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Effect of Consumption of Organically and Conventionally Produced Apples on Antioxidant Activity and DNA Damage in Humans

KARLIS BRIVIBA,*,† BERENIKE A. STRACKE,† CORINNA E. RÜFER,† BERNHARD WATZL,† FRANCO P. WEIBEL,‡ AND ACHIM BUB†

Institute of Nutritional Physiology, Federal Research Centre for Nutrition and Food, Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany, and Research Institute of Organic Agriculture, Frick, Switzerland

The present study was performed to compare the effects on antioxidant activity and on DNA damage of organic and conventionally produced apples grown under controlled conditions in human peripheral blood lymphocytes. Six healthy volunteers consumed either organically or conventionally grown apples (Golden Delicious, 1000 g) from two neighboring commercial farms in a double-blinded, randomized, cross-over study. The average content of total identified and quantified polyphenols in the organically and conventionally produced apples was 308 and 321 μg/g fresh weight, respectively. No statistically significant differences in the sum of phenolic compounds or in either of the polyphenol classes were found between the agricultural methods. Consumption of neither organically nor conventionally grown apples caused any changes in antioxidant capacity of low-density lipoproteins (lag time test), endogenous DNA strand breaks, Fpg protein-sensitive sites, or capacity to protect DNA against damage caused by hydrogen peroxide. However, a statistically significant decrease in the levels of endonuclease III sensitive sites and an increased capacity to protect DNA against damage induced by iron chloride were determined 24 h after consumption in both groups of either organic or conventionally grown apples, indicating the similar antigenotoxic potential of both organically and conventionally grown apples.

KEYWORDS: Apple; organic; conventional agricultural methods; antioxidant activity; DNA damage; lymphocytes

INTRODUCTION

Epidemiological and experimental data suggest that an increased intake of fruit and vegetables can reduce the risk of cancer (*1*). It has been hypothesized that organically grown fruit and vegetables are potentially healthier due to higher amounts of protective phytochemicals and lower levels of pesticides and fertilizers. Plant phytochemicals such as polyphenols are of special interest due to their wide-ranging biological activities, for example, antioxidant activity. In in vitro experiments, phenolic compounds have been shown to inactivate reactive oxygen and nitrogen species, which are able to generate DNA strand breaks and to oxidize DNA bases that can lead to the formation of mutations, hyperproliferation, and cancer (*2–4*).

The data on the content of phenolic compounds in fruits and vegetables grown organically vs conventionally are not consistent. A higher content was found in organically grown apples, some strawberry cultivars, marionberries, and pac choi (*5–8*). No consistent trend was found for the higher concentrations of phenolic compounds in plums, strawberries, and black currants grown organically vs conventionally (*8–11*). A lower content was found in organically grown broccoli (*12*). These data indicate that the organic production method alone does not necessarily increase the biosynthesis of phenolic compounds in the plants, but depending on cultivar, organic production provides an increased opportunity for environmental conditions to affect the levels of phenolic compounds in plants (*8*).

Several human intervention studies with organic and conventionally produced foods have been carried out. As in the case of the data on the content of phenolic compounds in fruits and vegetables, the effects in human intervention studies are not consistent. A study investigating the effect of conventionally and organically produced foods including meat, potato, wheat, and rye, and a number of fruits and vegetables on markers of antioxidant defense showed that although the organic diet caused higher urinary excretion of two polyphenols, quercetin and kaemferol, this diet was only one that led to an increased protein oxidation and decreased plasma antioxidant capacity (*13*). Organically produced tomatoes contained more polyphenols, carotenoids, and vitamin C than the conventionally produced

* To whom correspondence should be addressed. Tel: +49-721-6625-407. Fax: +49-721-6625-404. E-mail: karlis.briviba@bfel.de.
† Federal Research Centre for Nutrition and Food.
‡ Research Institute of Organic Agriculture.

