JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Bolus Consumption of a Specifically Designed Fruit Juice Rich in Anthocyanins and Ascorbic Acid Did Not Influence Markers of Antioxidative Defense in Healthy Humans

Sabine Ellinger,*[,†] André Gordon,[‡] Mira Kürten,[†,‡] Elvira Jungfer,[‡] Benno F. Zimmermann,[‡,§] Berndt Zur,[#] Jörg Ellinger,[⊥] Friedhelm Marx,[‡] and Peter Stehle[†]

[†]Department of Nutrition and Food Sciences, Nutritional Physiology, [‡]Department of Nutrition and Food Sciences, Food Chemistry, University of Bonn, Endenicher Allee 11-13, 53115 Bonn, Germany

[§]Institute Prof. Dr. Kurz GmbH, Eupener Strasse 161, 50933 Cologne, Germany

[#]Department of Urology and Children Urology and [⊥]Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany

ABSTRACT: Exotic fruits such as açai, camu-camu, and blackberries rich in natural antioxidants (ascorbic acid, anthocyanins) are marketed as "functional" foods supporting a pro-/antioxidant balance. Confirming data from human studies are lacking. Within a randomized controlled crossover trial, 12 healthy nonsmokers ingested 400 mL of a blended juice of these fruits or a sugar solution (control). Blood was drawn before and afterward to determine antioxidants in plasma, markers of antioxidant capacity [trolox equivalent antioxidant capacity, Folin−Ciocalteu reducing capacity, total oxidant scavenging capacity (TOSC)] and oxidative stress [isoprostane, DNA strand breaks in leukocytes in vivo], and their resistance versus H_2O_2-induced strand breaks. Compared with sugar solution, juice consumption increased plasma ascorbic acid and maintained TOSC and partly Folin−Ciocalteu reducing capacity (both P values < 0.05). Strand breaks in vivo increased after ingestion of both beverages (P < 0.001), probably due to postprandial and/or circadian effects. This anthocyanin-rich fruit juice may stabilize the pro-/antioxidant balance in healthy nonsmokers without affecting markers of oxidative stress.

KEYWORDS: *exotic fruit juice, ascorbic acid, anthocyanins, antioxidant properties, intervention study*

INTRODUCTION

There is convincing epidemiological evidence that regular fruit and vegetable consumption contributes to decrease the risk of several chronic diseases such as coronary heart disease[1] and probably certain kinds of cancer.[2] It is hypothesized that antioxidative ingredients such as polyphenols and water-soluble vitamins are the decisive factors explaining the health-promoting properties of fruits and vegetables.[3]

This scientific evidence is worldwide translated into public health initiatives such as "5 a day" and school fruit programs with the goal to increase daily consumption of fruits and vegetables. Encouraged by these policy-driven actions, the food industry is strongly engaged in launching novel fruit (and vegetable)-based products on the market. Blended juices, juice concentrates, and smoothies rich in polyphenols and water-soluble vitamins such as ascorbic acid are marketed as health-supporting foods specifically preventing radical-driven chronic diseases.

In this respect, economic and scientific interests are focused on fruits frequently consumed in South America. Camu-camu (*Myrciaria dubia*) grows in the Amazon region and contains anthocyanins (30−50 mg/100 g; cyanidin-3-glucoside accounts for 89% of total anthocyanin), and an extraordinarily high amount of ascorbic acid (up to 3.0 g/100 g pulp).[4] Another popular fruit in Central and South America is açai (*Euterpe oleraceae* Mart.). It is also rich in anthocyanins, especially in cyanidin-3-glucoside (up to 456 mg/L pulp).[2,5] These ingredients are thought to contribute to the health-promoting effects of these exotic fruits, partly due to their high antioxidant capacity in vitro.[4−6] Andean blackberries (*Rubus ssp.*) similarly present a high antioxidant capacity because of their high content in ellagtannins.[7]

