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ORIGINAL CONTRIBUTION

No effect of the farming system (organic/conventional) on the bioavailability of apple (*Malus domestica* Bork., cultivar Golden Delicious) polyphenols in healthy men: a comparative study

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Abstract
Background The organic food sales have been increasing during the recent years. It has been hypothesised that organically grown fruits are healthier based on their higher content of phytochemicals. However, data on the bioavailability of phytochemicals from organically or conventionally produced plant foods are scarce.
Methods Two human intervention studies were performed to compare the bioavailability of polyphenols in healthy men after ingestion of apples from different farming systems. The administered apples were grown organically and conventionally under defined conditions and characterised regarding their polyphenol content and antioxidant capacity. No significant differences in the polyphenol content and the antioxidant capacity from the organic and conventional farming system were observed.
Results In the *short-term intervention study,* six men consumed either organically or conventionally produced apples in a randomized cross-over study. After intake of 1 kg apples, phloretin (C_{max} 13 ± 5 nmol/l, t_{max} 1.7 ± 1.2 h) and coumaric acid (C_{max} 35 ± 12 nmol/l, t_{max} 3.0 ± 0.8 h) plasma concentrations increased significantly ($P < 0.0001$) in both intervention groups, without differences between the two farming systems. In the long-term intervention study, 43 healthy volunteers consumed organically or conventionally produced apples (500 g/day; 4 weeks) or no apples in a double-blind, randomized intervention study. In this study, 24 h after the last dosing regime, the apple intake did not result in increasing polyphenol concentrations in plasma and urine compared to the control group suggesting no accumulation of apple polyphenols or degradation products in humans.
Conclusion Our study suggests that the two farming systems (organic/conventional) do not result in differences in the bioavailability of apple polyphenols.

Keywords Apples · Bioavailability · Organic · Polyphenols · Antioxidant status · Human intervention study

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Introduction

Apple consumption has been linked to a reduced risk of lung cancer [1], asthma [2], cardiovascular diseases [3], and thrombotic stroke [4]. In animal studies, cloudy apple juice inhibited DNA damage and colon carcinogenesis induced by 1,2-dimethylhydrazine [5, 6]. These associations can partly be attributed to a high content of phytochemicals (e.g. flavonoids) [7]. Apples contain about 2 g polyphenols per kg fresh weight, depending on the cultivar.

They are rich in flavonoids (e.g. flavanols, flavonols, and dihydrochalcones) and they contain high amounts of hydroxycinnamic acid derivatives, mainly chlorogenic acid [8, 9]. Some secondary metabolites (e.g. flavonoids) exhibit stronger antioxidant activities in vitro than classic antioxidant vitamins such as vitamins C and E [10].

It has been hypothesised that organically grown fruits and vegetables could be healthier due to a higher content of phytochemicals. This might be caused by more pathogen stress during the ripening period than in conventionally produced fruits [11, 12]. Several studies have been conducted to gain information regarding the impact of the production method on polyphenols in apples, but the results are still inconsistent [13]. If significant differences between the organic and conventional farming system were observed, the organically produced ones tended to have higher polyphenol contents [14].

Few human intervention studies have been conducted to investigate the bioavailability of phenolic compounds from a diet based on organic compared to conventional foods [15–17]. A diet based on organically produced food (meat, potato, wheat, rye, fruits, and vegetables) resulted in a higher urinary excretion of quercetin and kaempherol, increased protein oxidation, and decreased plasma antioxidant capacity [15]. The ingestion of conventionally produced red wine leads to a decrease in copper-induced formation of thiobarbituric acid-reactive substances. In contrast, the consumption of organically produced red wine increased the catalase activity in erythrocytes [16]. Consumption of apples from conventional and organic farming systems showed no effect on the antioxidant capacity of LDL (lag-time test), endogenous DNA strand breaks, Fpg protein-sensitive sites or capacity to protect DNA against damage caused by hydrogen peroxide. However, 24 h after apple consumption decreased levels of endonuclease III sensitive sites and an increased capacity to protect DNA against damage induced by iron chloride were observed in both intervention groups [17].

However, there are no data on whether the farming system affects the bioavailability of polyphenols and the antioxidant status, as well as on immunological effects after consumption of organically and conventionally produced apples.

Therefore, the aim of the present study was to compare the bioavailability of apple polyphenols, the antioxidant status, as well as immune parameters in healthy men after ingestion of apples (cultivar Golden Delicious) from different farming systems in two human intervention studies. The apples (cultivar Golden Delicious) were characterised based on their polyphenol content and the antioxidant capacity of the administered apples, grown under defined organic and conventional conditions (harvest years 2004 and 2007) in Switzerland.