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tomatoes (*14*). However, the consumption of organic tomatoes did not lead to a higher plasma concentration of these antioxidants than the consumption of conventionally produced tomatoes (*14*). A conventionally produced red wine exhibited a polyphenol content about two-fold higher than an organic one. The consumption of the conventional but not of the organic red wine led to a decrease in copper-induced formation of thiobarbituric acid reactive substances, while the organic but not the conventional red wine caused an increase in catalase activity in erythrocytes (*15*). These inconsistencies indicate that agricultural practices can change the chemical composition of food, but modulation of physiological processes in humans cannot be predicted by the chemical analysis of food only. Physiological processes such as absorption, metabolism, excretion, and others can modulate the expected linear relationship between dose and effect.

Epidemicological studies have linked apple consumption with a reduced risk for cancer (*16, 17*). Apples are rich in biologically active compounds including polyphenols and are a major source of flavonoids in the Western diet (*18, 19*). Depending on cultivar, apples contain about 2 g polyphenols/kg fresh weight. Apple polyphenols were shown to be effective antioxidants in in vitro experiments (*20*). In animal studies, cloudy apple juice inhibited DNA damage and colon carcinogenesis induced by 1,2-dimethylhydrazin (*21, 22*). However, apple consumption induced an increase in antioxidant capacity in human plasma measured by the FRAP antioxidant assay, not because of a high flavonoid content but because of increases in the plasma urate concentrations caused by the metabolic effect of apple fructose (*23*).

It is not known whether apples from different production systems differentially affect markers of carcinogenesis. Here, we compare the levels of phenolic compounds in organic and conventionally produced apples from controlled production and their effects on oxidative stress and DNA damage in humans.

MATERIALS AND METHODS

Chemicals. Chlorogenic acid, phloretin 2′-xyloglucoside, phloretin 2′-glucoside, catechin, and epicatechin were purchased from Fluka (Taufkirchen, Germany), and procyanidin B_1 and B_2 were purchased from LGC Promochem GmbH (Wesel, Germany). Quercetin and quercetin-glycosides were obtained from Roth (Karlsruhe, Germany). Unless otherwise stated, all chemicals were purchased from Merck (Darmstadt, Germany).

Apple Production. Organic and conventional apples of the Golden Delicious cultivar originated from an existing network for system comparison studies that includes 10 commercial farms (five comparison pairs) in Switzerland. (*24*) At five locations in all important apple-producing areas of Switzerland, there are neighboring commercial orchards with certified organic and conventional productions, respectively. The apples used in the present study originated from one of the five neighboring commercial farm pairs.

The organically and conventionally grown apples were cultivated according to the requirements of "Bio Suisse" (predominant label organization for certified organic production in Switzerland) and "Suisse Garantie" (predominant label organization for integrated production in Switzerland), respectively. Orchard, soil, and microclimate conditions per farm pair were comparable. Measures for plant protection, fertilization, and crop load regulation corresponded to the usual production practice and were recorded in detail. The fruits were harvested according to a strict protocol by scientists of the Research Institute of Organic Agriculture (FiBL). Per orchard, approximately 50 kg or 350 fruits were sampled randomly at the first picking passage and at optimal maturity. Per tree, two fruits were sampled from the middle canopy zone. The fruits were checked to be without wounds and to fulfill table fruit quality. The sampled fruits of all orchards were immediately transported to the cold-storage room at FiBL, where they remained stored under normal atmosphere at 2 °C and 93% relative humidity.

Apple Sample Preparation for High-Performance Liquid Chromatography (HPLC) Analysis. From 50 apples, eight fruits were randomly chosen from each cultivar. Apples were cut into quarters, eliminating seeds and core, and samples were homogenized using a blender. Three to four grams were extracted with 10 mL of methanol in an ultrasonic bath for 1 h at 50 °C. After centrifugation [3000*g* for 10 min in a CR422 centrifuge (Thermo Electron Co, Fernwald, Germany)], the supernatant was filtered through a filter paper (no. 595, Schleicher & Schuell, Dassel, Germany) using a funnel to obtain a clear solution. The residue was washed three times with 5 mL of methanol and again filtered, and the organic extracts were combined. Methanol was evaporated at 30 °C using a rotavapor, and the aqueous residue was filled up to 5 mL with distilled water for HPLC analysis.

