

Effect of Diets Based on Foods from Conventional versus Organic Production on Intake and Excretion of Flavonoids and Markers of Antioxidative Defense in Humans

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Different food production methods may result in differences in the content of secondary metabolites such as polyphenolic compounds. The present study compared conventionally (CPD) and organically produced (OPD) diets in a human crossover intervention study ($n = 16$) with respect to the intake and excretion of five selected flavonoids and effect on markers of oxidative defense. The urinary excretion of quercetin and kaempferol was higher after 22 days of intake of the OPD when compared to the CPD ($P < 0.05$). The excretions of flavonoids in urine as a percentage of intake (0.6–4%) were similar after both interventions. Most markers of antioxidative defense did not differ between the diets, but intake of OPD resulted in an increased protein oxidation and a decreased total plasma antioxidant capacity compared to baseline ($P < 0.05$). Some varietal difference was seen in the study, and because selection of more resistant varieties is of central importance to organic farming, it cannot be excluded that the observed effects originate from these differences. The food production method affected the content of the major flavonoid, quercetin, in foods and also affected urinary flavonoids and markers of oxidation in humans.

KEYWORDS: Flavonoids; humans; organic food production; conventional food production; urinary excretion; antioxidative defense

INTRODUCTION

Flavonoids are a group of polyphenolic secondary metabolites that occur ubiquitously in all plants and are an integral part of the human diet. They are found in large amounts in vegetables, fruits, tea, and wine (1–3). Many isolated polyphenolic compounds show strong antioxidative properties *in vitro* (4–6), which is suggested to be one of the potential beneficial actions of these compounds in humans.

Polyphenolic compounds are a part of the plant defense system and have a variety of functions (7). The content in plants is influenced by cultivation and harvesting conditions such as growing conditions, degree of ripeness, size of the fruit, and variety of the plants (8). Organic food production is characterized by the absence or limited use of synthetic herbicides, pesticides, and insecticides and a lower use of fertilizers. Depending on the chemical substance, these chemicals may both

decrease and increase the production of polyphenolic compounds in plants (9, 10). In addition, organically produced plants have a longer ripening period compared to conventional plants because of a slower release of the supplied nutrients (11), and as flavonoids are formed in the ripening period, one could expect a higher content of these compounds in organically grown plants. Only a limited number of studies have investigated the effect of cultivation technique on the content of flavonoids, and the results are inconsistent. In a study of three different strawberry cultivars no difference between the organic and conventional systems was seen (12), whereas a study of marionberries showed a significantly higher content of total phenols in fruit varieties that were organically produced compared to conventionally produced fruits (13). It is also possible that cultivation conditions affect the absorption and availability of polyphenolic substances through effects on cell wall structure.

To date, flavonoid studies have mainly concentrated on the effect of single flavonoids given in large doses, either as pure compounds or from specific food sources eaten in large amounts (14). The present study focuses on the excretion of a number of flavonoids at a realistic dietary intake and derived from a variety of flavonoid sources.

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Table 1. Composition of the Intervention Diet (Grams per Day) at an Energy Intake of 10 MJ

meal	component	menu 1	menu 2	menu 3	menu 4
breakfast	bread with carrot	80	80	80	80
	butter	8	8	8	8
	strawberry jam	20	20	20	20
	semiskimmed milk	250	250	250	250
lunch	rye bread	100	100	100	100
	butter	8	8	8	8
	semiskimmed milk	250	250	250	250
	egg	50		50	
	meat pâté	50		50	
	carrot, raisins, and apple juice	120		120	
	meat sausage		60		60
	liver pâté		50		50
	green cabbage		50		50
	peas		30		30
dinner	ham fricassee	350			
	mashed potatoes	300			
	meal loaf		150		
	sauce		147		
	potatoes		200		
	broccoli		100		
	carrots		100		
	meat sausage			150	
	roasted vegetables ^a			391	
	meat sauce				280
snack	pasta				80
	apple cake	100		100	
	carrot cake		108		108
	bread with carrot	80	80	80	80
	strawberry jam	20	20	20	20
	butter	8	8	8	8
	apple juice	250		250	

^a Roasted vegetables: potato, 190 g; cabbage, 100 g; leek, 50 g; onion, 40 g.