Materials and methods

Chemicals

All chemicals were purchased from Carl Roth (Karlsruhe, Germany), Sigma-Aldrich (Taufkirchen, Germany), or Merck (Darmstadt, Germany).

Apple samples

Organically and conventionally produced apples (cultivar Golden Delicious) were harvested in 2004 and 2007 from an existing network for system comparison studies that includes ten commercial farms (five comparison pairs) in Switzerland [18]. A detailed description of the apple production has been published previously [14, 17].

The apples used in the human intervention studies originated from one of the five neighbouring commercial farm pairs (2004 for the *short-term intervention study* and 2007 for the *long-term intervention study*). Apple samples were stored at 2 °C and 93% relative humidity until the beginning of the human intervention studies (approximately 6 weeks).

Polyphenol concentration and antioxidant capacity of the apples

The polyphenol concentrations and the antioxidant capacity (FRAP, ORAC, and TEAC assays) of apples have been determined as described previously [14].

Subjects

Six non-smoking men, between 23 and 32 years of age, were recruited for the *short-term intervention study*. In the *long-term intervention study* 43 non-smoking men participated, aged 22–40 years.

The subjects were healthy according to clinical examination and medical history. They refrained from taking vitamin supplements or any medication for 6 months before and during the studies. The studies were approved by the Landesärztekammer Baden-Württemberg, and all participants gave their written consent.

Study designs

Short-term intervention study (3 days)

The study design of the *short-term intervention study* has been described previously [17]. The study was conducted in 2004 with the apples harvested in the same year.

Long-term intervention study (5 weeks)

The study was performed as a randomised, double-blind intervention study in 2007. The volunteers were divided into three groups. The human intervention study consisted of a 5-week experimental period, divided into a 1-week depletion period and a 4-week intervention period. During the entire study the volunteers were asked to consume no apples and apple products (e.g. apple juice). Furthermore, the volunteers had to refrain from products with high polyphenol contents like onions, red wine, chocolate, tea, coffee as well as whole grain products. A list of products which should be avoided was provided. During the 4-week intervention period the volunteers of the intervention groups consumed 500 g of apples (harvest 2007) daily from organic ($n = 16$) or conventional ($n = 16$) farming systems. The third group served as a control group for time effects ($n = 11$), and maintained an apple- and polyphenol-restricted diet throughout the total study period of 5 weeks.

Collection and preparation of blood samples (*long-term intervention study*)

After 1-week depletion, fasting blood samples were taken between 7 and 10 a.m. before apple consumption (day 0) as well as on day 28 (24 h after the last apple consumption). The preparation of blood samples was described previously [17].

Analyses in blood and urine samples (*short- and long-term intervention studies*)

Serum parameters

Glucose, triacylglycerol, cholesterol, and uric acid were determined using enzymatic kits (Boehringer, Mannheim, Germany).

Polyphenol concentration in plasma and urine samples (short- and long-term intervention studies)

Apple polyphenols and their metabolites in plasma and urine were analysed by HPLC/MS using an internal standard (2,4-dihydroxy-phenylpropionic acid). Total polyphenol concentrations were determined after extraction and enzymatic hydrolysis of phase-II-conjugates with a mix of β-glucuronidase and sulfatase.

The internal standard (25 µl of 1.25 µmol/l for plasma and 12.5 µl of 0.5 µmol/l for urine) was added to 1,000 µl plasma and to 500 µl urine, respectively. Samples were diluted with 500 µl sodium-acetate buffer (0.15 M; pH 5) and hydrolysed with 4,000 U of β-glucuronidase and 150 U of sulfatase for 60 min at 37 °C. After addition of ice-cold acetonitrile the samples were centrifuged at 13,000*g* for 10 min. The supernatants were collected, and the organic solvent was evaporated under a gentle stream of nitrogen. Afterwards, the samples were extracted three times with 500 µl ethyl acetate. The combined organic extracts were evaporated to dryness under a stream of nitrogen gas. For HPLC/MS analysis, the residue was dissolved in 250 µl (urine) and 100 µl (plasma) methanol:water (1:1 v/v), and 20 µl was used for HPLC/MS analysis.