HPLC Analysis of Phenolic Compounds in Apples. Analyses were performed on a high-pressure gradient system from Shimadzu (Duisburg, Germany) equipped with an autoinjector, a photodiode array detector, and a fluorescence detector. Separation was carried out on a Prontosil (250 mm × 4.6 mm i.d., particle size 3 μm) reversed-phase column (Bischoff, Leonberg, Germany). The solvent system consisted of 0.1% formic acid in water (pH 3) (A) and acetonitrile (B) with the following linear gradient: from 15 to 30% B in 50 min, from 30 to 50% in 10 min, and from 50 to 56.5% in 20 min. For analysis of the flavanols (+)-catechin and (–)-epicatechin, elution was effected using a linear gradient with 15% B for 25 min, from 15 to 30% B in 25 min, from 30 to 50% B in 10 min, and from 50 to 10% in 5 min. The flow rate was 0.8 mL/min, and the injection volume was 50 μL. The eluent was recorded with a diode array detector at 280 nm for quantification of the flavanols procyanidin B_1 and B_2, at 290 nm for the dihydrochalcones, at 320 nm for the hydroxycinnamates, and at 350 nm for the flavonols. Observed peaks were scanned between 190 and 500 nm. The fluorescence detector settings for quantification of (+)-catechin and (–)-epicatechin were an excitation wavelength of 280 nm and an emission wavelength of 320 nm.

HPLC/MS analysis was performed on a HP 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with an autoinjector, quaternary HPLC pump, column heater, UV detector, and HP Chem Station for data collection and handling. The HPLC was interfaced to an HP series 1100 mass selective detector equipped with an atmospheric pressure ionization–electrospray (API-ES) chamber. For the analysis of the apple polyphenols, conditions in the positive mode were the following: capillary voltage, 3.5 kV; fragmentor voltage, 150 V; nebulizing pressure, 50 psi; drying gas temperature, 350 °C; and drying gas flow, 12.5 L/min. Data were collected using both the scan and the selected ion monitoring mode. Spectra were scanned over a mass range of m/z 100–600 at 0.98 s per cycle. For LC, the same conditions as described were used.

Quantification was performed according to an external standard method using the commercial available reference compounds. Quantification of those polyphenols, which are not commercially available, was based on a representative standard of the same polyphenol class. The concentrations of the coumaroyl derivates and 4-caffeoylquinic acid were calculated using chlorogenic acid and of the quercetin glycosides using quercetin. The UV–vis absorptive spectra show that this is a reasonable assumption since the effect of conjugation on the UV–vis spectra is of minor importance. The identification of the formed metabolites has been elucidated using HPLC/diode array detection, HPLC/API-ES MS, and references compounds.

Study Design. A double-blind, randomized, cross-over study design was used. On the basis of a physical examination and medical history, six nonsmoking male volunteers were included in the study and randomly assigned to two groups. The mean age of participants was 27 years [standard deviation (SD) = 3], the mean height was 181 cm (SD = 8), the mean body mass was 75 kg (SD = 9), and the mean BMI was 23 kg/m^2 (SD = 3). The participants were instructed to avoid food high in polyphenols within 3 days before and during intervention. On two experimental days, each volunteer consumed after an overnight fast 1000 g of either organically or conventionally produced apples

