinical trials

See Table 4.10 (web only; available at: http://publications.iarc.fr/566).

The effect of roasting was studied on a range of inflammatory markers in subjects who drank 3–4 cups/day of light- or medium-roasted coffee (150 mL/cup) for 4 weeks. Only three markers changed: soluble vascular cell adhesion molecule-1 (sVACM-1) increased after both the light- and medium-roasted coffee; fibrinogen increased only after the medium-roasted coffee; and sE-selectin increased only after the consumption of the light-roasted coffee (Corrêa et al., 2013).

Kempf et al. (2010) studied the effect of coffee (4 and 8 cups/day) drinking in subjects with an elevated risk of type 2 diabetes, and measured six inflammatory markers; 1 month of coffee drinking was followed by 1 month of abstinence. Only IL-18 was significantly lower at the end of the coffee-drinking period.

A study of the acute effects of caffeinated and decaffeinated coffee (200 mL) found no effect on plasma/serum IL-6 and IL-18 (Gavrieli et al., 2011).

(ii) Human cells in vitro

Coffee extract and a synthetic mixture of roasting products both induced the nuclear translocation of nuclear factor κB (NF-κB) in macrophages (NR8383) and intact human gut tissue, whereas only the roast products had an effect on Caco-2 cells (Sauer et al., 2011).

Filtered and unfiltered coffee extracts inhibited LPS-induced activation of NF-κB in U937 cells transfected with a NF-κB–luciferase construct (Paur et al., 2010). Dark-roasted coffee extracts had a larger effect than light-roasted extracts. In agreement with changes in luminescence, NF-κB protein and mRNA levels changed together with the mRNA of several NF-κB target genes. [The Working Group noted that these results were obtained after direct exposure to coffee extracts.]

(iii) Experimental systems

See Table 4.11 (web only; available at: http://publications.iarc.fr/566).

In the rat, the effects of coffee on the expression and tissue concentration of several inflammatory cytokines were studied after the induction of inflammation using a variety of stressors: a mutant strain that accumulates iron and copper in the liver (Katayama et al., 2014); a high-fat diet (Vitaglione et al., 2010); DMN to induce liver fibrosis (Shin et al., 2010); and LPS (Sakamoto et al., 2001). In the liver of the Long Evans Cinnamon (LEC) rat, coffee suppressed IL-6 protein and mRNA levels as well as TNF-α mRNA. However, it did not affect TNF-α protein levels or IL-1β mRNA expression (Katayama et al., 2014). Decaffeinated coffee significantly lowered hepatic concentrations of TNF-α and IFN-γ, and increased those of IL-4, IL-6, and the anti-inflammatory IL-10 in Wistar rats fed a high-fat diet (Vitaglione et al., 2010). LPS-induced serum changes in TNF-α and IL-6 were not inhibited by coffee (Sakamoto et al., 2001).

In mice, coffee decreased mRNA levels of IL-6 in adipose tissue (Matsuda et al., 2011) and reduced serum levels of IL-1α, IL-6, and TNF-α (Guo et al., 2014). Other experiments evaluated coffee on inflammation induced by LPS (Paur et al., 2010), a high-fat diet (Fukushima et al., 2009), and diabetes (Yamauchi et al., 2010) in the mouse. In mice transfected with a NF-κB–luciferase construct, coffee reduced whole-body luminescence that had been induced with LPS (Paur et al., 2010). Coffee and pure caffeine reduced mRNA levels of various inflammatory cytokines in fat (MCP-1, IL-6, and TNF-α) and in serum (TNF-α) in diabetic mice (Yamauchi et al., 2010). Fukushima et al. (2009) showed that the increased expression in MCP-1 and IL-1β that is induced by a high-fat diet is partly inhibited by coffee. There were no clear differences between caffeinated and decaffeinated coffee.

Rat macrophages were exposed to roasted and non-roasted coffee in studies in vitro; only the roasted coffee increased the expression of NF-κB (Muscat et al., 2007). In mouse splenocytes, freeze-dried coffee attenuated the induction of interleukins by ovalbumin (Goto et al., 2011).

(b) Immunosuppression

(i) Exposed humans

See Table 4.12 (web only; available at: http://publications.iarc.fr/566).

In a cross-sectional study of 1728 older United States non-Hispanic white people (age, 55–74 years), a large number (77) of immune and inflammatory markers was compared between coffee drinkers and non-drinkers of coffee (Loftfield et al., 2015). The immune markers interferon gamma (IFNγ), fractalkine (CX3CL1), microphage inflammatory protein-1β (MIP-1β/CCL4), fibroblast growth factor-2 (FGF-2), and sTNFRII were found to be lower in coffee drinkers.

In an exploratory study with 15 subjects, consumption of 5 cups/day of coffee for 5 weeks had no effect on total T- and B-cell counts, but increased the counts of natural killer cells (Melamed et al., 1990). Coffee drinking suppressed lectin-stimulated transformation of lymphocytes, and stimulated the chemotaxis activity of mononuclear leukocytes.

(ii) Experimental systems

Goto et al. (2011) exposed splenocytes from mice to coffee extracts and observed a decrease in ovalbumin-induced cell proliferation.