Differences between the contents of many different antioxidants and other compounds affecting health could result from the differences in organic and conventional farming and the flavonoids may serve as markers for such differences in content and intake. In the present study we have therefore investigated the effect of conventionally (CPD) and organically produced diets (OPD) on the intake and excretion of five selected flavonoids and on markers of the antioxidative defense in humans. This is the first fully controlled intervention study to investigate the antioxidative effects of a complete OPD.

MATERIALS AND METHODS

Study Design. The study was a double-blinded randomized, crossover design with two intervention periods, each lasting 22 days with a strict control of dietary intake. The intervention periods were separated by a washout period of 3 weeks with habitual diet. Before each intervention period there was a 1 week run-in when the subjects were instructed to exclude flavonoid-containing foods from their diet. Blood samples were collected in the morning on days -1, 0, 22, and 23 in each intervention period; that is, on the two days before, on the last day, and the following day in each intervention period. The subjects were instructed to abstain from heavy physical exercise for 36 h, not to consume alcohol for 24 h, and to be fasting (0.5 L of water was allowed) for 12 h before blood sampling. Twenty-four hour urine samples were collected on days 0 and 22 in each intervention period, that is, on the day before and on the last day in each intervention period. On each urine collection day, the subjects were given 3 × 80 mg of *p*-aminobenzoic acid (PABA, Pharmacy of The Royal Veterinary and Agricultural University, Denmark), that is, one for each main meal to validate the completeness of the urine collection (15).

Diet. The intervention diets included four different menus consumed each week on days 1 and 5, 2 and 6, 3, and 4 and 7, respectively. The menus and the food quantities used in the two diets were identical. The composition of the menus is shown in **Table 1**. The calculated

Table 2. Varietal and Race Characteristics of CPD and OPD

	CPD	OPD
potato	Ukama	Revelino
	Imperia	Sava
	Nicola	
carrot	Premino F1	Napoli F1
	Bolero F1	Nanda
	Nicola	Maetro
leek	Prelina	Imperial
cabbage	Impala F1	Scandic
onion	Hygro	Hyssam
broccoli	Marathon	Matathon
wheat	Unknown	Ure
rye	Unknown	Petkus
apple	Golden Delicious	Golden Delicious
egg	Lohman Brun	Isam Brown
pig		
	sow	Landrace/Yorkshire
boar	Duroc	Duroc

daily intakes of fat, carbohydrate, and protein at an energy intake of 10 MJ were 92 g (35E%), 297 g (51E%), and 84 g (14E%), respectively [calculated from Dankost, a computer program, which is based on the Danish Veterinary and Food Administration food composition database (16)]. Individual portions of the meals were weighed according to estimated energy requirement (17). No foods or drinks other than those provided from the department were allowed. All intake of water was bottled water, and the subjects were allowed to drink coffee prepared from freeze-dried organic or conventional coffee powder. On weekdays, the lunch meal was eaten at the department, whereas breakfasts, snacks, and dinners were handed out and eaten at home. Friday afternoon, all foods for the weekend days were handed out with instructions and suggestions for preparation and consumption. The dinner meal and the water for coffee were heated in a microwave oven, which was supplied by the department.

Intervention Foods. Pork was the only meat used in the study, and the pigs were bred on the same location in Jutland on the Danish Institute of Agricultural Sciences (Research Centre Foulum, Tjele, Denmark). The pigs originated from the same litter and were divided into two groups at weaning, that is, either conventional or organic breeding. The race characteristics are shown in **Table 2**. The sow and the boar were both conventionally bred. All of the manufacturing of the meat, that is, minced meat, meat sausage, and liver pâté, was conducted at The Danish Meat Research Institute (Roskilde, Denmark). Identical recipes omitting additives were used for both the organic and conventional meat products. The conventionally produced dairy products, that is, butter and semiskimmed milk, were supplied by a small dairy (Borup Andelsmejeri, Gørløse, Denmark) where farmers were known to farm conventionally. The organic dairy products were delivered from an organic dairy (Thiese Mejeri, Thiese, Denmark). Eggs were collected directly from organic or conventional farmers by a consultant employed by the Department of Agricultural Systems (Danish Institute of Agricultural Science, Research Center Foulum, Tjele, Denmark). The collection was managed by the Department of Agricultural Systems in cooperation with the Department of Plant Research (Risoe National Laboratory, Roskilde, Denmark). One distributor (Frukt og Grønt rådgivning, Odense, Denmark) collected organically as well as conventionally grown vegetables from fields within a similar geographic location. The aim was to collect vegetables from fields located (1) >3 km from cities with >10000 inhabitants, (2) >3 km from major roads, (3) >10 km from industries with extensive omission, and (4) >15 km from highways. The organically and conventionally grown vegetables used in each intervention period were sowed and harvested within the same week. The organically grown apples were bought from a small shop specializing in organically grown foods. The remaining fruits and groceries, sugar, salt, etc., were bought in a local supermarket. The varietal differences between the organically and conventionally produced foods are shown in **Table 2**. The different varieties used in the study reflected the fruits and vegetables available on the market. Conventionally and organically produced wheat and rye seeds were purchased directly from the farmers by one bakery