Controlled clinical trials to evaluate the protective effects of these fruits are scarce. Daily intake of 70 mL of camu-camu juice for 1 week reduced urinary 8-hydroxydesoxyguanosine, a biomarker of DNA damage, which did not occur after ingestion of equal amounts of isolated ascorbic acid (1050 mg/day).[8] Intervention studies in healthy nonsmokers have shown that bolus consumption of açai (pulp or juice)[9] or a juice blend with açai as predominant ingredient[10] increased antioxidant capacity in plasma[9] and erythrocytes,[10] respectively, and decreased lipid peroxidation.[10] Even if individual markers on antioxidant capacity and oxidative stress were improved in these studies, a comprehensive picture concerning antioxidant defense is lacking.

The aim of our study was, thus, to investigate the effects of a bolus consumption of a blended juice made of açai, Andean blackberries, and camu-camu on the concentrations of plasma antioxidants, plasma antioxidant capacity, and markers of oxidative stress in healthy nonsmokers. A secondary goal was

Received: February 27, 2012
Revised: September 20, 2012
Accepted: October 17, 2012
Published: October 17, 2012

to detect and characterize metabolites of anthocyanins with reducing properties in plasma using a HPLC technology.

MATERIALS AND METHODS

Chemicals. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), 2′2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), catechin, Folin−Ciocalteu's phenol reagent, metaphosphoric acid, phosphate-buffered saline, hydrogen peroxide, sodium lauryl sulfate, *N*-lauroylsarcosine sodium salt, EDTA, and propidium iodide were purchased from Sigma-Aldrich, Taufkirchen, Germany. α-Keto-γ-methiolbutyric acid (KMBA) (≥97.5%), diethylenetriaminepentaacetic acid (DTPA) (≥99%), 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1), 2′-azobis(2-methylpropionamidine) dichloride (ABAP) (≥97%), gallic acid (≥97.5%), vanillic acid (≥97%), ferulic acid (≥99%), and formic acid were obtained from Sigma-Aldrich, Steinheim, Germany. L-Ascorbic acid, protocatechuic acid (≥97%), butylhydroxytoluol, perchloric acid, sodium dihydrogen phosphate-hydrate, ethanol, borate, and orthophosphoric acid were purchased from Merck, Darmstadt, Germany. Retinol, β-carotene, apocarotenal, α-tocopherol, and sodium carbonate were from Fluka, Buchs, Switzerland. Caffeic acid (purum) and low-melting agarose were maintained from Serva, Heidelberg, Germany, trifluoroacetic acid (extra pure, 99%) was obtained from Acros Organics, Geel, Belgium, and tris(hydroxymethyl)aminomethane was obtained from Applichem, Darmstadt, Germany. Acetone, ethanol, hexane, and isopropanol were received from Roth, Karlsruhe, Germany. Methanol, acetonitrile, and sodium dihydrogen phosphate were purchased from Fisher Scientific, Loughborough, UK.

Subjects and Study Design. To reach these study goals, we performed a randomized, controlled trial with crossover design. The primary end point was the assessment of total antioxidative capacity in blood. Sample size calculation was based on data from a preliminary trial with three healthy nonsmokers ingesting a bolus of 400 mL of the juice blend under standardized conditions. Trolox equivalent antioxidant capacity (TEAC) increased from 1.25 mmol/L trolox equivalents (TE; baseline) to 1.36 mmol/L TE (0.5 h; maximal TEAC). Considering a standard deviation of 0.075 mmol/L TE, a difference of 0.11 mmol/L TE could be detected with $\alpha = 0.05$ and a power of 90% if 11 participants were recruited. To account for dropouts, 12 participants were included in our study.

Participants (18−50 years, body mass index between 18.5 and 24.9 kg/m^2, nonsmokers for at least 6 months) were recruited within our staff. Exclusion criteria (questionnaire) were known hepatic/gastrointestinal disorders, pregnancy or breastfeeding, or regular use of vitamin or flavonoid-rich supplements. Participants were asked about their usual intake of fruits and vegetables (portions per day).