4.2.4 Receptor-mediated mechanisms

(a) Nuclear receptor signalling pathways

(i) Humans

No data from exposed humans were available to the Working Group.

In studies in vitro, treatment with regular and decaffeinated coffee for 24 hours significantly increased expression of aryl hydrocarbon receptor (AhR) in hepatoma (HepG2), colon carcinoma (Caco-2), and oesophagus carcinoma (KYSE70) cells (Kalthoff et al., 2010). Similarly, Ishikawa et al. (2014) reported that coffee is a strong activator of AhR expression in vitro.

The coffee component cafestol, at a concentration of 20 μM activated the farnesoid X receptor (FXR) and pregnane X receptor (PXR) in human liver HepG2 cells (Ricketts et al., 2007).

The coffee component HHQ was a putative ligand of the peroxisome proliferator-activated receptor γ (PPARγ) Shashni et al. (2013). Coffee treatment of human MCF-7 and MDA-MB-231 breast cancer cells inhibited PPARγ-dependent glycolytic enzymes.

(ii) Experimental systems

Decaffeinated coffee increased the level of PPARα in the livers of male Wistar rats fed a high-fat diet (Vitaglione et al., 2010).

In mouse 3T3-L1 cells, coffee extract (1.25%, 2.5%, and 5.0% v/v for 6 days) reduced *Pparγ* gene expression in a dose-dependent manner (Aoyagi et al., 2014). PPARγ protein was reduced in cells treated with 2.5% (v/v) coffee extract.

In a model system in vitro, cafestol activated human FXR in the monkey kidney CV-1 cell line (Ricketts et al. (2007).

(b) Sex hormone pathways

Kotsopoulos et al. (2009a) reported an inverse correlation between coffee intake and the level of luteal and free estradiol in 524 premenopausal women from the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII), but not luteal progesterone level. No association between coffee intake and estrogen and androgen levels was found in 713 postmenopausal women from the NHS and NHSII. In contrast, a significant increase in the level of estradiol associated with coffee consumption in women aged > 40 years who consumed > 1 cup/day of coffee (Lucero et al., 2001).

Several studies found a positive association between coffee and/or caffeine intake and the level of sex hormone-binding globulin (SHBG) in postmenopausal women. In the largest study involving 13 547 postmenopausal women from the Women's Health Initiative, intake of regular coffee, but not decaffeinated coffee, was positively associated with the SHBG plasma level Goto et al. (2014). Similarly, in the Rancho Bernardo Study of 728 postmenopausal women, caffeine intake increased plasma level of SHBG and estrone. In contrast, Wedick et al. (2012) did not find an association between caffeinated coffee consumption and SHBG; however, the sample size in that study was small (n = 42). Svartberg et al. (2003) reported a positive association between coffee consumption and the levels of total testosterone and SHBG. In contrast, as part of a Danish pregnancy study, the sons of women who consumed 4–7 cups/day of coffee during pregnancy had lower testosterone levels than the sons of mothers drinking 0–3 cups/day (P = 0.04) Ramlau-Hansen et al., 2008).

Sisti et al. (2015) demonstrated that coffee consumption modulates the 2-hydroxylation pathway, the major pathway in estrogen metabolism. This was evidenced by a positive association between coffee intake of > 4 cups/day and the

levels of 2-hydroxyestrone and 2-hydroxyestradiol in urine of premenopausal women.

Several studies in vitro demonstrated that coffee is a potent inhibitor of the estrogen SULT reaction, a major pathway for the inactivation of estrogens (Kauffman, 2004), in human colon carcinoma Caco-2 cells (Okamura et al., 2005; Saruwatari et al., 2008; Isshiki et al., 2013). In two separate studies, incubation of human colon carcinoma Caco-2 cells with coffee extract resulted in a dose-dependent inhibition of SULT activity (Okamura et al., 2005) in general, and estrogen SULT sulfation activity towards 17β-estradiol in particular (Saruwatari et al., 2008). In addition, treatment of Caco-2 cells with 2.5% (v/v) coffee extract for 24 hours resulted in a 60% reduction of *SULTE1* gene expression and a 25% reduction in cytosolic estrogen SULT activity (Isshiki et al., 2013).

In the treatment of estrogen receptor α (ERα) -positive human breast cancer MCF7 cells with coffee constituents, caffeine at concentrations of 0.2, 1.0, and 5 mM or caffeic acid at concentrations of 2, 10, and 50 μM for 72 hours suppressed the expression of ERα (Rosendahl et al., 2015). In contrast, Ezechiáš et al. (2016) did not detect antiestrogen or antiandrogen effects of caffeine at a concentration of 8 μM on the human breast cancer T47D cell line.

(c) Glucocorticoid hormone pathways

Humans

Consumption of regular coffee (with a caffeine concentration of 3.0 mg/kg bw) increased plasma cortisol concentration at 60 minutes and thereafter in healthy young men Gavrieli et al. (2011). In contrast, in a randomized pilot crossover study, consumption of 4 cups/day of green coffee by healthy volunteers for 2 weeks significantly decreased urinary free cortisol level; it was also found that both black coffee and green coffee reduced urinary cortisol/cortisone ratio (Revuelta-Iniesta & Al-Dujaili, 2014).

