Carcinogenesis vol.28 no.8 pp.1800–1806, 2007
doi:10.1093/carcin/bgm145
Advance Access publication June 29, 2007

Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on *ex vivo* induced lymphocytic DNA damage in human volunteers

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Consumption of fruits and vegetables has been associated with a decrease in cancer incidence and cardiovascular disease, presumably caused by antioxidants. We designed a human intervention study to assess antioxidative and possible anti-genotoxic properties of fruit-borne antioxidants. We hypothesized that individuals bearing genetic polymorphisms for genes related to quercetin metabolism, benzo[*a*]pyrene metabolism, oxidative stress and DNA repair differ in their response to DNA protective effects of increased antioxidant intake. In the present study, 168 healthy volunteers consumed a blueberry/apple juice that provided 97 mg quercetin and 16 mg ascorbic acid a day. After a 4-week intervention period, plasma concentrations of quercetin and ascorbic acid and trolox equivalent antioxidant capacity (TEAC) were significantly increased. Further, we found 20% protection ($P < 0.01$) against *ex vivo* H_2O_2-provoked oxidative DNA damage, measured by comet assay. However, the level of *ex vivo* induced benzo[a]pyrene-diol-epoxide (BPDE)-DNA adducts was 28% increased upon intervention ($P < 0.01$). Statistical analysis of 34 biologically relevant genetic polymorphisms revealed that six significantly influenced the outcome of the intervention. Lymphocytes from individuals bearing variant genotype for *Cyp1B1*5* seemed to benefit more than wild-types from DNA damage-protecting effects upon intervention. Variants for *COMT* tended to benefit less or even experienced detrimental effects from intervention. With respect to *GSTT1*, the effect is ambiguous; variants respond better in terms of intervention-related increase in TEAC, but wild-types benefit more from its protecting effects against oxidative DNA damage. We conclude that genotyping for relevant polymorphisms enables selecting subgroups among the general population that benefit more of DNA damage-modulating effects of micronutrients.

Introduction

Numerous epidemiological studies have associated consumption of fruits and vegetables with decreased cancer incidence and cancer mortality rates (1,2); for instance, between the intake of flavonoids via fruits and vegetables and several other degenerative diseases like cardiovascular disease and ageing (3,4). Fruits and vegetables represent the most important source of dietary anticarcinogens (5), and animal studies have consistently shown antitumourigenic effects of commonly consumed vegetables (1). However, epidemiological evidence for this association has been weakened by some recent prospective studies (6–8).

Scavenging of reactive oxygen species and reactive carcinogen metabolites, as well as induction of phase II detoxification enzymes (9) may explain the suggested anticarcinogenic potential of fruits and vegetables. (10,11).

Oxidative stress leads to the induction of cellular oxidative damage, which may cause adverse modifications of DNA, proteins and lipids (12). *In vitro*, a flavonoid such as quercetin has already shown its ability of protecting human lymphocyte DNA against hydrogen peroxide treatment (13). Consequently, a decrease in oxidative damage upon flavonoid intervention may be interpreted as a plausible explanation for the protective effects of fruits and vegetables on cancer risk. Similarly, dietary antioxidants may modulate other biomarkers for cancer risk, such as aromatic DNA adducts formed by polycyclic aromatic hydrocarbons, which have been established as predictors of lung cancer risk (14). In earlier studies, we tested the efficacy of protecting lymphocyte DNA *in vitro* by quercetin and ascorbic acid before adding H_2O_2 or benzo[*a*]pyrene (B[*a*]P). Subsequently, in a pilot study on supplementation of healthy volunteers by administering a quercetin-rich blueberry/apple juice, we demonstrated that a 4-week intervention period is suitable for enhancing antioxidant defence, and that a quercetin plasma level was reached that reduced *ex vivo* induced DNA damage (15).

Genetic polymorphisms of genes involved in, for instance, biotransformation, DNA repair and oxidative stress are expected to influence the antioxidative and anti-genotoxic efficacy of intervention by micronutrients. Indeed, Mooney *et al.* (16) have shown that the association between smoking-adjusted plasma β-carotene levels and DNA damage appeared only significant in those subjects lacking the *GSTM1* detoxification gene. Moreover, Palli *et al.* (17) concluded that *GSTM1* nulls showed strong inverse associations between DNA adduct levels and vegetable intake.

We therefore hypothesized that within the general population, subgroups exist that based on genetic predisposition, benefit relatively more from chemoprevention by an antioxidant-rich diet. Correspondingly, the present large-scale intervention study has been carried out in order to assess prevention of *ex vivo* induced DNA damage by increased intake of fruit-borne antioxidants. Healthy volunteers consumed 1 l of blueberry/apple juice per day, leading to a dose of 16 mg ascorbic acid and 97 mg of quercetin bound to a sugar moiety that ensures biological availability. Quercetin concentration, ascorbic acid concentration and TEAC were monitored. Lymphocytes were treated *ex vivo* with H_2O_2 or B[*a*]P before and after the intervention period, and differences in induced single-strand breaks in DNA and BPDE-DNA adducts assessed. Subjects were genotyped for 34 relevant polymorphisms selected on the basis of their hypothesized interaction with applied biomarkers in blood.