HPLC/MS analysis of phenolic compounds in plasma and urine (short- and long-term intervention studies)

HPLC analysis was performed on a HP 1200 system (Agilent Technologies, Waldbronn, Germany) equipped with an auto-injector, column oven and photodiode array detector. The auto-injector was set to 10 °C and the column oven to 37 °C. Separation was carried out on a Prontosil (150 mm × 4 mm i.d., particle size 3 µm) reversed-phase column (Bischoff, Leonberg, Germany). Solvent A consisted of 0.1% formic acid in water (pH 3) and solvent B of acetonitrile. A linear gradient was used: from 10 to 38% B in 15 min, from 18 to 47% in 10 min, from 47 to 80% in 4 min, and from 80 to 10% in 2 min. The flow rate was set to 1.0 ml/min, and the injection volume was 20 µl.

The HPLC system was directly coupled to a hybrid triple quadrupole/linear in trap (QTrap®, Applied Biosystem, Darmstadt, Germany) equipped with a TurboIonSpray source. Analytes were detected in the negative ion mode at a vaporiser temperature of 500 °C and ion spray voltage of –4 kV. Spectral data were recorded with N_2 (CAD = 4) as collision gas and a declustering potential of –61 V. Data acquisition was performed in the MRM mode $[M–H]^-$. The transitions and the collision offset energies of each identified polyphenol in plasma as well as urine samples are listed in Table 1. Data collection and handling was done with Analyst 1.4.2.

Quantification was performed by external calibration using commercially available reference compounds. Calibration curves for the different polyphenols were constructed in the range of 0.01–1 µmol/l in which the linearity of the response was given. The recovery for all polyphenols was greater than 90%. The variation coefficient of the method was below 10% (intra assay). The limits of detection ranged from 24 fmol to 7 pmol.

Antioxidant activity in urine and plasma samples (long-term intervention study)

Total polyphenol concentration in urine was measured by the Folin–Ciocalteau method as described previously [19]. Results are expressed as total polyphenols (gallic acid

Table 1 Transitions and the collision offset energies of the identified polyphenols

	Q1 → Q3 (m/z)	Collision offset energy (V)
Caffeic acid	179 → 135	−20
Catechin	289 → 245	−24
Chlorogenic acid	353 → 191	−24
Coumaric acid	163 → 119	−22
Dihydrocaffeic acid	181 → 137	−18
Dihydroferulic acid	195 → 136	−12
3,4-Dihydroxy-phenylacetic acid	167 → 123	−20
2,4-Dihydroxy-phenylpropionic acid	153 → 109	−18
Epicatechin	289 → 245	−20
Ferulic acid	193 → 134	−22
Hippuric acid	178 → 134	−16
Homovanillic acid	181 → 137	−12
Isoferulic acid	193 → 134	−22
Isorhamnetin	315 → 300	−30
Isovanillic acid	167 → 108	−28
3-Hydroxy-benzoic acid	137 → 93	−16
3-Hydroxy-phenylacetic acid	151 → 107	−12
3-Hydroxy-phenylpropionic acid	165 → 121	−16
4-Hydroxy-phenylpropionic acid	166 → 122	−16
Phloretin	273 → 167	−20
Phloroglucinol	125 → 57	−20
Procyanidin B1	577 → 289	−32
Procyanidin B2	577 → 289	−34
Quercetin	301 → 151	−30
Sinapic acid	223 → 149	−28
Syringic acid	197 → 121	−24
Vanillic acid	167 → 152	−16

equivalent) per mg creatinine. Urinary creatinine was determined using an enzymatic kit (Boehringer, Mannheim, Germany). Total antioxidant status in plasma was analysed by using the FRAP, TEAC, and ORAC assays as described previously [20].

Carotenoids, vitamins E and C in plasma (long-term intervention study)

Concentrations of carotenoids (α-, and β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin), vitamin E (α-tocopherol) as well as vitamin C in plasma were determined according to the methods described previously [20, 21].

Isolation of peripheral blood mononuclear cells (PBMC; long-term intervention study)

Isolation of PBMC and the quantification of cytokine secretion were done as described previously [20].

Quantification of NK cells, NKT cells, and T lymphocytes (long-term intervention study)

The percentages of $CD3^{-}CD16^{+}CD56^{+}$ NK cells, $CD3^{+}CD16^{+}CD56^{+}$ NKT cells, and of $CD3^{+}$ T lymphocytes were measured by flow cytometry (FACSCalibur, BectonDickinson, Heidelberg, Germany). Whole blood samples (100 µl) were incubated with 4 µl of a mixture of fluorescein isothiocyanate-IgG_1 anti-human CD3 antibody and phycoerythrin-labelled IgG_{2a} anti-human CD16 and CD56 antibodies (Simultest, BectonDickinson) for 30 min in the dark at room temperature. Fluorescein isothiocyanate-labelled IgG_1 and phycoerythrin-labelled IgG_{2a} (Simultest, BectonDickinson) mouse monoclonal antibodies were used as isotype controls. Afterwards, the red blood cells were lysed with fluorescence-activated cell sorting lysing solution (BectonDickinson), and the samples were washed with phosphate-buffered saline (without Ca and Mg). The stained cells were stored on ice in 1% paraformaldehyde (Sigma-Aldrich), and fluorescence was measured within 24 h.