Oral consumption of caffeine at 3.3 mg/kg bw, which is equivalent to 2–3 cups of coffee, significantly elevated cortisol level after 60 minutes Lovallo et al. (1996).

In a study in vitro, treatment of human embryonic kidney HEK-293 cells with 0.5% coffee extract for 40 minutes inhibited endogenous 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) activity, resulting in blockage of 11β-HSD1-dependent cortisol formation and preventing nuclear translocation of glucocorticoid receptor (Atanasov et al., 2006).

(d) Gastrointestinal hormone pathways

(i) Humans

Acquaviva et al. (1986) studied the effect of coffee on the release of gastrin in healthy volunteers and demonstrated a strong gastrin-releasing property of coffee. Drinking 100 mL of decaffeinated coffee resulted in a prompt and lasting elevation of total gastrin. The stimulatory effect of coffee consumption, especially regular coffee, was reported on the release of three other gastrointestinal hormones, glucagon-like peptide 1 (GLP-1), and cholecystokinin (Douglas et al., 1990; Johnston et al., 2003; Olthof et al., 2011).

In a study in vitro, Fujii et al. (2015) demonstrated that treatment of human caecum NCI-H716 cells with 0.05% and 0.1% of extract of coffee polyphenols for 2 hours resulted in a dose-dependent increase of GLP-1 secretion.

(ii) Experimental systems

Treatment of male C57BL/6J mice with extract of coffee polyphenols by gavage increased GLP-1 in portal vein blood (Fujii et al., 2015).

(e) Adipose-derived hormone pathways

(i) Humans

A positive association between the consumption of ≥ 4 cups/day of regular coffee and plasma adiponectin level has been reported in diabetic and non-diabetic women (Williams

et al., 2008). Several other independent studies have demonstrated a similar positive association between coffee consumption and plasma adiponectin concentrations (Imatoh et al., 2011; Pham et al., 2015). In a cross-sectional study comprising Japanese workers (2554 men, 763 women), coffee consumption was positively and significantly associated with adiponectin level (Yamashita et al., 2012). Specifically, individuals who consumed ≥ 4 cups/day of coffee had a significantly greater plasma adiponectin level as compared with those who consumed 1 cup/day. Furthermore, coffee consumption in Japanese men was not only associated with a greater adiponectin level, but that there was also a positive dose-dependent significant association between coffee consumption and plasma adiponectin level (Imatoh et al., 2011). Indeed, individuals who consumed 1–2 cups/day of coffee had a greater plasma adiponectin level (6.43 μg/mL; n = 220) than individuals who consumed 1–5 cups/week of coffee (5.91 μg/mL; n = 181). In a randomized parallel-arm controlled-intervention trial, consumption of regular coffee (5 cups/day for 8 weeks) increased plasma adiponectin levels (Wedick et al., 2011). Contrary to the positive association between coffee consumption and greater adiponectin level, several reports have shown that coffee consumption was linked to low leptin levels in plasma (Yamashita et al., 2012; Imatoh et al., 2015).

(ii) Experimental systems

In male Wistar rats fed a high-fat diet for a month and decaffeinated coffee or solutions of coffee polyphenols in drinking-water (the daily amount of coffee or coffee polyphenols corresponded to 6 cups of espresso coffee or 2 cups of filtered coffee), the expression of adiponectin receptor 2 (*Adipo-R2*) in the livers was increased as compared with rats fed a high-fat diet alone (Vitaglione et al., 2010).

Treatment of mouse 3T3-L1 cells with 2.5 or 5% (v/v) coffee reduced the adiponectin gene in a dose-dependent manner (Aoyagi et al., 2014).

4.2.5 Alterations of cell proliferation, death, or nutrient supply

(a) Coffee, cell death, and cell proliferation

(i) Humans

Grubben et al. (2000) studied the effect of unfiltered coffee on the extent of cell proliferation in colorectal mucosa in healthy volunteers in a crossover randomized trial. A total of 64 healthy volunteers (31 men and 33 women; age, 43 ± 11 years) were randomly assigned to two groups. The study consisted of two intervention periods of 2 weeks each separated by a washout period of 8 weeks. One group drank 1 L/day each (6 cups/day) of unfiltered regular coffee; the other group did not drink coffee. Colorectal biopsies were taken on day 15 of each intervention period. When comparing proliferation cell nuclear antigen (PCNA) immunostaining results from the control and experimental groups, no effect of coffee drinking on cell proliferation in colorectal mucosa was found.

In vitro, an antiproliferative effect of various dilutions of four different regular or decaffeinated coffee brands was shown in human ovarian carcinoma A2780 cells after 48 hours of treatment. The magnitude of inhibitory activity varied among the different brands of coffee (Tai et al., 2010).