Materials and methods

Study population

The study population consisted of 168 healthy volunteers, 114 female and 54 male, aged 18–45 years. Volunteers were recruited through advertisements in local newspapers. Volunteers were considered healthy based on self-experienced health status and were included if they met with the following basic requirements: non-smokers, no use of medication (except for oral contraceptives) and no use of vitamin supplementation at the moment of intervention. Further, if it was expected to interfere with the study, subjects were excluded from the study based on dietary habits, profession or other lifestyle factors, for instance excessive polycyclic aromatic hydrocarbon exposure at work or high antioxidant intake due to personal dietary habits. All this information was gathered through questionnaires. Subjects were fully informed about the details of the study and gave their written informed consent. The Medical Ethical

Abbreviations: B[*a*]P, benzo[*a*]pyrene; BPDE, benzo[*a*]pyrene-diol-epoxide; *COMT1*, catechol *O*-methyltransferase 1; PBS, phosphate-buffered saline; SNP, single-nucleotide polymorphism; TEAC, trolox equivalent antioxidant capacity.

Committee of Maastricht University and the Academic Hospital Maastricht approved the protocol. Demographic information of the study population is listed in Table I.

Washout period

As we were particularly interested in raising plasma flavonoid levels, prior to the intervention period, volunteers were given a list of food ingredients rich in flavonoids in general and quercetin in particular, which they had to avoid during a 5-day washout period. Items on this list were onions, apples, red wine, tea, biological and freshly pressed fruit juices, berries (e.g. blueberries and elderberries), grapes, cherries, raisins, parsley, broccoli, cabbage, beans and tomatoes (18). Next to avoiding food items on the list, subjects were asked to minimize the use of spices and herbs (19,20). The design of this intervention as well as the efficacy of the washout period was based on a pilot study, described earlier (15).

Intervention

In a paired design, each subject acted as his or her own control. As mentioned before, based on the results from our pilot study, it was concluded that best results were obtained after 4 weeks of intervention. The 5-day flavonoid washout period was followed by a 4-week intervention period with a custom-made blueberry/apple juice mixture, produced specifically for this study by Riedel Drinks (Riedel, Ede, The Netherlands). This mixture of which subjects consumed 1 l/day, contained ∼97 mg of quercetin per litre, most of it bound to a glucoside or a galactoside at the 3-position, which is known to facilitate its biological availability (21). The ascorbic acid content of the juice was 16 mg/l. The impact of seasonal variation in dietary habits or increased sensitivity was overcome by year-round random sampling.

Collection of samples

After the 5-day washout period, the first blood sample, before intervention, was drawn between 8 and 9 AM. Volunteers were allowed to have breakfast before sampling, but were not allowed to drink any fruit juice. After the 4-week intervention period, the second blood sample was drawn between 8 and 9 AM, and treated the following way. Again, breakfast but not any juice was allowed. Venous blood samples were drawn into one 10 ml ethylenediaminetetraacetic acid vacuum tube for plasma analyses and into two 10 ml vacuum lithium heparin tubes (venoject II, Terumo-Europe, Leuren, Belgium) for isolation of lymphocytes. The ethylenediaminetetraacetic acid tubes were kept at 4°C; subsequently, tubes were centrifuged for 10 min at 265*g* at 4°C to separate plasma. For analysis of ascorbic acid, plasma was deproteinized and stabilized using 10% of trichloroacetic acid (final concentration 5%). For TEAC and quercetin determination, aliquots were frozen without any treatment. All plasma samples were kept at −80°C until analysis. For isolation of lymphocytes, heparinized blood was diluted by adding phosphate-buffered saline (PBS, pH 7.4) and by layering this mixture over Lymphoprep™ (Axis-shield, Oslo, Norway) in a leucosep tube (Greiner Bio-one, Frickenhausen, Germany). Centrifuging for 20 min at 860*g* at room temperature separated lymphocytes, which were thereupon washed and re-suspended in PBS.

Analytical methods

Total quercetin concentration in plasma, defined as the sum of quercetin glucuronides and sulphates, was analysed by means of high-performance liquid chromatography with coulometric array detection after enzymatic hydrolysis, as described by De Boer *et al.* (14). The concentration of ascorbic acid was analysed by means of high-performance liquid chromatography as described earlier (22). Calibrators containing the same amount of trichloroacetic acid as the samples were prepared freshly. TEAC assay was used to determine the total antioxidant capacity in plasma of all volunteers, according to procedures as described by Fischer *et al.* (23).