PBMC proliferation and lytic activity of NK cells (long-term intervention study)

Proliferation of PBMC and the NK cell activity of PBMC were determined as described previously [20, 22].

Determination of plasma pharmacokinetics

Peak plasma concentration (C_{max}) and the time required to attain C_{max} (t_{max}) were obtained directly by visual inspection of each subject's plasma concentration–time profile. The area under the plasma concentration–time profile from 0 h to infinity (AUC_{inf}) was calculated by a non-compartmental approach using PK SOLUTIONS™ 2.0 (Summit Research Services, Montrose, CO, USA, 1999) computer software.

Statistical analysis

All statistical calculations were performed using the STATVIEW program version 5.0 (SAS Institute, Cary, NC, USA, 1998). Results are reported as means ± SD. Differences were considered significant at $P < 0.05$. Normality was checked by means of the Kolmogorov–Smirnov test.

Apples of 2007

Differences between the mean values of the polyphenol concentrations and the antioxidant capacity in apples were statistically analysed using the unpaired Student's *t* test.

Short-term intervention study

Changes between the baseline (0 h) and the following time points among the two treatment groups were tested for significance by repeated measures analysis of variance (ANOVA) and Tukey–Kramer post hoc test. The differences between the mean values of C_{max}, t_{max}, and AUC_{inf} after administration of the apples were statistically analysed using the unpaired Student's t test.

Long-term intervention study

Treatment effects were tested by subtracting the data of day 28 and day 0 and subsequently analysed using analysis of variance (ANOVA).

Results

Polyphenol concentration and antioxidant capacity in apples

The polyphenol concentrations in organically and conventionally grown apples (cultivar Golden Delicious) were as follows: in 2004: 307.8 ± 70.9 µg/g and 320.6 ± 64.3 µg/g as published previously [16] and 2007: 518.5 ± 40.4 µg/g and 508.4 ± 76.6 µg/g (Fig. 1). No statistical differences between the organic and conventional farming system were observed.

The results of the antioxidant capacity of the apples (at harvest) are listed in Table 2. In contrast to 2004 the organically produced apples exhibited a significantly higher antioxidant capacity (FRAP and TEAC assays) than the conventionally produced ones in 2007. No other significant differences were observed.

Human intervention studies

All participants of the *short-term intervention study* ($n = 6$) and of the *long-term intervention study* ($n = 43$) completed the intervention studies.

Short-term intervention study (3 days)

Serum parameters before and after apple consumption of the six men were reported previously [17].

Apple polyphenol concentrations in plasma (short-term intervention study)

In Fig. 2a, b, the plasma appearance and disappearance curves for phloretin and coumaric acid are shown. After consumption of 1 kg organically as well as conventionally produced apples, phloretin and coumaric acid concentrations increased significantly. The plasma pharmacokinetic variables for coumaric acid and phloretin are listed in Table 3. Furthermore, the vanillic acid concentrations increased significantly after lunch (6 h after apple intake) in both intervention groups (data not shown). No other changes in the plasma polyphenol concentrations were observed. There were no further significant differences between the organic and conventional group in any of the analysed polyphenols.

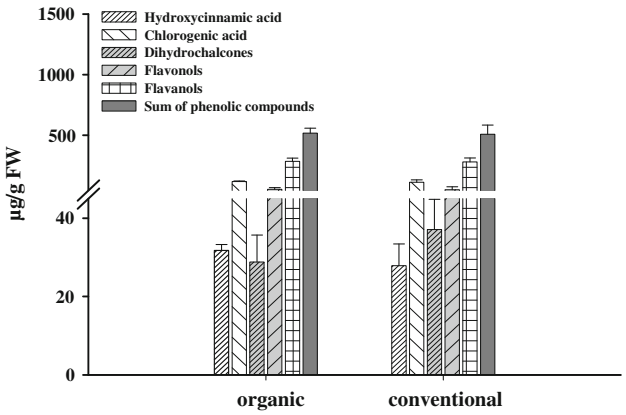

Fig. 1 Polyphenol concentrations of organically and conventionally produced apples (cultivar Golden Delicious; harvest 2007). Values are means ± SD; $n = 5$ per group; *FW* fresh weight. No significant differences were observed (unpaired Student's t test). The sum of phenolic compounds is calculated as the sum of the results for the individual polyphenols: chlorogenic acid, hydroxycinnamic acids (4-caffeoylquinic acid, 3-coumaroylquinic acid, 4-coumaroylquinic acid, and 5-coumaroylquinic acid), dihydrochalcones (phloretin 2′-xylog-lucoside and phloretin 2′-glucoside), flavanols (catechin, epicatechin, procyanidin B1, and procyanidin B2), and flavonols (quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-xyloside, quercetin 3-arabinoside, and quercetin 3-rhamnoside)