Table 1. Concentrations of Phenolic Compounds and Relative Fraction Percentage of Each Polyphenol Class in Organically and Conventionally Produced Golden Delicious[a]

	organically produced ($n = 8$)		conventionally produced ($n = 8$)	
phenolic compounds	μg/g fw	relative weight percent	μg/g fw	relative weight percent
chlorogenic acid	104.2 ± 25.5 (72.6 − 149.8)	33.8	96.7 ± 10.8 (85.4 − 119.5)	30.1
hydroxycinnamates	132.5 ± 28.9 (98.3 − 156.2)	43.0	128.3 ± 13.8 (111.0 − 132.6)	39.9
flavanols	120.8 ± 30.5 (72.2 − 174.6)	39.3	122.2 ± 25.5 (88.2 − 166.7)	38.1
dihydrochalcones	25.3 ± 5.2 (19.57 − 31.6)	8.2	24.3 ± 5.1 (17.1 − 33.4)	7.6
flavonols	29.2 ± 10.5 (18.5 − 50.6)	9.5	46.3 ± 27.7 (16.1 − 106.9)	14.4
sum of phenolic compounds	307.9 ± 70.6 (225.1 − 440.8)	100.0	320.9 ± 66.5 (243.8 − 455.1)	100.0

[a] Chlorogenic acid is listed in an extra row and is taken into account in the class of the hydroxycinnamates. The sum of phenolic compounds is calculated as the sum of the single phenolic acids, dihydrochalcones, and flavonoids. Data are means ± SD. In parentheses, the minimum and maximum values are shown.

Table 2. Serum Parameters before and after Consumption of Either Conventionally or Organically Grown Apples[a]

	baseline	1 h	2 h	3 h	4.5 h	6 h	9 h	12 h	24 h
					glucose (mmol/L)				
conventional	4.50 ± 0.49	4.60 ± 1.28	4.94 ± 0.91	4.87 ± 0.82	4.69 ± 0.45	4.51 ± 0.38	5.04 ± 0.71	4.65 ± 0.63	4.70 ± 0.53
organic	4.71 ± 0.23	4.80 ± 0.54	4.96 ± 0.42	5.02 ± 0.34	4.67 ± 0.30	4.51 ± 0.22	4.65 ± 0.52	4.76 ± 0.67	4.58 ± 0.44
					triacylglycerol (mmol/L)				
conventional	1.10 ± 0.34	0.96 ± 0.28	0.88 ± 0.26	0.92 ± 0.37	1.06 ± 0.40	1.07 ± 0.32	1.31 ± 0.28	1.24 ± 0.14	1.13 ± 0.23
organic	1.10 ± 0.20	1.00 ± 0.20	0.96 ± 0.19	0.97 ± 0.25	1.12 ± 0.46	1.28 ± 0.48	1.49 ± 0.39[b]	1.33 ± 0.25	1.19 ± 0.28
					uric acid (mmol/L)				
conventional	0.37 ± 0.11	0.41 ± 0.12[b]	0.40 ± 0.11	0.39 ± 0.11	0.38 ± 0.11	0.38 ± 0.11	0.40 ± 0.11	0.39 ± 0.10	0.38 ± 0.10
organic	0.38 ± 0.11	0.41 ± 0.12	0.41 ± 0.12	0.39 ± 0.11	0.39 ± 0.12	0.38 ± 0.11	0.40 ± 0.10	0.40 ± 0.11	0.39 ± 0.12

[a] Values are means ± SD ($n = 6$). [b] Within group treatment effects; significantly different from baseline (repeated measures ANOVA, Dunnett posthoc test; $p < 0.05$). There were no significant differences between groups (conventional vs organic; ANOVA Tukey–Kramer posthoc test).

without seeds and core along with one white roll. There was a washout period of 1 week between each experimental day. The study was approved by the ethical committee of the Landesärztekammer Baden-Württemberg, and all participants gave written consent.

Blood Samples. Fasting venous blood samples were collected in EDTA containing tubes (Sarstedt-Monovette, Nümbrecht, Germany) before consumption of apples and then after 1, 2, 3, 4.5, 6, 9, 12, and 24 h. The samples were immediately placed on ice. Plasma was collected after centrifugation at 1500*g* for 10 min at 4 °C. For serum measurements, blood was collected in a "Serum-Z-Monovette" (Sarstedt-Monovette) and after complete clotting separated by centrifugation at 1500*g* for 10 min at room temperature. Plasma and serum were stored at −80 °C until analysis.