Oxidative treatment of samples

Before and after intervention, quiescent peripheral blood lymphocytes were exposed *ex vivo* and in duplicate to oxidative stress. For this, immediately upon isolation, lymphocytes at a concentration of 1×10^6/ml PBS were exposed to 25 μM of H_2O_2 for 1 h at 37°C. Dose and duration had previously been established in a dose range-finding experiment (Wilms, L.C unpublished data). In all subjects, viability of lymphocytes turned out to be >95% after treatment (assessed by trypan blue exclusion). We used a slightly modified alkaline (pH > 12) comet assay (24–26), implemented according to the recent guidelines (25), to assess the level of single-strand breaks in DNA. After the incubation, cells were washed once in PBS (pH 7.4) and then taken up in PBS again at a cell concentration of 1×10^6/ml. The cell suspension was mixed with low melting point agarose (Sigma, St Louis, Mo) and positioned on 1.5% agarose-coated slides. Further procedures were as described earlier (15). Comets were stained with ethidium bromide (50 μl, 1 mg/ml) for fluorescence microscopy; per blindly scored slide, a total of 50 cells was scored using the software program Comet assay III (Perceptive Instruments Ltd, Suffolk, UK), background levels of single-strand breaks being corrected by subtracting the levels in non-exposed lymphocytes of each individual from that in H_2O_2-exposed lymphocytes of the same subject.

Table I. Description of study population: number of subjects per sex, their mean age and lifestyle

Sex	*N*	Age [mean (min;max)]	Lifestyle
Male	54	29 (18;45)	Healthy, non-smoking, no use of medication
Female	114	28 (18;45)	Healthy, non-smoking, no use of medication

B[a]P treatment of samples

Before and after intervention, quiescent peripheral blood lymphocytes were exposed *ex vivo* to the food carcinogen B[*a*]P in duplicate. Immediately after isolation, lymphocytes were taken up in RPMI 1640 at a concentration of two million cells/ml culture medium. Cells were exposed to 1 μM B[*a*]P in 0.5% dimethyl sulfoxide for 18h at 37°C. As was investigated before (15), this concentration of B[*a*]P was sufficient to produce well-detectable levels of adducts in quiescent lymphocytes for analysis by ^{32}P-post-labelling. Control samples were treated with 0.5% dimethyl sulfoxide. Both B[*a*]P and dimethyl sulfoxide were applied at non-toxic concentrations; viability of lymphocytes turned out to be >95% after treatment in all subjects (trypan blue exclusion). After incubation, cells were re-suspended carefully and centrifuged for 20 min at 860*g*. Cells were washed twice using PBS. The remaining lymphocyte pellet was kept at −20°C until DNA isolation. Phenol extraction was used to isolate DNA from lymphocytes. BPDE-DNA adducts were assessed by ^{32}P-post-labelling following the nuclease P1 enrichment technique as described by Reddy *et al.* (27) with some modifications (28). In all experiments, two BPDE-DNA standards with known adduct levels (1 adduct/10^7 and 1 adduct/10^8 nucleotides) were analysed in parallel for quantitation purposes. Quantitation was performed using Phosphor-Imaging technology (Fujifilm FLA-3000). BPDE-DNA adduct levels were corrected for the amount of DNA in the reaction, which was analysed by high-performance liquid chromatography–Ultraviolet analysis of an aliquot of the digested DNA.

Genotyping

DNA for genotyping was isolated from lymphocytes by standard phenol extraction procedures. All 168 volunteers were genotyped for a total of 34 single-nucleotide polymorphisms (SNPs). The SNPs were selected based on a known or an expected association with oxidative stress, biotransformation of quercetin and B[*a*]P, as well as DNA repair. The Cancer SNP 500 database was used to obtain DNA sequences and allele frequencies (http://snp500cancer.nci.nih.gov). SNPs were analysed using a multiplex polymerase chain reaction method developed by Knaapen *et al.* (29) and further developed by Ketelslegers *et al.* (30) who describes 17 of 34 SNPs analysed here. All analysed SNPs are located in the encoding region, which implies they are all functional.

Required group size

The experimental set-up described here was tested before in a pilot study supplementing eight healthy volunteers with a blueberry/apple juice. Information from this study, namely a reduction of oxidative DNA damage of 59% and a reduction in BPDE-DNA damage of 11%, in combination with the observed variation between subjects was used as input for a power calculation. The power calculation (power 0.9; confidence interval 95%) performed on data obtained in a pilot study revealed that a group size of 12–21 subjects would suffice in order to obtain statistically significant results (15).

Statistical analyses

Paired samples *t*-test was used to assess the efficacy of the intervention on the concentration of quercetin and ascorbic acid in plasma, on plasma TEAC, on the level of *ex vivo* induced oxidative DNA damage and on the level of *ex vivo* induced BPDE-DNA adducts. Multivariate, stepwise linear regression was used to assess the impact of sex, age and the different relevant polymorphisms on the efficacy of the intervention. Parameters rendering a statistically significant outcome of the stepwise linear regression were further analysed by paired samples *t*-test. Pearson's correlations were calculated between increase in ascorbic acid, quercetin and TEAC, respectively, versus *ex vivo* induction of oxidative DNA damage and B[*a*]P adduct level, respectively, corrected for the polymorphism involved. SPSS software (12.0.1 for Windows) was used for all statistical analyses of the data.

Results

SNPs

Table II lists all analysed SNPs, universal ID codes, the amino acid change related to the polymorphism, enzyme function as well as the expected effect of the polymorphism. The last three columns of this table represent the frequency of wild-types, heterozygous and variants of these polymorphisms in the current intervention population.