Several studies have examined the antiproliferative and cytotoxic effects of the coffee-specific diterpenes kahweol and cafestol in various human cancer cell lines. Kahweol (20–80 μM for 24 hours and 48 hours) treatment of human HN22 and HSC4 oral squamous cancer cell lines significantly decreased cell viability in a dose- and time-dependent manner (Chae et al., 2014). Cárdenas et al. (2014) showed a potent proapoptotic effect of kahweol in several human cancer

cell lines (HT-29 colon adenocarcinoma, HL-60 leukaemia, and MDA-MB-231 breast cancer cells). In MDA-MB-231 breast cancer cells, a dose-dependent increase of the subG1 cell population was accompanied by a dose-dependent decrease of cells in the G2/M phase. Additionally, treatment of MDA-MB-231 breast cancer cells with kahweol induced caspase 3/7 activity. Several independent studies (e.g. Oh et al., 2009; Choi et al., 2015) reported similar proapoptotic effects of kahweol on various human cancer cells.

A proapoptotic activity in human cancer cells was also reported for another coffee-specific diterpene: cafestol. Choi et al. (2011) demonstrated dose-dependent cafestol-induced antiproliferative and proapoptotic effects in human Caki renal carcinoma cells. Kotowski et al. (2015) reported a dose-dependent reduction in cell viability and the induction of apoptosis in three cafestol-treated human head and neck squamous cell carcinoma cell lines: SCC25, CAL27, and FaDu.

(ii) Experimental systems

Lina et al. (1993) showed that drinking coffee diluted 10 times (10%) or undiluted coffee brew (100%) for 2 weeks and 6 weeks did not alter cell proliferation in the urinary bladders of male Wistar rats. Miura et al. (2004) investigated the effect of instant coffee on the growth of rat hepatoma AH109A cells using a tumour-implant model in vivo. Donryu rats with subcutaneously implanted AH109A cells fed a diet containing 0.1% of instant coffee powder for 2 weeks exhibited a suppressive effect on the in vivo growth of AH109A cells, with significantly smaller tumour sizes in coffee-fed rats.

Chlorogenic acid (30 µM and 60 µM for 24 hours) significantly decreased the cell viability of B16 murine melanoma cells (Li et al. (2014). Instant coffee inhibited the proliferation of rat hepatoma AH109A cells assessed by [methyl-^{3}H]-labelled thymidine incorporation (Miura et al. (1997). Moreover, an antiproliferative effect on AH109A cells was reported for the serum obtained from rats given instant coffee solution at 100 mg/mL per 100 g bw by gavage. In a subsequent study, Miura et al. (2004) instant coffee was proapoptotic in AH109A cells.

(b) Autophagy

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Two studies investigated the effect of coffee and caffeine on autophagy in vivo. In the first study, short-term administration of 3% (w/v) regular or decaffeinated coffee by gavage to female C57BL/6 mice rapidly induced autophagy in multiple organs, including liver, heart, and muscle (Pietrocola et al., 2014). A similar autophagy-inducing effect of regular or decaffeinated coffee in the livers was also observed after the longer-term (for 2 weeks) administration of 3% (w/v) coffee in drinking-water. Autophagy induced by coffee was independent of caffeine content and accompanied by the inhibition of the enzymatic activity of mTORC1. In a second study, administration of 0.05% (w/v) of caffeine for 4 weeks in the drinking-water of male C57/BL6 mice maintained on a high-fat diet resulted in a marked increase in LC3-II protein levels (Sinha et al., 2014).

(c) Angiogenesis

(i) Humans

No data in exposed humans were available to the Working Group.

An antiangiogenic effect of cafestol (Wang et al., 2012) and kahweol (Cárdenas et al., 2011) was reported in human umbilical vein endothelial cells (HUVEC) and human HT-1080 fibrosarcoma cells.

(ii) Experimental systems

Using the mouse aortic ring assay, 5 μM of kahweol inhibited microvessel sprouting by 40% after 10 days of treatment, whereas 25 μM almost completely inhibited this angiogenic effect (Cárdenas et al., 2011).

In zebrafish (*Danio rerio*), 75 μM of kahweol inhibited intersegmental vessel formation after 24 hours of treatment (Cárdenas et al., 2011). Similarly, kahweol at 50 μM inhibited angiogenesis in treated eggs in the chicken chorioallantoic membrane assay (Cárdenas et al., 2011).

4.2.6 Other mechanisms

(a) DNA repair

No human data on coffee were available to the Working Group.

In male ICR mice given 0.1% instant coffee solution in drinking-water for 35 weeks, no changes in the hepatic expression of 8-OHdG repair-associated genes was found (Morii et al., 2009). In male Fischer rats given kahweol and cafestol in the diet for 10 days, a marked and dose-dependent increase in the hepatic levels of O^6-methylguanine-DNA methyltransferase (MGMT) was seen (Huber et al., 2003). Similarly, "Turkish" coffee given in drinking-water for 10 days significantly increased hepatic MGMT activity.

Several studies in vitro have shown that caffeine inhibits DNA repair. Caffeine was shown to inhibit the ataxia telangiectasia mutated (ATM) activity in human HeLa cells and lymphoblasts by Blasina et al. (1999). In human fibroblasts, caffeine compromised the non-homologous end-joining pathway and sensitized the cells to X-ray exposure (Kawata et al., 2005). In rodent cells, caffeine inhibited DNA replication (Schlegel & Pardee, 1986) and the homology-directed repair of DNA double-strand breaks (Wang et al., 2004), and delayed replication fork progression (Johansson et al., 2006).