Table 2 Antioxidant activity of apples (cultivar Golden Delicious, harvest 2004 and 2007)

	Organic		Conventional	
	2004	2007	2004	2007
FRAP (µmol TE/g FW)	4.1 ± 1.1	5.4 ± 0.4*	4.5 ± 1.0	4.3 ± 0.4
ORAC (µmol TE/g FW)	9.0 ± 1.3	11.2 ± 0.6	7.9 ± 1.5	10.4 ± 1.3
TEAC (µmol TE/g FW)	7.9 ± 2.2	10.3 ± 0.6*	8.8 ± 2.3	7.8 ± 0.7

Values are means ± SD ($n = 5$). Significant differences between the organically and conventionally produced apples (harvest 2007) were observed

TE trolox equivalent; *FW* fresh weight

*$P < 0.05$, unpaired Student's t test

Long-term intervention study (28 days)

The characteristics of the 43 volunteers at days 0 and 28, namely age, BMI, blood glucose, lipid profile, uric acid

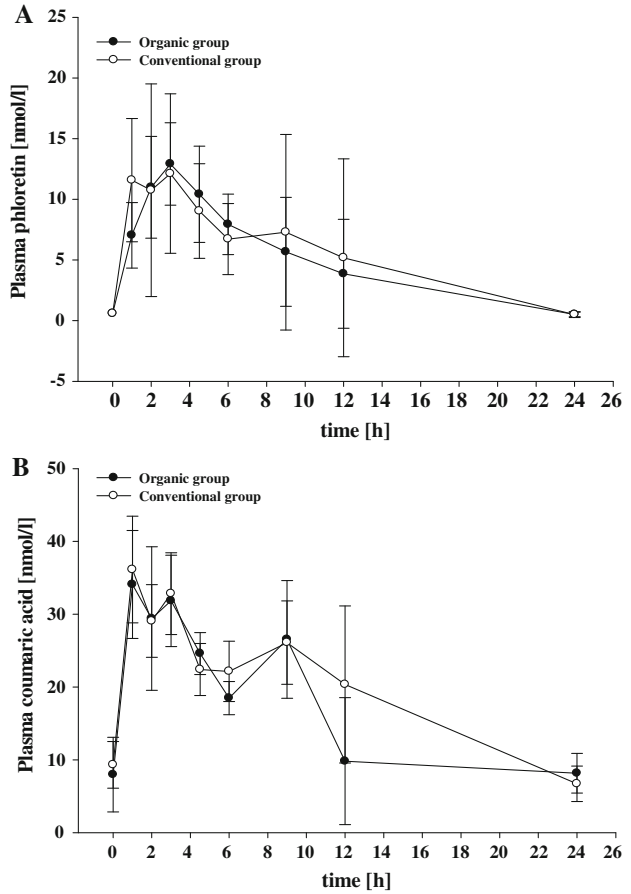

Fig. 2 Mean plasma appearance and disappearance curves (±SD) for phloretin (**a**) and coumaric acid (**b**) in six men after consumption of 1 kg apples (cultivar Golden Delicious; after hydrolysis of the phase II conjugates to the aglycones; short-term study). Plasma phloretin and coumaric acid concentrations increased significantly after apple consumption ($P < 0.001$; repeated-measure ANOVA, Tukey–Kramer post hoc test) with no differences between groups

Table 3 Plasma pharmacokinetic variables for coumaric acid and phloretin after consumption of 1 kg apples (short-term study)

	AUC_{inf} (nmol h/l)	C_{max} (nmol/l)	t_{max} (h)
Coumaric acid			
Organic group	695.5 ± 446.3	34.1 ± 7.4	1.7 ± 1.4
Conventional group	517.2 ± 334.8	36.1 ± 17.9	1.7 ± 1.0
Phloretin			
Organic group	117.7 ± 49.8	12.9 ± 3.4	3.2 ± 0.9
Conventional group	129.3 ± 88.4	12.1 ± 6.6	2.8 ± 0.8