Effects on quercetin concentration

Four-week intervention with a blueberry/apple juice significantly increased mean (±SE) plasma quercetin levels from 28.8 nM (±1.05) to 79.2 nM (±5.14). Regression analysis showed that the level of increase significantly depended on *NQO1*2* ($P = 0.000$). Sex and age did not have any impact. As can be seen in Figure 1a, *NQO1*2* heterozygous subjects showed a significantly larger increase in plasma quercetin concentration than their wild-type counterparts; homozygous subjects were, however, not present in this particular population.

Effects on ascorbic acid concentration

The mean (±SE) plasma concentration of ascorbic acid was significantly increased by the intervention, from 58 μM (±1.19) to 61 μM (±1.07) ($P = 0.001$). Sex and age were no confounding factors for this parameter. The change in plasma concentration of ascorbic acid was strongly influenced by *Cat1* ($P = 0.006$; Figure 2). Carriers of the *Cat1* wild-type showed hardly any increase in ascorbic acid concentration (1.27 ± 1.13 μM), whereas heterozygous and variants show a large (6.80 ± 2.25 and 6.68 ± 2.26 μM, respectively) increase in AA concentration.

Effects on antioxidant capacity

The mean (±SE) TEAC value was significantly ($P = 0.000$) increased by the intervention from 781 μM (±3.95) to 800 μM (±4.02) trolox equivalent. Sex and age had no effect on the TEAC outcome. Increases of plasma TEAC levels appeared to be partly associated with observed increases in plasma quercetin levels. Stepwise regression indicates that only *GSTT1* ($P = 0.045$) was significantly involved in the effect of a 4-week intake of blueberry/apple juice on plasma TEAC: persons with the *GSTT1* deletion showed the largest increase in plasma antioxidative capacity (Figure 3).

Effects on oxidative DNA damage

Overall, paired analysis of background corrected samples revealed a significant ($P = 0.006$) protection of 20% against *ex vivo* induced oxidative DNA damage upon intervention by blueberry/apple juice intake. Before intervention, the mean tail moment was 9.9 [±0.51 (mean ± SE)]; after intervention, the mean tail moment was reduced to 8.0 [±0.52 (mean ± SE)]. The impact of all genetic polymorphisms, as well as sex and age, was assessed by stepwise regression analysis. *GSTT1* ($P = 0.004$), *XRCC1*4* ($P = 0.006$) and sex ($P = 0.018$) were significant predictors of the intervention effect on comet assay. Lymphocytes obtained from persons with *GSTT1* wild-type showed a larger intervention-related decrease in single-strand breaks than lymphocytes from those carrying the *GSTT1* deletion. However, this was not associated with intervention-related changes in plasma levels of quercetin and ascorbic acid, or plasma TEAC levels. Finally, men showed a larger protective effect upon blueberry/apple juice intervention than women.

Table II. List of SNPs, their position and the amino acid change that is related to the polymorphism

SNP	Amino acid change	dbSNP ID	Function	Effect on enzymatic function	Frequencies wt/hz/v[a]		
*CYP1A2*1F*		rs762551	Phase I bioactivation	Higher inducibility	86	69	13
*GSTM1*0*	Deletion		Phase II detoxification	No enzyme activity	90	—	78
*GSTP1*2*	I105V	rs947894	Phase II detoxification	Decreased enzyme activity	61	85	22
*GSTP1*3*	A114V	rs1799811	Phase II detoxification	Decreased enzyme activity	140	28	0
*GSTT1*0*	Deletion		Phase II detoxification	No enzyme activity	139	—	29
*NAT2*5*	I114T	rs1801280	Phase II detoxification	Decreased enzyme activity	66	76	26
*NAT2*6*	R197Q	rs1799930	Phase II detoxification	Decreased enzyme activity	84	67	17
*NAT2*7*	G286E	rs1799931	Phase II detoxification	Decreased enzyme activity	161	7	0
*XRCC1*2*	R194W	rs1799782	DNA repair	Increased enzyme activity	147	20	1
*XRCC1*3*	R280H	rs25489	DNA repair	Decreased enzyme activity	142	25	1
*XRCC1*4*	Q399R	rs25487	DNA repair	Decreased enzyme activity	66	77	25
*XRCC3*1*	T241M	rs861539	DNA repair	Decreased enzyme activity	71	71	26
*XPD*5*	K751Q	rs1052559	DNA repair	Decreased enzyme activity	27	85	56
*XPD*6*	R156	rs238406	DNA repair	Decreased enzyme activity	35	85	48
*OGG1*2*	S326C	rs1052133	DNA repair	Decreased enzyme activity	100	60	8
*BrCA2*1*		rs1799943	DNA repair	Decreased enzyme activity	102	55	11
*BrCA2*3*	N372H	rs144848	DNA repair	Decreased enzyme activity	90	57	21
*GPX1*1*	P198L	rs1050450	Oxidative stress	Decreased enzyme activity	71	80	17
*APEX1*1*	D148E	rs3136820	Oxidative stress	Decreased enzyme activity	48	87	33
*mEH*2*	Y113H	rs1051740	Phase II detoxification	Decreased enzyme activity	79	77	12
*mEH*3*	H139R	rs2234922	Phase I bioactivation	Increased enzyme activity	105	55	8
*CAT*1*		rs1001179	Oxidative stress	Decreased enzyme activity	104	48	16
*MnSOD2*1*	V16A	rs1799725	Oxidative stress	Decreased enzyme activity	46	81	41
*NQO1*2*	R139W	rs4986998	Phase II detoxification	Decreased enzyme activity	153	15	0
*NQO1*1*	P187S	rs1800566	Phase II detoxification	Decreased enzyme activity	95	67	6
*CYP1A1*2A*		rs5030838	Phase I bioactivation	Decreased enzyme activity	145	22	1
*CYP1A1*2C*	I462V	rs1048943	Phase I bioactivation	Decreased enzyme activity	159	8	1
*CYP1A1*4*	T461N	rs1799814	Phase I bioactivation	Decreased enzyme activity	160	8	0
*CYP1B1*5*	V432L	rs1056836	Phase I bioactivation	Decreased enzyme activity	55	83	30
*CYP1B1*7*	N453S	rs1800440	Phase I bioactivation	Decreased enzyme activity	115	46	7
*CYP2E1*5*		rs6413420	Phase I bioactivation	Decreased enzyme activity	151	16	1
*CYP3A4*1B*		rs2740574	Phase I bioactivation	Decreased enzyme activity	154	12	2
MPO		rs2333227	Oxidative stress	Decreased enzyme activity	94	64	10
*COMT*1*	V158M	rs4680	Phase II detoxification	Decreased enzyme activity	41	84	43