(Bageriet Aurion, Hjørring, Denmark), specializing in organic bread production. The seeds were ground into flour, and identical recipes were used for the organic and conventional breads. The breads were produced in large batches and frozen until use.

Subjects. Six males and 10 females, 21–35 years of age with a mean body mass index (BMI) of 23.4 kg/m², volunteered for the study. All subjects were apparently healthy, none of the subjects were pregnant, lactating, or took medicine regularly, and all were nonsmokers. The subjects were instructed not to take dietary supplements or to give blood for 2 months before and during the study. Subjects received oral and written information about the study and gave their written consent. The study was approved by the Research Ethics Committee of Copenhagen and Frederiksberg (J. no. KF01-221/98).

Food Analysis. Duplicate portions of each of the four menus were collected on days 1–4 in the first week of both intervention periods and prepared for analysis as described by Knudsen et al. (18). Each sample was frozen at –20 °C until analysis. For analysis of polyphenols, 10 g of freeze-dried material was extracted twice with 50 mL of methanol and centrifuged at 3000 rpm for 10 min. The collected methanol fraction was washed with 75 mL of heptane three times, and the heptane fractions were discarded. The methanol was evaporated to dryness under reduced pressure. Further cleanup by solid phase extraction proceeded as follows: A 5 g reversed phase C18 Mega Bond Elut cartridge (Varian, Harbor City, CA) was conditioned by passing 10 mL of methanol followed by 20 mL of water. The sample was resuspended in 30 mL of aqueous solution and applied to the cartridge. After passing, the cartridge was rinsed with 20 mL of water. The rest of the sample was dissolved in 10 mL of methanol, which was applied to the cartridge and then washed with 40 mL of methanol. The collected methanol fractions were evaporated to dryness, and 5 mL of 1.2 M HCl (50% MeOH) was added. The mixture was refluxed for 2 h while hydrolyzed at 90 °C on a steam bath and subsequently allowed to cool in a refrigerator. The round-bottom flask used for hydrolysis was washed with 5 mL of methanol, and the final extract was filtered through a 0.45 μm filter (Sartorius AG, Göttingen, Germany).

The high-performance liquid chromatography (HPLC) system consisted of a Waters (Milford, MA) 717 autoinjector, a Waters 616 pump, and a Waters 996 PDA detector. The column was a Phenomenex Prodigy (Torrance, CA) RP C18 column (250 × 4.6 mm, 5 μm) protected by a Phenomenex Securityguard guard column. The mobile phase consisted of 30% methanol/70% water (A) and 100% methanol (B). The gradient was 25–86% B in 50 min at a flow rate of 1 mL/min (isocratic 25% B for 1 min and then a linear gradient changing from 25 to 40% B between 1 and 10 min and from 40 to 43% B between 10 and 24 min, and from 43 to 86% B between 24 and 30 min). For the last 20 min the column was eluted isocratically with 86% B. Each sample was injected three times (20, 50, and 100 μL), and calculations were based on detection at 289 or 368 nm.

Blood Sampling and Analysis. Blood samples were collected with minimal stasis from an antecubital vein in the morning from resting individuals (15–20 min of recumbent rest) with 20 G needles into evacuated EDTA-coated tubes (Becton Dickinson Vacutainer Systems, Becton Dickinson, Plymouth, U.K.). Plasma samples were stored at –80 °C until analysis (maximum 12 months). Erythrocytes were washed three times with 0.9% NaCl, hemolyzed, and stored at –80 °C until analysis (maximum 6 months).