Values are means ± SD. No significant differences between the groups and no significant intervention effect were observed (unpaired Student's *t* test)

AUC_{inf} area under the plasma concentration time profile from 0 h to infinity; C_{max} peak plasma maximum concentration of polyphenols; t_{max} time required to attain C_{max}

Table 4 Characteristics of study participants ($n = 43$) on day 0 (after 1-week polyphenol depletion) and day 28 of the long-term intervention study

	Organic group ($n = 16$)	Conventional group ($n = 16$)	Control group ($n = 11$)
Age (years)			
Day 0	29.4 ± 5.6	28.5 ± 5.8	27.4 ± 3.1
BMI (kg/m^2)			
Day 0	23.5 ± 3.2	23.6 ± 2.5	23.8 ± 2.0
Blood parameters (mg/dl)			
Triacylglyceride			
Day 0	102.6 ± 37.1	89.8 ± 25.4	88.4 ± 39.8
Day 28	111.8 ± 43.8	96.8 ± 26.8	98.8 ± 29.5
Cholesterol			
Day 0	185.5 ± 35.0	177.0 ± 25.9	176.0 ± 37.1
Day 28	177.4 ± 28.5	177.7 ± 34.2	171.1 ± 34.7
Glucose			
Day 0	69.2 ± 5.3	71.7 ± 5.2	74.1 ± 5.0
Day 28	74.1 ± 5.2	71.7 ± 6.1	75.8 ± 7.4
Uric acid			
Day 0	5.7 ± 1.0	5.4 ± 0.9	5.7 ± 1.6
Day 28	5.7 ± 1.2	5.4 ± 0.7	5.6 ± 1.2
Vitamins (μmol/l)			
α-Tocopherol			
Day 0	20.3 ± 5.7	22.4 ± 10.3	19.2 ± 6.5
Day 28	18.2 ± 5.7	17.7 ± 5.6	17.7 ± 3.4
Vitamin C			
Day 0	49.9 ± 8.8	53.1 ± 9.0	56.2 ± 19.4
Day 28	52.8 ± 2.4	53.2 ± 2.9	37.3 ± 19.1
Sum of carotenoids			
Day 0	1.7 ± 1.1	1.7 ± 0.7	1.6 ± 1.1
Day 28	1.4 ± 0.8	1.4 ± 0.8	1.4 ± 0.7

Values are means ± SD. No significant differences between the groups and no significant intervention effect were observed (ANOVA)

as well as plasma vitamins C and E, and the sum of carotenoids are summarised in Table 4. No significant differences of these parameters were observed between the three groups. The 4-week lasting intervention with apples did not affect concentration of plasma glucose, uric acid, triacylglycerol, cholesterol, vitamins C and E, as well as the sum of carotenoids significantly (Table 4).

Apple polyphenol concentrations in plasma and urine (long-term intervention study)

The apple consumption had no effect on the polyphenol concentrations in plasma and urine of the volunteers (data not shown).

Physiological effects in the short- and long-term intervention studies

In both studies apple consumption had no effect on the antioxidant status in plasma as determined by the TEAC and ORAC assays (data listed only for the *long-term intervention study* in Table 5) as well as by the Folin–Ciocalteau method in urine of the volunteers (Table 5). In the FRAP assay, statistically significant differences were observed in *the short-term* but not in the *long-term intervention study*. The FRAP values increased significantly after 1 h after the apple consumption (1 kg) in both groups: from 1,045 ± 238 to 1,126 ± 231 μmol Fe^{2+}/l in the organic group and from 1,005 ± 197 to 1,129 ± 204 μmol Fe^{2+}/l in the conventional group. No statistically significant differences between the organic and conventional group were seen.

Immune parameters were measured only in the *long-term intervention study*. No statistically significant changes (time and treatment) were observed with regard to mitogen-activated PBMC proliferation and cytokine secretion as well as to the percentage and lytic activity of NK cells at day 0 and after 28 days of intervention with organically and conventionally produced apples compared to the control group (data not shown).

Discussion

Data of the influence of the farming system on phytochemical concentrations, their bioavailability, and physiological effects are scarce. Therefore, the aim of the present study was to compare the bioavailability, the antioxidant status, and immune parameters in healthy men after ingestions of apples (cultivar Golden Delicious) from different farming systems in two human intervention studies.