The SNPs were selected based on a known or an expected association with oxidative stress, biotransformation of quercetin and B[*a*]P, as well as DNA repair.
[a]wt = homozygous wild-type, hz = heterozygous, v = homozygous variant. The numbers reflect the numbers of subjects carrying that genotype; a hyphen indicates that the method was not able to distinguish between heterozygous or homozygous wild-type (in case of a deletion), therefore both of these polymorphisms are gathered under homozygous wild-type.

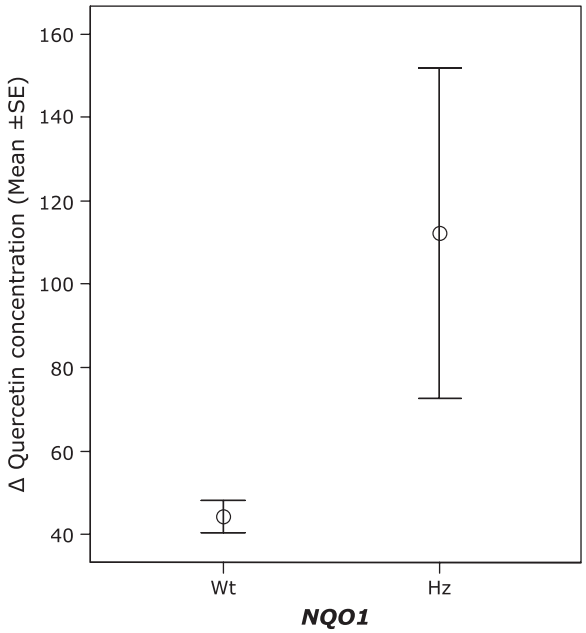

Fig. 1. Effect of *NQO1*2* polymorphism on the increase of plasma quercetin concentration upon intervention.

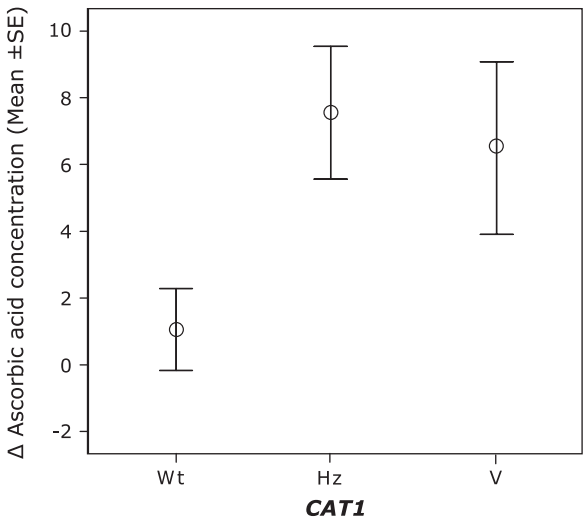

Fig. 2. Effect of CAT1 on the increase in plasma ascorbic acid concentration.