(b) Epigenetic alterations

No data for coffee were available to the Working Group.

In human MCF-7 and MDA-MB-231 breast cancer cells in vitro, Lee & Zhu (2006) reported demethylation of the promoter region of the retinoic acid receptor β (*RARβ*) gene by caffeic acid and chlorogenic acid.

In rodents, prenatal caffeine exposure induced epigenetic alterations. When given to pregnant Wistar rats, caffeine reduced hepatic methylation of DNA and histones in the offspring (Tan et al., 2012) and induced the expression of DNA methyltransferase and histone deacetylase genes in fetal adrenals (Ping et al., 2014).

Lee & Zhu (2006) demonstrated concentration-dependent inhibition of DNA methylation catalysed by prokaryotic SssI DNA methyltransferase and human DNMT1 by caffeic acid and chlorogenic acid.

4.3 Genetic susceptibility

The literature on the genetic modifiers of coffee consumption-associated traits is diverse and can be subdivided into two broad categories: studies of polymorphisms that are associated with coffee consumption patterns and coffee drinking preference; and studies of genetic variants as factors of susceptibility or resistance to certain cancers in humans. While the data for the latter category are sparse and come from a relatively small number of molecular epidemiology studies, there is strong evidence from several large-scale genome-wide association studies (GWAS) and meta-analyses that habitual coffee consumption is associated with a limited number of modifier alleles.

4.3.1 Genetic mediators of habitual coffee consumption

Coffee and caffeine consumption patterns are highly heritable (as high as 58%) traits (Yang et al., 2010). Coffee consumption habits are strongly associated with polymorphisms in genes involved in metabolism and pharmacological mechanisms of the action of caffeine. Specifically, cytochrome P450 (CYP)1A2, which is almost exclusively responsible for the oxidative metabolism of caffeine in humans (Kot & Daniel, 2008b), and AhR, a nuclear receptor that is responsible for the upregulation of xenobiotic metabolizing enzymes by coffee (Kalthoff et al., 2010), are two genes that exhibit strong associations with coffee and caffeine consumption. For instance, a meta-analysis (Sulem et al., 2011) of four GWASs of coffee consumption assessed from a questionnaire (0 to ≥ 4 cups/day) completed by around 6000 coffee drinkers from Germany, Iceland, the Netherlands, and the USA found two sequence variants to be significantly associated with increased coffee consumption: rs2472297-T located between *CYP1A1* and *CYP1A2* at 15q24; and rs6968865-T near *AHR* at 7p21. The association of these SNPs with coffee consumption was observed in both smokers and non-smokers. [The Working Group noted that the lack of effect of smoking indicates that, even though components of cigarette smoke may affect the same metabolism pathways, the effect of caffeine alone is pronounced.] Similarly, Amin et al. (2012) reported a significant association for two SNPs in the 15q24 region between *CYP1A1* and *CYP1A2* genes in a meta-analysis of GWASs, as assessed by questionnaires, from eight Caucasian cohorts (over 18 000 individuals). Importantly, significant associations between SNPs in *AHR* and *CYP1A1-CYP1A2* and caffeine and coffee consumption from GWASs in European populations were also replicated in an ethnically distinct Costa Rican population (Josse et al., 2012).

A more recent genome-wide meta-analysis of over 100 000 coffee consumers and non-consumers of European and African-American ancestry, in which intake was assessed in terms of the number of cups of predominantly regular coffee consumed per day, Cornelis et al. (2015) confirmed eight loci, including six novel loci, that are located in or near genes potentially involved in the pharmacokinetics (*ABCG2*, *AHR*, *POR*, and *CYP1A2*) and pharmacodynamics (*BDNF* and *SLC6A4*) of caffeine. [The Working Group noted that these studies demonstrate that coffee consumption is strongly associated with polymorphisms in genes that are involved in metabolism and the pharmacological mechanisms of the action of caffeine.]

4.3.2 Genetic modifiers of cancer-associated effects of coffee

(a) Cancer of the breast

Rabstein et al. (2010) studied the modifier effects of *N*-acetyltransferase 2 (*NAT2*) polymorphisms and several lifestyle factors, including coffee consumption, on the risks of developing estrogen receptor (ER) and progesterone receptor (PR) -positive or -negative breast tumours in 1020 cases and 1047 population controls in Germany. In slow acetylators, frequent consumption of coffee (> 4 cups/day vs none) was associated with higher risks of receptor-negative tumours [risk of developing ER-negative tumours: OR, 2.55; 95% CI, 1.22–5.33].

Two studies investigated whether the variation in *CYP1A2* modifies associations between caffeine and coffee consumption and breast cancer risk. In a cohort of 3062 cases and 3427 controls, Lowcock et al. (2013) found that while high coffee consumption, but not total caffeine intake, may be associated with reduced risk of ER-negative and postmenopausal breast cancers, these effects were independent of *CYP1A2* genotype. Similarly, the *CYP1A2* genotype did not

affect breast cancer risk in *BRCA1* mutation carriers (Kotsopoulos et al., 2007).