Apples

The concentrations of the polyphenols and the antioxidant capacity of the analysed apples from 2004 and 2007 are similar to those previously reported by others [23–25]. In 2007, however, not in 2004, the antioxidant capacity of the organically produced apples was significantly higher than those of the conventionally ones. It has been reported that the phytochemical concentrations are positively correlated with the antioxidant capacity of fruits, including apples [23, 26]. However, in our studies the polyphenol concentrations did not differ. Therefore, it might be speculated that other unidentified antioxidant substances (such as complex procyanidins) are responsible for the significantly higher antioxidant capacity in organically grown apples in 2007. The statistically significant year to year variations in the polyphenol concentration and in the antioxidant capacity of the apples can be attributed to climate variations as has been shown previously by our group [14].

Short-term intervention study

After consumption of 1 kg apples, the volunteers ingested 308 ± 71 mg apple polyphenols from organically and 321 ± 66 mg apple polyphenols from conventionally produced apples. The intake of apple polyphenols resulted in a significant increase of peak plasma phloretin and coumaric acid concentrations in both intervention groups (Fig. 2a, b). Marks et al. [27] reported on a C_{max} of 73 ± 11 nmol/l of phloretin 2′-O-glucuronide in nine healthy men after consumption of 500 ml cider (92 μmol/l

Table 5 Antioxidant status of the study participants ($n = 43$) at days 0 and 28 after consumption of 500 g apples daily (long-term study)

	Organic group ($n = 16$)	Conventional group ($n = 16$)	Control group ($n = 11$)
Plasma antioxidant parameters			
FRAP (μmol Fe^{2+}/l)			
Day 0	920.5 ± 139.8	893.8 ± 121.2	929.1 ± 109.1
Day 28	904.5 ± 151.1	870.3 ± 96.6	904.7 ± 116.9
ORAC (μmol TE/l)			
Day 0	12,335.6 ± 1,327.4	11,621.3 ± 1,767.5	11,449.1 ± 2,067.8
Day 28	12,196.9 ± 1,407.5	11,868.8 ± 1,671.7	11,408.2 ± 2,309.7
TEAC (μmol TE/l)			
Day 0	2,396.6 ± 84.6	2,374.4 ± 78.9	2,399.3 ± 95.6
Day 28	2,384.0 ± 74.6	2,372.9 ± 72.0	2,396.4 ± 106.2
Urinary antioxidant parameters (nmol GAE/mg Creatinine)			
Folin–Ciocalteau method			
Day 0	150.1 ± 45.5	155.7 ± 53.6	126.5 ± 39.5
Day 28	163.9 ± 49.1	171.1 ± 35.1	186.1 ± 38.8

Values are means ± SD. No significant differences between the groups and no significant intervention effect were observed (unpaired Student's *t* test)

TE trolox equivalent; *GAE* gallic acid equivalent

dihydrochalcones). The apples in the present study contained 50 μmol/kg dihydrochalcones and resulted in plasma concentrations of around 12.5 nmol/l. Therefore, the bioavailability of phloretin seems to be greater after cider consumption, which might be attributed to the ethanol content. It has been shown that ethanol can increase the absorption of flavonoids [28]. Comparable studies on the pharmacokinetic of coumaric acid after consumption of apples or apple products have not yet been published.

The t_{max} of phloretin and coumaric acid was observed around 1–3 h after apple consumption. Other studies also reported on t_{max} of apple polyphenols after 1–2 h after consumption of cloudy apple juice (1 l) or apple cider (500 ml) [27, 29]. After intake of green coffee extract, t_{max} of coumaric acid in plasma of the volunteers was 2.5 ± 1.8 h [30]. These studies are in agreement with our results. After the main meal, the vanillic acid plasma concentrations increased significantly in both intervention groups, although the lunch (rice and chicken) was low in polyphenols. Our own analysis revealed that rice contains ferulic and caffeic acid (data not shown). It has been reported that caffeic acid is metabolised to vanillic acid in the human body [31]. Therefore, the increase in the plasma vanillic acid concentration can be attributed to the intake of rice in the present study, and may not be caused by apple polyphenol consumption.

Long-term intervention study

During the 4-week intervention period, the volunteers ingested 259 ± 20 mg total polyphenols per day in the organic group and 254 ± 38 mg in the conventional group. Apple intake did not result in increased polyphenol concentrations in plasma and urine compared to the control group. In the *short-term intervention study*, however, we were able to show that polyphenols are quickly absorbed; the maximal plasma concentrations of phloretin and coumaric acid were achieved after 1–3 h. 24 h after dosing, plasma concentrations returned to the baseline values as depicted in Fig. 2. Furthermore, it has been reported that this holds good not only for plasma concentrations but also for urinary excretion [27]. Since in our study blood and urine samples of the volunteers were collected after an overnight fasting period, the fast elimination of the apple polyphenols might be the reason for the absence of increased polyphenol concentrations in plasma as well as in urine of the volunteers after daily apple consumption. Our results shows further that none of the polyphenols degradation products (e.g. phenolic acids and phloroglucinol) accumulated in plasma or urine after regular apple consumption.