Effects on BPDE-DNA adduct level

Upon intervention, in the whole study population, levels of *ex vivo* induced BPDE-DNA were significantly increased from 1.48 (±0.07; mean ± SE) adducts per 10^7 normal nucleotides to 1.90 (±0.12; mean ± SE) adducts per 10^7 nucleotides. Sex and age did not influence the level of BPDE-DNA adducts. Stepwise regression revealed that *CYP1B1*5* ($P = 0.015$) and catechol *O*-methyltransferase 1 (*COMT1*) ($P = 0.038$) significantly predicted these increases in *ex vivo* induced adduct levels (Figure 5a and b, respectively). *Cyp1B1*5* wild-types showed the largest increase in B[*a*]P-induced DNA adducts whereas *Cyp1B1*5* variants showed intervention-related absence of *ex vivo* increased BPDE-DNA adduct level, however, without association with increases in plasma quercetin, ascorbic acid and TEAC levels. As for *COMT1*, levels of adducts after the intervention increased with the number of polymorphic alleles, meaning that *COMT1* variants show the largest increase in *ex vivo* induced BPDE-DNA adducts upon intervention. The difference in induced BPDE-DNA adduct

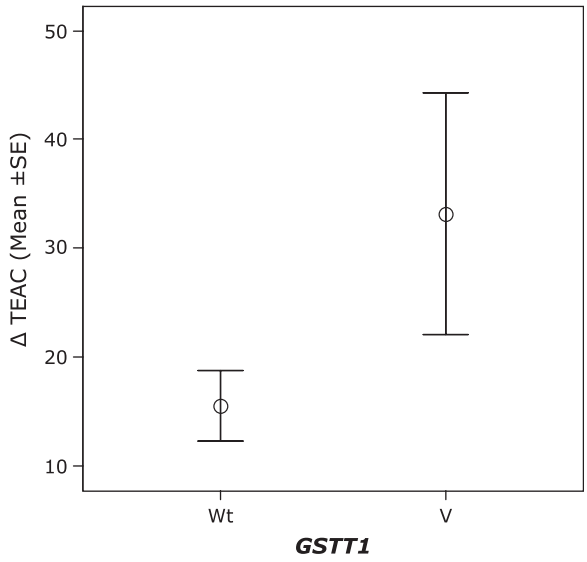

Fig. 3. Effect of *GSTT1* polymorphism on the increase of plasma TEAC upon intervention.

levels and the increase in plasma TEAC appear inversely correlated in *COMT1* wild-type subjects ($R = -0.317$; $P = 0.043$).

Discussion

Several *in vitro* studies have showed that quercetin and other flavonoids, as well as ascorbic acid, possess a strong antioxidant activity related to a strong scavenging capacity (31–38). Further, quercetin has been investigated *in vitro* for its anti-genotoxic effects (39–41), partly related to its antioxidative capacity. Although biologically relevant, *in vitro* models have limitations with respect to extrapolating results to the *in vivo* situation. In earlier studies, we have found that quercetin or ascorbic acid pretreatment of human lymphocytes *in vitro* before adding a stressor like H_2O_2 or B[*a*]P significantly reduced the level of single-strand breaks and BPDE-DNA adduct levels, respectively. Subsequently, we have assessed whether supplementation of healthy volunteers with a quercetin-rich fruit juice also results in prevention of *ex vivo* provoked DNA damage (22). From this pilot study, we concluded that a 4-week intervention period is suitable for enhancing antioxidant defence, as was supported by the increase in quercetin plasma concentrations, an increase in TEAC value and a decrease in H_2O_2-provoked DNA damage *ex vivo*. However, observed large inter-individual variations in this pilot study required a more in-depth investigation in a larger study population.

Aim of the present intervention study therefore was to assess effectiveness of chemoprevention of an increased intake of fruit-borne antioxidants, by evaluating effects on biomarkers of oxidative or genotoxic risk. For that purpose, lymphocytes were treated *ex vivo* with an oxidative stressor or a carcinogenic compound before and after a 4-week intervention period with a daily intake of quercetin-rich blueberry/apple juice. Since hypothetically, genetic variation may underlie the observed inter-individual variations in the DNA-protecting effect of an antioxidant intervention, a large set of genetic polymorphisms of genes involved in metabolism, DNA repair and oxidative stress was taken into account.

This blueberry/apple juice intervention significantly increased plasma quercetin concentration, ascorbic acid concentration and concomitantly, plasma antioxidant capacity. Neither increases in plasma ascorbic acid nor increases in plasma quercetin concentration appeared exclusively responsible for the increase of plasma TEAC. In contrast, in a blueberry and cranberry juice intervention study by Pedersen *et al.* (42), an increase of plasma antioxidant capacity was caused solely by the increase of plasma ascorbic acid concentration

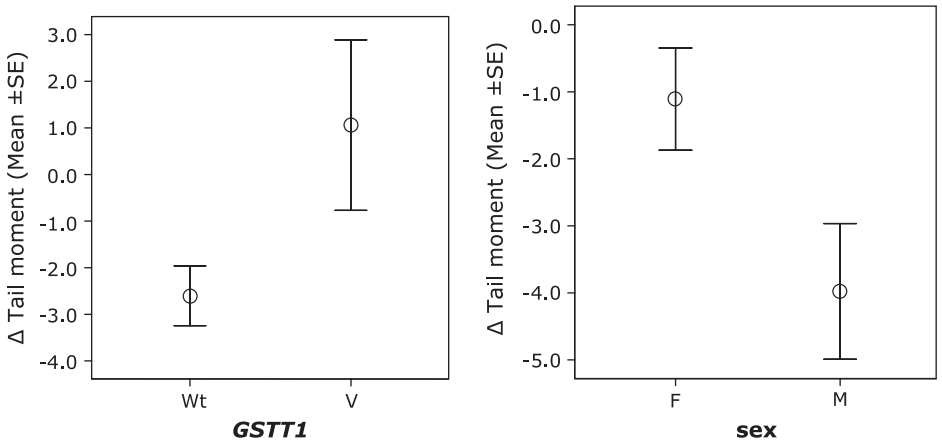

Fig. 4. Effects of *GSTT1* polymorphism and sex on the level of induced oxidative DNA damage in human lymphocytes.