(b) Cancer of the ovary

Goodman et al. (2003) published the results of a small molecular epidemiology study that examined genetic modifiers of risk of cancer of the ovary (164 cases of epithelial cancer of the ovary and 194 controls) in association with coffee consumption; subjects were stratified into non-drinkers, and moderate (< 7 cups/week) and heavy (> 7 cups/week) drinkers. A modest positive association between caffeine and coffee consumption and an increased risk of ovarian cancer was reported, as well as some evidence that the risk may be modified by *CYP1A2* genotype. A positive significant trend (P = 0.04) in the odds of ovarian cancer associated with coffee (using a threshold of 7 cups/week) and caffeine intake was observed among women with the *CYP1A2* A/A genotype but not among women with any C allele. [The Working Group noted that this small study would not change the overall evaluation of inadequate evidence for the carcinogenicity of coffee.]

Kotsopoulos et al. (2009b) used data and biological specimens from the Nurses' Health Studies and the New England-based case–control study of ovarian cancer (1354 ovarian cancer cases and 1851 controls) to investigate the relationship between genetic polymorphisms in caffeine-metabolizing enzymes, coffee consumption (evaluated using a dietary questionnaire; subjects stratified as consuming < 2.5 cups/day or ≥ 2.5 cups/day of coffee), and the risk of ovarian cancer. The study found no relationship between coffee consumption and ovarian cancer risk in the overall population. Two SNPs in CYP19 (CYP19013 A and CYP19027 G) were found to be associated with an 18% increased (P for trend = 0.02) and 15% decreased (P for trend = 0.05) risk of ovarian cancer, respectively. However, variants in CYP1A1, CYP1A2, or CYP2A6 could not account for the inconsistent reports of coffee intake and ovarian cancer risk.

(c) Cancer of the bladder

A hospital-based case–control study of association between genetic polymorphisms, coffee drinking, and risk of cancer of the bladder (197 cases and 211 controls) (Covolo et al., 2008) found no association between the genetic polymorphisms in NAT1, NAT2, GSTM1, GSTT1, GSTP1, SULT1A1, XRCC1, XRCC3, and XPD, risk of bladder cancer, and coffee consumption (evaluated from the dietary questionnaire). The only positive finding in this study was a significantly increased risk of bladder cancer (OR, 3.18; 95% CI, 1.06–9.55) among GSTP1 105–114 Val carriers who regularly consumed large quantities of coffee (> 3 cups/day).

A hospital-based case–control study of bladder cancer risk factors (185 cases and 180 controls, all Caucasian men) found no interaction between polymorphisms in CYP1A2, risk of bladder cancer, and coffee consumption (determined in cups/day) from a lifetime dietary questionnaire (Pavanello et al., 2010).

(d) Cancer of the colorectum

A study of 1579 incident cases of adenocarcinoma of the colon and 1898 population-based controls showed that consumption of coffee (intake was evaluated from a questionnaire as part of the diet history) was not associated with colon cancer, and that GSTM1 variants did not modify this association (Slattery et al., 2000).

A nested case–control study of 1252 cases and 2175 controls from 477 071 participants (70.2% women) of the European Investigation into Cancer and Nutrition (EPIC) cohort examined potential effect modification by CYP1A2 and NAT2 for the relationship between colorectal cancer and coffee consumption (based on the recorded number of cups per day/week/month) from a country-specific dietary questionnaire (Dik et al., 2014). In this study, total coffee

consumption (high vs zero/low) was not associated with risk of colorectal cancer (HR, 1.06; 95% CI, 0.95–1.18) or subsite cancers. High-consumption subjects with slow CYP1A2 or NAT2 activity had a similar risk compared with non-consumers/low-consumption subjects with a fast CYP1A2 or NAT2 activity.

(e) Leukaemia

A hospital-based case–control study of 280 cases of acute childhood leukaemia and 288 controls examined various gene–environment interactions for the polymorphisms of CYP1A1, GSTM1, GSTP1, GSTT1, and NQO1 and maternal coffee consumption during pregnancy identified from a dietary questionnaire; subjects were stratified into three groups: never drinkers, < 3 cups/day, and ≥ 3 cups/day (Clavel et al., 2005). Overall, the polymorphisms were not associated with the risk of leukaemia; however, it was observed that the association between maternal coffee consumption during pregnancy and leukaemia was weaker among children with the heterozygous or homozygous mutant *NQO1* genotype than for those with the wildtype genotype. No *P* value for interaction was given.

Another study of the associations between childhood acute leukaemia and maternal caffeinated beverage consumption during pregnancy (764 acute leukaemia cases and 1681 controls in France) also explored the interactions between caffeinated beverage consumption and polymorphisms of metabolism enzymes (NAT2, ADH1C, CYP2E1) (Bonaventure et al., 2013). While it was found that regular maternal coffee consumption during pregnancy was weakly associated with childhood acute leukaemia (OR, 1.2 [95% CI, 1.0–1.5]; $P = 0.02$) no significant gene–environment interactions with coffee drinking were observed.