The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and catalase (CAT) in erythrocytes, the Trolox equivalent antioxidant capacity (TEAC), and the ferric reducing ability of plasma (FRAP) were measured by automated assays on a Cobas Mira Plus analyzer (Roche, Diagnostic Systems, Basel, Switzerland). The activities of the enzymes were expressed as per milligram of hemoglobin in the blood samples. SOD, GSH-Px, and TEAC were determined using commercially available kits (SD125, RS506, and NX2332, respectively; Randox, Crumlin, U.K.), whereas GR and CAT activities were determined according to methods described by Wheeler et al. (19). FRAP was determined as described by Benzie and Strain (20). Hemoglobin was determined on a Cobas Minos analyzer (Roche, Diagnostic Systems). Glutathione, flavin adenine dinucleotide, purpald, and potassium periodate were purchased from Sigma Chemical Co. (St. Louis, MO). An internal erythrocyte pool was used as standard

for the analysis of antioxidative enzymes and analyzed in duplicate with every person–series. The accepted interval was the established mean ± 2 standard deviations (SD) for each of the four enzyme activities; otherwise, the person–series was reanalyzed. The within-run coefficient of variation (CV) for a standard sample was ≤7% ($n = 24$), and the between-run was ≤11% for all four enzyme activities ($n = 16$). A standard plasma sample analyzed together with the test samples from the current study had a CV for TEAC of 5.7% ($n = 15$) and a CV for FRAP of 0.85% ($n = 14$).

Malondialdehyde (MDA) was determined in plasma by using a thiobarbituric acid-reactive substance (TBARS) HPLC method as previously described in Young et al. (21). All analyses were performed on the same day, and the mean intraday variation between double determinations was 5.8%. Determination of 2-amino adipic semialdehyde (2-AAS) in plasma was performed as previously described by Daneshvar et al. (22). Mean intraday variation for a standard plasma sample analyzed together with the samples from the current study was <7% ($n = 4$).

Urine Sampling and Analysis. Twenty-four hour urine samples were collected on days 0 and 22 in acid-washed plastic bottles containing 50 mL of 1 M HCl and 10 mL of 10% (w/v) ascorbic acid. Urine samples were weighed, density was measured, and pH was adjusted to 3–4 with 1 M HCl. Aliquots of 250 mL were stored at –20 °C until analysis. The following flavonoids were quantified in the urine samples by LC-MS as described by Nielsen et al. (23): quercetin, kaempferol, and isorhamnetin. The flavanones naringenin and hesperetin were also quantified. In brief, 250 ng of 5,7,8-trihydroxyflavone was added to 2 mL of urine sample as internal standard and enzymatically hydrolyzed as described elsewhere (23). After hydrolysis, 2 mL of ice-cold methanol was added to stop the reaction, and the samples were evaporated to dryness under vacuum. The hydrolyzed samples were redissolved in 10% aqueous methanol, and 250 ng of morin was added as an additional internal standard to assess the performance of the mass spectrometer, giving a final volume of 250 μL. The sample was then centrifuged at 10000g for 5 min at 4 °C, and the entire amount of the supernatant was injected onto the LC-MS system. Prior to and after each series of analysis the performance of the entire LC-MS assay was controlled by injections of aliquots containing all employed flavonoid standards, including the internal standards. Determinations were carried out singly. Two control urine samples spiked with 250 ng of all flavonoids included in the assay were included in each series of analyses. The recovery was 248.2 ± 18 ng (mean ± SD, $n = 13$) with an intraday CV% of 11.3% ± 4.2.

Statistical Analysis. Biomarker analyses were performed on duplicate blood samples (taken on two successive days) before and after each intervention period. Flavonoid analysis on urine samples was performed on single samples (before and after each intervention period). Food analyses were performed on duplicate portions. The means of duplicates were used in the statistical analyses. All of the measured erythrocyte and plasma biomarkers showed a normal distribution, whereas the content of investigated flavonoids in the diet and excretion of flavonoids in urine did not. The effect of period and the presence of carry-over with respect to biomarkers and excretion of the investigated flavonoids in urine were determined according to the methods of Woods et al. (24) and Armitage and Berry (25). TEAC showed clear evidence of carry-over into the second period, and the effect of intervention was consequently determined by comparing the two groups by unpaired *t* test in the first period only. Paired and unpaired comparisons, using the Wilcoxon signed rank test and the Mann–Whitney test, respectively, were performed for the flavonoids in diet and urine. The SPSS statistical package (SPSS Inc., Chicago, IL) was used to perform all analyses.