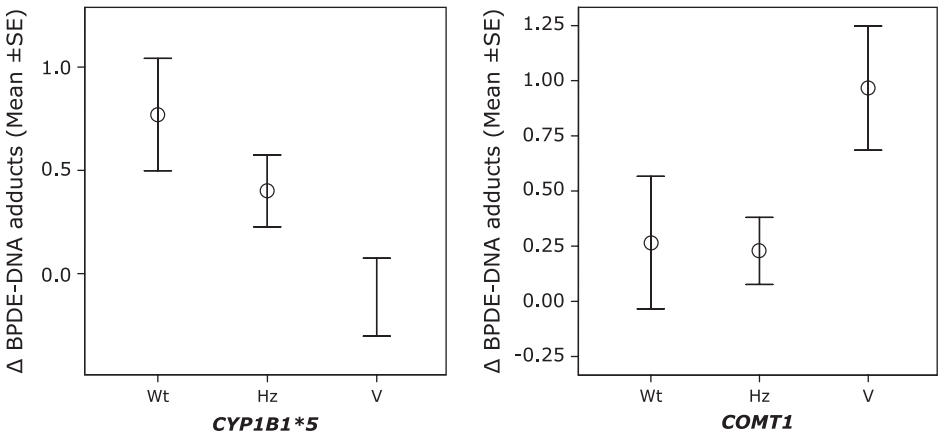

Fig. 5. Effect of *Cyp1B1*5* (a) and COMT1 (b) on the intervention related change in BPDE-DNA adduct level.

and not by increase of phenols. Obviously, the specific blueberry/apple juice mixture we applied may contain other, hitherto unidentified, antioxidants that contribute to the increase in TEAC.

With respect to protection by 4-week dietary intake of this fruit juice against *ex vivo* H_2O_2- or B[*a*]P-induced DNA damage, within the whole study population effects were modest and strongly biased by large inter-individual differences. Despite this, we did find a significant protection against H_2O_2-induced oxidative DNA damage. However, we also observed a significant increase in BPDE-DNA adducts induced *ex vivo* upon intervention. Upon classification of subjects according to genetic polymorphisms, of the 34 polymorphisms investigated in the present study, six appeared to influence the outcome of the intervention.

*NQO1*2* heterozygous subjects showed a larger increase in plasma quercetin levels by the intake of blueberry/apple juice than their wild-type counterparts. This may well be explained by the fact that the *NQO1*2* wild-types have a higher enzyme activity, and therefore probably higher metabolism of quercetin (43), which results in a lower end concentration of free quercetin in plasma. Further, quercetin has been associated with an induction of *NQO1* gene expression (44), resulting in an even more enhanced metabolism of quercetin.

As for *Cat1*, the increase of plasma ascorbic acid concentration increases with the number of polymorphic alleles. Wild-types show no increase, whereas heterozygous and homozygous variants show a rather large increase in plasma AA acid concentration. Catalase is an endogenous antioxidant enzyme and together with superoxide dismutase and glutathione peroxidase, it serves as primary defence against oxidative stress in the antioxidant network. It can be suggested that the lack of endogenous antioxidant defence is compensated by a more efficient uptake of exogenous ascorbic acid.

GSTT1 polymorphism had a significant impact on the effect of this fruit juice intervention on plasma TEAC. Human *GSTT1* encodes for a phase II enzyme involved in detoxification of various electrophilic compounds, and plays an important role in antioxidative defence. In our study, *GSTT1* variants showed the largest increase in plasma TEAC ($P = 0.045$). Since *GSTT1* deletion is associated with a reduced enzyme activity, it is expected to cause lower plasma TEAC as compared with *GSTT1* wild-type. Presumably, by taking in relatively larger amounts of exogenous antioxidants, *GSTT1* variants may possess a certain very effective mechanism to compensate for this loss of activity. Another explanation for this finding is that antioxidants contributing to TEAC value are more efficiently metabolized and excreted in *GSTT1* wild-type.

In contrast, it appeared to be *GSTT1* wild-type subjects who benefited most from antioxidant treatment with regard to protection against *ex vivo* induced oxidative DNA damage in their lymphocytes (Figure 4). It is suggested that absence of functional *GSTT1* shifts the oxidant/antioxidant balance towards more pro-oxidative conditions that may lead to oxidation of polyphenols and possibly other antioxidants taken in through the blueberry/apple juice. In this respect, it is of interest that *GSTT1*-null individuals are not more susceptible to oxidative damage in a non-challenged situation (45).