(f) Melanoma

A hospital-based case–control study of 304 incident cases of cutaneous melanoma and 305 controls explored the relationship between *GSTM1* and *GSTT1* positive and null individuals and coffee consumption (evaluated from a dietary questionnaire as never/occasional, 1, 2, or > 2 cups/day) (Fortes et al., 2013). A high frequency of coffee drinking (more than once per day) was associated with a protective effect for cutaneous melanoma (OR, 0.46; 95% CI, 0.31–0.68) after adjusting for sex, age, education, hair colour, common naevi, skin phototype, and sunburn episodes in childhood. When the subjects were stratified by *GSTM1* and *GSTT1* genotype, the inverse association for coffee was high for subjects with both *GSTM1* and *GSTT1* null polymorphisms.

4.4 Other effects

4.4.1 Humans

(a) Preneoplastic lesions

(i) Adenoma of the colorectum

Several studies have reported a decreased risk of adenomas of the colorectum with coffee drinking (Kato et al., 1990; Almendingen et al., 2001; Budhathoki et al., 2015). However, other reports have found no association (Baron et al., 1997; Nagata et al., 2001), or have suggested increased risks (Lee et al., 1993). Only two studies considered how coffee was prepared. One US-based investigation (Baron et al., 1997) considered caffeinated versus decaffeinated coffee, and found no association with consumption of either beverage. An investigation in Japan (Kono et al., 1991) reported a borderline significant trend of decreasing adenoma risks with increasing intake of instant (but not brewed) coffee.

One large investigation that had no evident selection biases reported significant trends of decreased risks with increased coffee intake. The

trends became apparent only after controlling for confounding factors (Budhathoki et al., 2015). The odds ratio for drinking > 291 mL/day of coffee versus < 26 mL/day was 0.67 (95% CI, 0.48–0.93).

[The Working Group noted that many of the studies regarding coffee and adenomas are subject to possible selection bias in the choice of controls (Kato et al., 1990; Olsen & Kronborg, 1993; Hoshiyama et al., 2000; Almendingen et al., 2001; Nagata et al., 2001) and/or insufficient adjustment for likely confounding factors such as cigarette smoking (Kato et al., 1990; Lee et al., 1993; Hoshiyama et al., 2000). Additionally, no studies addressed the association of coffee drinking with preinvasive lesions in the pathway to serrated colorectal cancer (Bettington et al., 2013).]

One case–control study of adenoma (Lee et al., 1993) assessed the association between colorectal cancer and estimated total caffeine intake from coffee, tea, and carbonated beverages. Although this study reported an association with coffee drinking in women, there was no association with caffeine intake. [The Working Group noted the inadequate control for possible confounding factors, such as cigarette smoking, in this study.]

(ii) Barrett oesophagus

One multicentre hospital-based case–control study investigated the association between coffee drinking and biopsy-confirmed Barrett oesophagus in patients admitted for non-neoplastic, non-gastroenterological conditions (Conio et al., 2002). In unadjusted analyses, there was no difference in the prevalence of coffee drinking between cases and controls (Conio et al., 2002). A second study investigated the association between coffee drinking and Barrett oesophagus in patients who underwent oesophagogastroduodenoscopy; controls without Barrett oesophagus underwent colonoscopy or oesophagogastroduodenoscopy. The authors found an association between Barrett oesophagus and coffee drinking in unadjusted analyses, but no association after multivariable adjustment (Sajja et al., 2016). [The Working Group noted that both studies were susceptible to selection bias in the choice of controls.]

(b) Metabolic effects

Multiple single-dose clinical trials have shown that caffeinated coffee increases insulin resistance and impairs glucose homeostasis (Beaudoin & Graham, 2011). However, the few trials that have investigated longer-term (≥ 1 month) consumption did not observe such metabolic impairments (Kempf et al., 2010; Wedick et al., 2011). Studies that investigated decaffeinated coffee have reported conflicting findings (see review by Beaudoin & Graham, 2011).

Clinical trials that have manipulated caffeine intake have also found that caffeine alone (MacKenzie et al., 2007) or caffeine added to decaffeinated coffee (Gavrieli et al., 2013; Robertson et al., 2015) interferes with glucose homeostasis. It is not clear if the effects of caffeine on glucose regulation are dependent upon dose (Gavrieli et al., 2013; Robertson et al., 2015).

One clinical trial (van Dijk et al., 2009) assessed the effects of the coffee constituents chlorogenic acid (1 g) and trigonelline (500 mg) in a glucose tolerance test. Both compounds reduced early circulating glucose and insulin levels compared with placebo, with no effect on the areas under the concentration curves.

Observational studies clearly show an inverse association between diabetes and coffee intake (Higdon & Frei, 2006; Natella & Scaccini, 2012; Cano-Marquina et al., 2013; Jiang et al., 2014). A meta-analysis of 26 cohort studies involving 50 595 cases of type 2 diabetes reported that risk decreased by 12% (95% CI, 10–14%) and 11% (95% CI, 2–18%) for every 2 cups/day increment in coffee and decaffeinated coffee intake, respectively (Jiang et al., 2014). [The Working Group noted that the differences between the acute and chronic effects may involve acclimation to caffeine and/or the effects of other substances in coffee that improve insulin resistance.]