RESULTS

The body weights of the subjects were 71.8 ± 12.9 kg (mean ± SD), and no significant changes during the study were observed. The average daily energy intake was 12.0 ± 2.7 MJ. Recovery of PABA in urine was 99.6 ± 6.9% (mean ± SD).

The contents of the examined flavonoids in the CPD and OPD are shown in **Table 3**. The OPD was found to contain

Table 3. Content of Flavonoids in the Organically and Conventionally Produced Intervention Diets (Micrograms per 10 MJ)

flavonoid	intervention period ^a	
	CPD	OPD
quercetin	2632 ± 774 ^b	4198 ± 1370 ^b
kaempferol	333 ± 328	608 ± 352
hesperetin	31 ± 330	0 ± 547
naringenin	0 ± 133	0 ± 603
isorhamnetin	496 ± 93	0 ± 327

^a Median ± SD determined in dietary samples based on 7 days of dietary intake in each intervention period. ^b Contents of flavonoids in the two dietary treatments were significantly different (Mann–Whitney *U* test): *P* < 0.01.

Table 4. Excretion of Flavonoids in Urine Samples^a

flavonoid	baseline ^c	intervention period ^b	
		CPD	OPD
quercetin			
μg/24 h	6 ± 3	19 ± 2 ^{d,g}	27 ± 3 ^{e,g}
% of intake		0.57 ± 0.07	0.48 ± 0.08
kaempferol			
μg/24 h	0.0 ± 0.3	2 ± 1 ^{d,g}	5 ± 4 ^{f,g}
% of intake		0.6 ± 0.2	0.7 ± 0.6
hesperetin			
μg/24 h	3 ± 3	4 ± 43	10 ± 31
% of intake		0.9 ± 9.8	0.8 ± 3.1
naringenin			
μg/24 h	29 ± 7	29 ± 24	32 ± 50
% of intake		4.1 ± 3.1	1.9 ± 2.9
isorhamnetin			
μg/24 h	0.0 ± 0.3	0.0 ± 0.4	0.0 ± 0.9
% of intake		0.0 ± 0.05	0.0 ± 0.1

^a Median ± SEM; *n* = 16. Determined in 24-h urine samples from 16 subjects. ^b Values determined in urine samples from the last day in intervention (day 22). ^c Baseline values determined in urine samples from day 0; i.e., on subjects' habitual diet without tea, wine, spices, vegetables, and fruit. ^{d–f} Significantly different on last day in intervention from baseline (Wilcoxon rank scores): ^d, *P* < 0.05; ^e, *P* < 0.01; ^f, *P* ≤ 0.01. ^g The effects of the dietary treatments are significantly different (Wilcoxon rank scores): *P* < 0.05.

significantly higher amounts of quercetin (*P* < 0.01) compared to the CPD, and there was a trend toward a higher content of isorhamnetin in the CPD (*P* = 0.07) and a higher content of kaempferol in the OPD (*P* = 0.10). The contents of the five selected flavonoids in the two intervention periods were similar for both diets.

The urinary excretion (micrograms per 24 h) of quercetin and kaempferol was significantly higher after intake of the OPD compared to the CPD (*P* < 0.05) (Table 4), whereas no differences were seen between the two intervention periods with respect to the other measured flavonoids. The production method did not affect the average urinary excretion of the measured flavonoids as a percentage of intake (Table 4). Furthermore, a high interindividual variation was observed. Especially one individual excreted a high amount of the investigated flavonoids compared to the other subjects (data not shown).