Our study showed no decrease but rather an increase in BPDE-DNA levels *ex vivo* induced in lymphocytes upon intervention in the whole study population. This corresponds to findings by Anderson *et al.* (46) who investigated several flavonoids for protective properties against food mutagens like 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine and 2-amino-3-methylimidazo (4,5-f) quinoline. At high doses, quercetin reduced genotoxic damage by these compounds

in both lymphocytes and sperm whereas at low doses, these flavonoids tended to have exacerbating effects, the mechanism responsible for this effect, however, is unknown. Plasma quercetin levels as achieved by our blueberry/apple juice intervention were within the nanomolar range, and therefore may have resulted in an increase in BPDE-DNA adducts. Effects on the amount of BPDE-DNA adducts can be explained by the inhibition of phase I enzymes, like CYP1A1, CYP1B1 and other CYPs, by quercetin (40,47,48). In our study, the effect of *CYP1B1*5* polymorphism on *ex vivo* formation of DNA adducts is obviously intervention related, since this polymorphism does not influence levels of BPDE-DNA adducts induced before intervention (data not shown). Variants who may be associated with a relatively lower CYP1B1*5 activity show lower levels of BPDE-DNA adduct formation *ex vivo* upon intervention, possibly due to inhibition by quercetin of the remaining enzyme activity.

Furthermore, we have found that *COMT1* variants demonstrated the most profound increase in *ex vivo* induced BPDE-DNA adduct levels. This is probably due to the lower activity of that is inherent to the variant genotype (49). COMT is a phase II enzyme that is involved in eliminating B[*a*]P metabolites (50), and has been shown to be inhibited by tea catechins (51). None of the *COMT1* genotypes are associated with intervention-related protection against adduct formation, but in *COMT1* wild-type subjects, there only is a mild increase in induced DNA adducts in lymphocytes which is inversely correlated with increases in plasma TEAC. This seems to imply that the increase in antioxidant status lowers the intervention-related increase in BPDE-DNA adducts. A remarkable finding is that an increase in plasma TEAC, clearly indicating an antioxidant effect, is not correlated with a decrease in oxidative DNA damage in either genotype. Taking several genotypes into account by grouping individuals by calculating the sum of relevant risk alleles did not further establish intervention effects.

In line with previous findings (52–56), we confirmed absence of antioxidative effects by micronutrients on baseline DNA damage; quercetin appeared to only exert its protective effects upon oxidative challenge *ex vivo*. Moller *et al.* (57) also argued that dietary interventions with antioxidants showed higher beneficial outcomes in oxidatively stressed subjects like diabetics or HIV-infected patients.

Supplementation with ascorbic acid turned out to be effective in oxidatively challenged lymphocytes. Duthie *et al.* (58) have investigated oxidative DNA damage in smokers and non-smokers by means of comet assay. A 20-week supplementation with vitamin C (100 mg/day), vitamin E (280 mg/day) and β-carotene (25 mg/day), resulted in a significant decrease in endogenous oxidative damage. Further, lymphocytes of these treated subjects showed an increased resistance *in vitro* to H_2O_2-provoked oxidative DNA damage. Mooney *et al.* (59) investigated the impact of vitamin C and vitamin E supplementation for >12 months in smokers. Their study revealed that smoking women, not men, showed a significant 31% decrease in BPDE-DNA adducts in leukocytes upon vitamin C/vitamin E intervention. An interesting finding was that this decrease was even more pronounced (43%) in women carrying the *GSTM1*-null genotype.

Altogether, in this intervention study, we demonstrated that 4-week intake of a quercetin-rich blueberry/apple juice was sufficient to increase plasma concentrations of quercetin, ascorbic acid and other unidentified antioxidants. Furthermore, the antioxidative capacity of plasma increased significantly, as reflected by the increased TEAC value. These results show that the blueberry/apple intervention induced antioxidative defence mechanisms. Despite the fact that plasma quercetin concentrations did not reach the level at which quercetin is known to exert protective effects *in vitro* (15), protective effects were clearly observed in H_2O_2-exposed lymphocytes following intervention. In contrast, *ex vivo* induced levels of BPDE-DNA adducts in B[*a*]P-exposed lymphocytes increased upon intervention suggesting increased risk of blueberry/apple juice intake. Overall, out of 168 subjects, 139 *GSTT1* wild-types (83%) and 30 *CYP1B1*5* variants (18%) may benefit most from increased fruit-borne antioxidant intake with respect to reducing risks of DNA damage, whereas for 43 carriers of the *COMT1* variants (26%), risks may be elevated. Evaluation of the role of genetic polymorphisms thus appears to provide a helpful tool in assessing susceptible groups and groups that benefit from specific dietary interventions. However, more integrated approaches are required for definitely assessing health costs and benefits of micronutrients.

Funding

The Netherlands Organisation for Health Research and Development. Nutrition: Health, Safety and Sustainability program. Grant number 01412012.

Acknowledgements

We would like to thank Riedel Drinks for providing the fruit drink.

Conflicts of Interest Statement: None declared.

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Received February 1, 2007; revised May 30, 2007; accepted June 13, 2007