Low-Density Lipoprotein (LDL) Oxidation and Analysis of Plasma (Serum) Parameters. An ultracentrifugation method was used to isolate LDL from plasma (*25*). The ex vivo oxidation of isolated LDL was performed as described earlier (*26*). Serum glucose, triacylglycerol, and uric acid were determined by enzymatic kits (Boehringer Mannheim, Germany).

Single Cell Microgel Electrophoresis Assay (Comet Assay). DNA damage was measured by the single cell microgel electrophoresis assay (comet assay) before consumption of apples and after 4.5 and 24 h. Lymphocytes were isolated by density gradient centrifugation using Lymphoprep (Life Sciences, Eggenstein-Leopoldshafen, Germany). To investigate the capacity to protect DNA against damage caused by hydrogen peroxide or iron chloride, 2×10^5 cells were treated with 100 μM H_2O_2 or 1 mM $FeCl_3$ in PBS for 1 h at 37 °C. Control cells were incubated in PBS containing no additives. Twenty microliters of lymphocyte suspension (2×10^5 cells) was mixed with 75 μL of low melting point agarose and was placed between two layers of agarose on a microscope slide. Three slides/parameter from each volunteer were placed in lysis buffer (100 mM Na_2EDTA, 1% Triton X 100, 2.5 mM NaCl, 1% lauroyl sarcosine sodium salt, 10 mM Tris, and 10% DMSO) for 1 h. After lysis, the slides for detection of Endonuclease III (Endo III) or formamidopyrimidine-DNA glycosylase (Fpg)-sensitive sites were washed three times with enzyme treatment buffer (40 mM HEPES, 0.1 mM KCl, 0.5 mM EDTA, and 0.2 g/L BSA, pH 8.0), 50 μL of the Endo III (50 U/mL diluted in enzyme treatment buffer), or Fpg protein solution (8 U/mL diluted in enzyme treatment buffer) and were placed on the slides and incubated at 37 °C for 30 min. Then, slides were placed in an electrophoresis chamber containing alkaline buffer (1 mM Na_2EDTA and 300 mM NaOH) for 20 min to allow DNA unwinding. Electrophoresis was carried out at 25 V and 300 mA for 40 min.

Fifty cells of each slide (150 cells per subject) were analyzed using the imaging software of Perceptive Instruments (Halstead, United Kingdom). The amount of damaged DNA was expressed as the percentage of DNA in the tail (tail intensity).

Statistical Methods. Data are presented as means ± SD. All statistical calculations were performed with the StatView program (SAS Institute 1998, Cary, NC). Statistical analysis of the polyphenol content in organically and conventionally produced apples was performed by unpaired Student's *t* test. Repeated measures analysis of the variance (ANOVA) were used to evaluate time-dependent changes in blood parameters. If the repeated measures ANOVA was significant ($P < 0.05$), comparisons between baseline and treatment were made using the Dunnett's posthoc test.

RESULTS

HPLC Analysis of Phenolic Compounds in Apples. The average content of total polyphenols in the organically and conventionally produced apples was 307.9 and 320.9 μg/g fresh weight, respectively. There were no statistically significant differences between the agricultural methods with respect to the sum of phenolic compounds (**Table 1**). Hydroxycinnamates (chlorogenic acid and other caffeoyl quinic acid as well as coumaroyl quinic acid derivatives), which are the major class of apple polyphenols, accounted for up to 43 and 39.9%, respectively, of the polyphenolic compounds in apples (**Table 1**). Chlorogenic acid represents the main compound of all quantified polyphenols with 104.2 and 96.7 μg/g fresh weight, corresponding to 33.8 and 30.1% of the quantified polyphenols. Significant concentrations of flavanols [(+)-catechin and (−)-epicatechin as well as procyanidins B_1 and B_2] were also found with (−)-epicatechin and procyanidin B_2 being the major compounds. Further classes of polyphenols in apples are the flavonols (quercetin-glycosides) and the dihydrochalcones (phloretin-glycosides). As depicted in **Table 1**, those classes represent