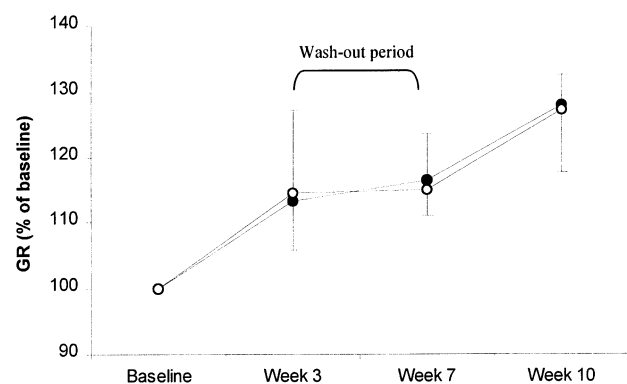
The effects of the two intervention diets on biomarkers of antioxidative status are shown in Table 5. For the majority of the markers there was no difference between diets. However, a significant carry-over effect was observed for TEAC. Consequently, the values are based on results from the first period only, which showed that TEAC was significantly higher after intake of the CPD (*P* < 0.05) compared to the OPD.

The excretion (micrograms per 24 h) of quercetin and kaempferol increased significantly after both diets when com-

Table 5. Effect of Dietary Treatment on Biomarkers of Antioxidative Status^a

blood parameter	baseline ^c	intervention period ^b	
		CPD	OPD
CuZn-SOD (units/g of Hb)	1321 ± 135	1297 ± 134	1294 ± 157
CAT (units/g of Hb)	19.76 ± 2.11	20.32 ± 3.08	20.06 ± 2.86
GSH-Px (units/g of Hb)	46.32 ± 10.27	44.94 ± 10.26 ^d	45.10 ± 9.79 ^e
GR (units/g of Hb)	10.21 ± 1.43	11.49 ± 1.92 ^f	11.30 ± 1.47 ^g
FRAP (nmol/L)	857 ± 195	823 ± 160	829 ± 127
TEAC (mmol/L) ^h	0.996 ± 0.119	1.029 ± 0.074 ^h	0.951 ± 0.056 ^h
2-AAS (pmol/mg of protein)	17.86 ± 1.95	18.99 ± 2.15	19.33 ± 2.17 ^e
MDA (pmol/mg of protein)	32.60 ± 6.51	32.93 ± 5.99	33.31 ± 5.84

^a Mean ± SD; *n* = 16. ^b Values are determined as mean values of blood samples from days 22 and 23. ^c Baseline values determined in blood samples from days -1 and 0, i.e., on subjects' habitual diet without tea, wine, spices, vegetables, and fruit. ^d TEAC values are based on results from the first intervention period. ^{e–g} Significantly different on the two last days in intervention from baseline (paired samples *t* test): ^e, *P* < 0.05; ^f, *P* < 0.01; ^g, *P* < 0.001. ^h The effects of the dietary treatments are significantly different (independent samples *t* test): *P* < 0.05.

**Figure 1.** Percentage change in the activity of GR at baseline and at weeks 3, 7, and 10 in the experiment in eight subjects from group A (●) (given the OPD in the first 22-day period) and eight subjects from group B (○) (given the CPD in the first 22-day period). There was a wash-out period between weeks 3 and 7. Values are means with standard deviation represented by vertical bars.

pared to baseline, whereas no significant change was seen for the remaining flavonoids (Table 4).

There was a significant increase in the activity of GR (*P* < 0.001 and *P* < 0.01 CPD and OPD, respectively) and a significant decrease in the activity of GSH-Px (*P* < 0.01 and *P* < 0.05 CPD and OPD, respectively) for both intervention diets compared to the baseline values, that is, activities at flavonoid-reduced diet. There was an increase in 2-AAS (*P* < 0.05) after intake of the OPD but not after the CPD. However, when the mean of both interventions was compared with baseline (data not shown), there was a significant increase in 2-AAS (*P* < 0.05). No statistically significant effects of dietary treatments when compared to baseline were observed for SOD, CAT, FRAP, and MDA.

During the whole experiment, a significantly increased activity of GR was observed when week 10 was compared with baseline (*P* < 0.001) (Figure 1).