(c) Liver diseases

Observational studies have found that coffee drinking protects against, or improves the prognosis of, liver diseases associated with hepatocellular carcinoma (Saab et al., 2014). A meta-analysis of coffee drinking and risk of hepatic fibrosis and cirrhosis included eight studies investigating cirrhosis, seven investigating advanced hepatic fibrosis, and one investigating both (Liu et al., 2015). Overall, 3034 coffee consumers and 132 076 non-consumers were studied in the investigations. The pooled odds ratio for hepatic cirrhosis in coffee consumers compared with non-consumers was 0.61 (95% CI, 0.45–0.84). For advanced fibrosis, the odds ratio was 0.73 (95% CI, 0.58–0.92). There were statistically significant inverse associations for both alcohol-associated cirrhosis and cirrhosis associated with hepatitis C. [The Working Group noted the heterogeneity in this meta-analysis.] Decaffeinated coffee does not appear to be associated with cirrhosis/liver fibrosis (Modi et al., 2010; Khalaf et al., 2015). [The Working Group noted the inadequate consideration of smoking in the paper by Khalaf et al. (2015), and the lack of control for smoking in the study by Modi et al. (2010).]

Coffee consumption may also be associated with lower severity of non-alcoholic fatty liver disease (NAFLD) (Chen et al., 2014a; Wadhawan & Anand, 2016). Decaffeinated coffee did not appear to have the same associations (Modi et al., 2010; Dickson et al., 2015; Khalaf et al., 2015). However, coffee consumption was not associated with the prevalence of ultrasound-diagnosed NAFLD (Zelber-Sagi et al., 2015). A meta-analysis of observational studies reported that caffeine consumption is not associated with the prevalence of NAFLD (Shen et al., 2016). However, caffeine is associated with a reduced severity of disease in affected patients (Molloy et al., 2012; Shen et al., 2016).

There are suggestions that coffee intake ameliorates the severity of chronic hepatitis C (Wadhawan & Anand, 2016). In cross-sectional studies of hepatitis C patients, coffee intake has been inversely associated with degree of fibrosis and other measures of liver injury (Liu et al., 2015). A cohort study showed that fibrosis in patients who drank coffee progressed less quickly than those who did not (Freedman et al., 2009); coffee-drinking patients also responded better to peginterferon and ribavirin therapy (Freedman et al., 2011). In a randomized open-label crossover trial, 40 patients with hepatitis C were randomized to either 4 cups/day of coffee for 1 month or abstinence. Coffee intake caused a reduction in plasma procollagen type III, a measure of fibrosis and collagen synthesis (Cardin et al., 2013). Inverse associations between caffeine intake and transaminase levels, fibrosis, and disease activity in hepatitis C patients have also been reported (Costentin et al., 2011; Khalaf et al., 2015).

4.4.2 Experimental systems

Most of the experimental animal studies on the effect of coffee and its ingredients on insulin resistance and insulin secretion were conducted in different mouse models of type 2 diabetes. Using spontaneously diabetic male KK-A^y mice, Yamauchi et al. (2010) demonstrated that ingestion of diluted black coffee as drinking-water (black coffee/water = 1:1 v/v) for 5 weeks improved insulin resistance. The similar effect of regular coffee, decaffeinated green coffee bean extract, and chlorogenic acid on improving insulin resistance have been reported in C57BL/6 mice (Rustenbeck et al., 2014; Song et al., 2014; Ma et al., 2015) and male Sprague-Dawley rats (Shearer et al., 2007) fed a high-fat diet. Coffee ingestion increased insulin sensitivity via the induction of Akt serine phosphorylation in liver and skeletal muscle (Kobayashi et al., 2012; Jia et al., 2014) and increasing insulin-receptor substrate-1 (IRS-1) tyrosine phosphorylation

(Jia et al., 2014). In contrast, Tan et al. (2012) reported that intragastrical administration of caffeine at 120 mg /kg bw per day to pregnant Wistar rats from gestational day 11 to 20 reduced the expression of insulin-like growth factor 1 receptor (IGF-1R) and IRS-1 in the fetal livers.

Potentiation of liver toxicity induced by carbon tetrachloride by intake of unfiltered coffee has been reported in Sprague-Dawley rats (Poyrazoglu et al., 2008). Another study found that coffee prevented liver toxicity in rats injected with lipopolysaccharide (Sakamoto et al., 2000), however. Both caffeinated and decaffeinated instant coffee protected rats against liver fibrosis after dimethylnitrosamine injection (Shin et al., 2010). Similar findings were reported for brewed coffee (but not instant coffee) in rats treated with diethylnitrosamine and carbon tetrachloride (Furtado et al., 2014). In a study where male Wistar rats were given an extract of Colombian coffee, the coffee-treated rats had lower liver weight, less portal fibrosis, and less collagen deposition than those given water (Panchal et al., 2012).

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