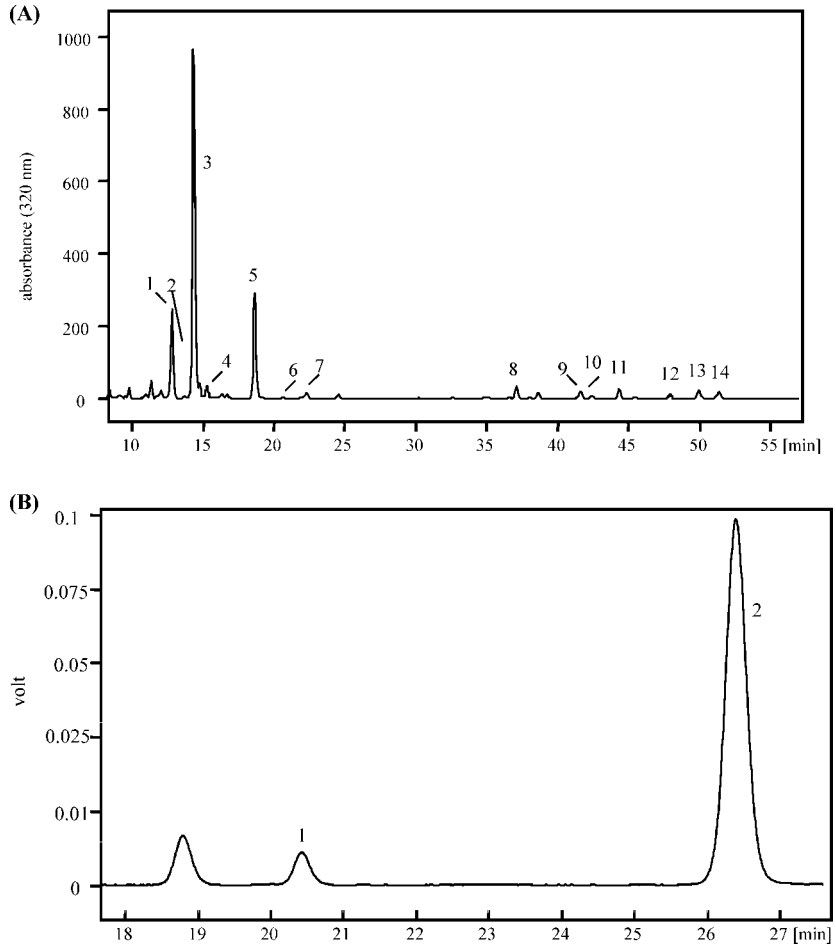

Figure 1. Representative HPLC chromatograms of apples (Golden Delicious) (**A**) absorbance at 320 nm. The numbered peaks are as follows: 1, 4-caffeoylquinic acid; 2, procyanidin B1, 3, chlorogenic acid; 4, 3-coumaroylquinic acid; 5, 4- coumaroylquinic acid; 6, procyanidin B2; 7, 5-coumaroylquinic acid; 8, phloretin 2′-xyloglucoside; 9, quercetin 3-galactoside; 10, quercetin 3-glucoside; 11, phloretin 2′-glucoside; 12, quercetin 3-xyloside; 13, quercetin 3-arabinoside; and 14, quercetin 3-rhamnoside. (**B**) Fluorescence detection at wavelength 280 (excitation) and 320 nm (emission). The numbered peaks are 1, catechin; and 2, epicatechin.