In both intervention studies no significant differences were observed between the organic and conventional group in any of the analysed polyphenols. However, it has to be pointed out that in our study the polyphenol content did not differ significantly between the samples. To date, no human intervention study has been published to analyse the bioavailability of apple polyphenols from organic and conventional farming systems. Caris-Veyrat et al. [32] and Stracke et al. [20] conducted human intervention studies to investigate the bioavailability of carotenoids from organically and conventionally produced tomatoes and carrots, respectively. The carotenoid concentration did also not differ between organic and conventional samples. The authors reported that plasma carotenoid concentrations increased significantly after consumption of these vegetables [20, 32]. This is in agreement with the present results. It might be postulated that similar carotenoid contents in carrots and tomatoes as well as similar polyphenol concentrations in apples are the reason for the comparable bioavailabilities of carotenoids and apple polyphenols from the different farming system. In consequence, differences in the plant matrix that might be caused by the different farming system are not relevant for the bioavailability of carotenoids as well as of apple polyphenols. Also, because no differences in functional measurements were observed, organically produced fruits and vegetables have basically no higher nutritional value than conventionally produced ones.

Grinder-Petersen et al. conducted a human cross-over intervention study with respect to the intake and excretion of five selected polyphenols from organic (OPD) and conventional (CPD) produced diets. The higher content of quercetin in the OPD compared to the CPD resulted in a higher excretion of quercetin. However, plant food diets were based on different cultivars. Therefore, the higher excretion of quercetin might be due to the differences in cultivars of fruits and vegetables in the diets [15].

A number of in vitro studies have demonstrated that apples, apple extracts as well as apple polyphenols possess high antioxidant capacities in vitro [33]. Lotito and Frei [34] were able to demonstrate that apple consumption increased the antioxidant capacity in human plasma measured by the FRAP assay. This increase is caused by the increase of plasma urate concentrations due to the metabolic effect of apple fructose. This is well in line with our results. After 1 kg intake of apples the antioxidant capacity in the FRAP assay and also the urate concentrations [17] increased significantly. Therefore, the increase of the antioxidant capacity in the FRAP assay in our study is also an effect of the metabolic effect of apple fructose and cannot be attributed to the intake of apple polyphenols. In the *long-term intervention study,* the functional measurements (antioxidant status) did also not differ between the intervention groups and the control group (no apples). Obviously, moderate apple consumption in general has no

measurable effect on the antioxidative status. An explanation for the missing effects on the antioxidant status (in both studies) might be the relatively low bioavailability (in most cases less than 1%) as well as the effective metabolism of the apple polyphenols in the human body [35]. Furthermore, in the case of quercetin it has been shown that the antioxidant capacity of quercetin conjugate is lower than that of the aglycone [36]. Almost 100% of quercetin is present as phase-II-conjugates in plasma [37–39]. The conjugation could also be an explanation for the missing antioxidant effects. Unfortunately, we were not able to investigate polyphenol-conjugates with our detection method. Therefore, these results suggest that moderate apple consumption is not sufficient to enhance the antioxidant status in healthy volunteers.

In vitro studies with pure flavonoids have demonstrated an impact on several immune mechanisms [40]. However, intervention studies investigating immunological effects of polyphenols or polyphenol-rich foods in healthy humans are scarce. In an intervention study with healthy volunteers on a low-polyphenol diet, a 2-week supplementation with polyphenol-rich juices from apples and other fruits enhanced lymphocyte responsiveness to mitogen-activation and NK cell lytic activity [19]. Since in both studies daily supply with polyphenols was similar (approximately 230–260 mg/day), the different outcomes most likely result from differences in the nature of the polyphenols applied and in the bioavailability from the varying food matrices.

In summary, these are the first intervention studies in which the bioavailability, the antioxidant status, as well as the effect on immune parameters of organically and conventionally grown apples were assessed. In this study with apples of similar polyphenol content, the farming system had no effect on the polyphenol concentration in the apples as well as on the bioavailability of polyphenols from apples. We were not able to identify any apple polyphenol or degradation products that accumulate in human plasma or urine after regular apple consumption. Finally, no antioxidant or immunological effects of apple consumption were observed.

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