DISCUSSION

The present study is the first to investigate the influence of growth conditions (conventional vs organic) on the levels of selected flavonoids in the diet and on urinary excretion of flavonoids. The present study showed a higher content of quercetin in the OPD than in the CPD, which was also reflected

in the urinary levels of quercetin with a higher excretion after the OPD. Similarly, the excretion of kaempferol in urine was significantly higher after intake of OPD than CPD, although there was only a nonsignificant trend for a higher content of kaempferol in the OPD. It cannot be excluded that the difference in quercetin content could be due to varietal differences because the flavonoid contents in different varieties of fruits and vegetables may show a large variation (2, 26, 27). Hence, any differences between the two types of diet could originate from varietal differences. The different varieties used in the present study reflect the organic and conventional varieties of fruits and vegetables available on the market and thus reflect a realistic composition of the diet seen from a consumer's perspective. Thus, it can be concluded that the growth conditions and varietal differences between conventional and organic fruits and vegetables significantly affect the content of the investigated flavonoids and may result in differences in the urinary excretion of flavonoids.

The decrease in TEAC with OPD and the increase with CPD seem to contradict the observations of a higher level of flavonoids in the OPD. A previous study with flavonoid-rich diets did not affect TEAC (21, 28), indicating that other factors in the OPD may have affected this marker.

In the present study, the proportions of the excreted flavonoids were similar in both interventions. Only a small fraction of the flavonoid intake was excreted in urine (0.0–4.1%). This is in agreement with other studies that have found an excretion of the flavonol quercetin between 0.3 and 1.4% after intake of onions, apples, and fruit juice (21, 29–31). The flavanones hesperetin and naringenin may be excreted in high amounts (up to 30% of intake) when ingested as large doses of grapefruit juice (1250 mL) and excreted as smaller quantities (up to 7% of intake) when ingested as orange juice (1250 mL) (32). If the urinary level of flavonoids is presumed to reflect the exposure dose, then, for example, the flavonol quercetin is apparently of minor quantitative importance, at a realistic dietary intake of fruits and vegetables. Other flavonoids, such as the citrus flavanones, may be more important to humans, because the urinary excretion is higher, even when they are present in limited amounts in the diet (33–35).

In the present study, an increased activity of GR was seen during the total experimental period (Figure 1), and it is in accordance with other studies, which included intervention diets with a high level of antioxidants (21, 28). A decreased activity of GR has previously been observed after depletion of fruits and vegetables during an intervention trial (33), indicating that the high intake of these food items in the present study rather than the intervention per se was responsible for the effect (28). In this study glutathione peroxidase activity decreased with the dietary change, which is not in accordance with previous observations after diets rich in secondary plant metabolites for which we have observed either no effect (21, 28) or an increase (36).

The increase in 2-AAS with the OPD when compared to baseline values indicates an increased oxidative damage to proteins after intake of organically produced foods. However, in this study no differences between the two types of intervention at the end of intervention were seen, and there was a significant mean increase in 2-AAS when CPD and OPD were combined. This suggests that the observed increase is a general effect of the intake of fruits and vegetables. We have earlier observed increases in 2-AAS after intake of fruits and vegetables (21) and decreases in 2-AAS after intake of a diet low in flavonoids (36). 2-AAS has been observed to correlate positively with

plasma ascorbate (28) and thus seems to paradoxically reflect a good status in water-soluble redox-active substances, including flavonoids and ascorbate.

A large interindividual variation in the urinary excretion of all of the investigated flavonoids was observed, which is in accordance with other studies on flavonoids (32, 33, 37). Seemingly the bioavailability of flavonoids is high in some individuals probably due to variations in intestinal physiology or microflora. However, when larger groups of subjects are studied, urinary flavonoid excretion is closely correlated to intake of fruits and vegetables (38).

In conclusion, the growing conditions of fruits and vegetables (conventional vs organic) affected the content of five selected flavonoids and resulted in differences in the urinary excretion of major dietary flavonoids. Also, markers of antioxidative defense were affected by the food production method. Because selection of more resistant varieties is of central importance to organic farming, it cannot be excluded that the observed effects originate from the varietal differences between the OPD and CPD rather than from the differences in handling procedures, including pesticide use.

ABBREVIATIONS USED

2-AAS, 2-aminoadipic semialdehyde; CAT, catalase; CPD, conventionally produced diet; FRAP, ferric reducing ability of plasma; GR, glutathione reductase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; OPD, organically produced diet; PABA, *p*-amino benzoic acid; SOD, superoxide dismutase; TEAC, Trolox equivalent antioxidant capacity.

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