Table 3. Ex Vivo LDL Oxidation before and after Consumption of Either Conventionally or Organically Grown Apples

	before	1 h	2 h	3 h	4.5 h	6 h	9 h	12 h	24 h
					initial CD (A_{234nm})				
conventional	0.65 ± 0.05	0.63 ± 0.04	0.62 ± 0.04	0.61 ± 0.02	0.60 ± 0.03	0.60 ± 0.03	0.63 ± 0.06	0.61 ± 0.04	0.69 ± 0.03
organic	0.70 ± 0.06	0.65 ± 0.05	0.68 ± 0.03	0.67 ± 0.03	0.67 ± 0.04	0.64 ± 0.03	0.70 ± 0.03	0.66 ± 0.06	0.65 ± 0.04
					change from initial CD (ΔA_{234nm})				
conventional	0.30 ± 0.11	0.31 ± 0.11	0.28 ± 0.12	0.30 ± 0.12	0.27 ± 0.09	0.29 ± 0.08	0.33 ± 0.10	0.27 ± 0.13	0.31 ± 0.17
organic	0.34 ± 0.19	0.33 ± 0.07	0.29 ± 0.12	0.41 ± 0.08	0.35 ± 0.14	0.39 ± 0.06	0.39 ± 0.16	0.36 ± 0.09	0.45 ± 0.14

between 7.6 and 14.4% of the total polyphenols. No statistically significant differences in any of the polyphenol classes were observed. Representative HPLC chromatograms of the polyphenols identification (apples; Golden Delicious) are shown in **Figure 1A**,**B**.

Intervention, Serum Parameters and Antioxidant Capacity. All six participants completed the study and tolerated the intervention with apples well. None of them reported any side effects during the entire study period. No study participant had to be excluded due to health problems or incompliance for other reasons. Intervention did not affect the plasma glucose concentration (**Table 2**). There was an increase in the plasma triacylglycerol concentration about 3 h after dinner (9 h after apple consumption). In the organic apples group, this increase was statistically significant. Apples are rich in fructose, the consumption of which can cause increased production of uric acid due to the increased degradation of AMP. Uric acid plasma concentrations peaked 1 h after apple consumption (**Table 2**). A statistically significant increase was observed in the conventionally produced apple group. Intervention had no effect on ex vivo LDL oxidation (**Table 3**).

DNA Damage in Peripheral Blood Lymphocytes. There were no statistically significant differences between groups in the baseline levels of endogenous DNA damage (stand breaks and endonuclease III- and Fpg protein-sensitive sites) or in the capacity to protect DNA against damage caused by hydrogen peroxide or iron chloride (**Table 4**). The consumption of apples grown by both agricultural methods did not affect any parameter of DNA damage after 4.5 h, while 24 h after consumption, a statistically significant decrease in the level of Endo III-sensitive sites (specific for oxidized pyrimidines) and an increased capacity to protect DNA against damage induced by iron chloride was determined. There were no statistically significant

Table 4. Effect of Apple Consumption on Endogenous DNA Damage and Capacity To Protect DNA against Strand Breaks Caused by H_2O_2 or $FeCl_3$ in Peripheral Blood Lymphocytes[a]

	organic ($n = 6$)	conventional ($n = 6$)
endogenous DNA strand breaks (TI %)		
baseline	2.70 ± 0.86	2.38 ± 1.76
4.5 h	2.53 ± 0.39	2.73 ± 1.50
24 h	2.41 ± 0.84	2.70 ± 1.88
endogenous oxidative DNA damage		
Endo III sites (specific for oxidized pyrimidines)		
baseline	3.69 ± 2.61	4.60 ± 1.23
4.5 h	2.97 ± 1.19	3.05 ± 1.87
24 h	1.10 ± 1.20[b]	1.19 ± 0.73[b]
Fpg sites (specific for oxidized purines such as 8-oxoguanosine)		
baseline	1.65 ± 1.51	2.04 ± 1.38
4.5 h	1.37 ± 1.55	1.53 ± 1.75
24 h	1.73 ± 1.53	1.03 ± 1.38
antioxidant capacity of lymphocytes to protect DNA		
H_2O_2-induced strand breaks		
baseline	46.72 ± 4.81	43.40 ± 8.04
4.5 h	45.55 ± 3.18	43.49 ± 3.59
24 h	41.75 ± 4.53	41.35 ± 3.01
$FeCl_3$-induced strand breaks		
baseline	9.38 ± 2.89	9.23 ± 3.05
4.5 h	6.92 ± 1.18	7.42 ± 2.67
24 h	5.12 ± 1.67[b]	5.63 ± 1.73[b]

[a] Blood samples were taken before (baseline), 4.5, and 24 h after consumption of apples. At baseline and at 24 h, fasting blood samples were obtained. [b] Significantly different from baseline ($p < 0.05$, repeated measures ANOVA, Dunnett posthoc test).

differences between the effects of organically or conventionally produced apples on DNA damage.

DISCUSSION

Although there is evidence that organic apples contain higher amounts of phenolic compounds (*27*), we did not observe any statistically significant differences between the agricultural methods with respect to the sum of phenolic compounds and each of the polyphenol classes. An explanation could be the relatively large variation in the data for the concentration of phenolic compounds in the present study. As not all compounds could be identified in the apples, we cannot exclude that the concentrations of some unmeasured compounds such as complex procyanidins could differ in apples from different agricultural practices. A number of in vitro studies have demonstrated that apples, apple extracts, and apple polyphenols possess high antioxidant capacity in vitro, including inhibition of copper-induced LDL oxidation (*20, 28*). In the present study with healthy volunteers, intervention with 1 kg of apples (organic or conventional) had no effect on LDL antioxidant capacity, and there were no differences between apples produced by different agricultural practices. A previous study using a similar amount of apples failed to detect any antioxidant effect using several test systems in human plasma (*23*). The explanation for the observed differences between in vitro and in vivo experiments could be the relatively low bioavailability and effective metabolic conversion of apple polyphenols, which are not able to improve the effective endogenous antioxidant network in healthy subjects. However, we can also not exclude that the myeloperoxidase-peroxynitrite used in the present study for LDL oxidation leads to different results than to the copper-induced LDL oxidation, which had been applied in the in vitro studies

The present study shows that consumption of either organic or conventionally produced apples decreased oxidative DNA damage (Endo III-sensitive sites) in a similar manner 24 h later. The coefficients of variation for the data on DNA damage in lymphocytes in our study are large. This can be a study limitation to observe a statistically significant effect between the groups. However, here, with samples of conventional and organic apples that did not differ in their content of investigated polyphenols, no trend for an effect of the growing method could be found.

The maximal plasma concentrations for most polyphenols are observed 1–8 h after consumption (for a review, see ref *29*). This indicates that a nontypical polyphenol compound or an indirect mechanism such as changes in gene expression could be responsible for the observed effect. Flavonoids are known to be able to interact with transition metal ions such as copper and iron, which can induce oxidative processes and DNA damage in vitro (*30, 31*). It is possible that flavonoids could affect the concentration of the so-called free transition metal ions pool or affect the endogenous mechanism responsible for controlling their concentrations.

Different types of fruit and vegetables exhibit large differences in capacity for inhibiting genotoxicity. Some types such as kiwi fruit, blood orange juice, tomato juice, tomato puree, spinach, and high amounts of mixed fruits and vegetables protected against DNA damage in lymphocytes in humans (*32–36*) However, several studies report no effect of several types of fruits and vegetables such as black currant juice, cooked carrots, tinned mandarins in orange juice, or mixed vegetables (for a review, see ref *37*). Thus, we have identified apples as another fruit to add to the already known types of fruits and vegetables with antigenotoxic properties in humans.

In summary, intervention with either organic or conventionally grown apples (1 kg) did not affect antioxidant capacity (LDL lag time test). There was no effect on endogenous DNA strand breaks and Fpg-sensitive sites in peripheral blood lymphocytes, but apples strongly decreased oxidative DNA damage recognized by Endo III and increased the capacity to protect DNA against damage induced by iron chloride, indicating that both organically and conventionally grown apples have antigenotoxic potential.

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