The randomization into two equal groups was done by lots. Randomization was stratified by sex to ensure an equal number of males and females in each group. Group A first ingested 400 mL of a fruit juice blend after a 12 h overnight fast; after a wash-out period of 2−3 weeks, they received 400 mL of a solution with equal amounts of monosaccharides (13.0 g of glucose, 7.2 g of fructose) as control. Group B consumed the test beverages in reversed order.

To avoid artifacts by other foods, subjects were instructed to abstain from foods rich in polyphenols (hand-out) starting 24 h before the first study day until completion. On the study day, participants received a standardized meal (two bread rolls with butter and cheese) 3 and 5 h after consumption of the study beverages. Ad libitum consumption of water was allowed.

Written informed consent was obtained from all participants. The study was conducted according to the Declaration of Helsinki and was approved by the Ethical Committee of the University of Bonn (No. 207/08).

Juice Composition. The fruit juice blend consisted of 44% açai, 12% camu-camu, and 44% blackberry juice. The fruit juice was produced according to the technological standards for the production of customary in trade from a commercially available frozen açai puree (açai juice pads; Açai GmbH, Berlin, Germany), camu-camu pulp [Brazilian Agricultural Research Corporation (Embrapa)], and Costa Rican blackberry juice [Centro National de Investigacion en Tecnologia de Alimentos (CITA), Costa Rica]. As the capacity to scavange peroxyl and hydroxyl radicals as well as peroxynitrite in vitro was different for these fruits (açai, high capacity to scavange peroxyl radicals; camu-camu, good scavenger for peroxyl radicals and peroxynitrite; blackberry, effective against hydroxyl radicals), maximum capacity in vitro could be achieved by the above-mentioned combination of fruits (data not shown). For this reason, this combination of fruit was used in our trial to obtain a strong antioxidant protection in vivo.

Genuine glucose and fructose contents of the juice were 8.0 and 7.2 g/400 mL, respectively, as determined by using the enzymatic D-glucose/D-fructose test kit (Boehringer, Mannheim, Germany). Five grams of glucose was added to 400 mL of juice to make it tastier. Glucose and fructose contents of the control beverage were adjusted to the juice values. This was important to avoid confounding effects by fructose, which is degraded endogenously to uric acid and can, thus, exhibit antioxidative effects.[11,12] The juice composition is described in Table 1. As expected, cyanidin-3-glucoside and cyanidin-3-rutinoside were the major anthocyanins, contributing 84% of the total peak area in the HPLC chromatogram. The remaining peak area is distributed over five minor peaks, which were identified as anthocyanins according to their UV/vis spectra, but not further characterized. In addition, the juice provided flavanols, flavanols, hydrolyzable tannins, and considerable amounts of ascorbic acid.

Table 1. Composition of a Single Portion (400 mL) of the Study Juice

	unit	juice	method	reference
cyanidin-3-glucoside	mg	11.9	HPLC-UV/vis[a]	
cyanidin-3-*O*-rutinoside	mg	16.0	HPLC-UV/vis[a]	
ascorbic acid	mg	936	HPLC-UV	42
total phenolic content	mg GAE[b]	1612	photometry	43
total flavan-3-ols	mg A2E[c]	3.49	photometry	44
total flavonols	mg QE[d]	27.9	photometry	45
total hydrolyzable tannins	mg TAE[e]	413	photometry	46

[a]Identified by LC-MS/MS, quantified using authentic reference compounds. [b]Using gallic acid as reference (GAE = gallic acid equivalents). [c]Using proanthocyanidin A2 as reference (A2E = proanthocyanidin A2 equivalents). [d]Using quercetin as reference (QE = quercetin equivalents). [e]Using tannic acid as reference (TAE = tannic acid equivalents), samples and standard solutions were cooled in an ice bath and for reaction incubated at 30 °C for 30 min; a juice sample without addition of the reagent was used as blank. Investigations were done at least in duplicate. Data are means.

Blood Sampling. Blood samples (7.2 mL each) were collected before and 30, 60, 90, 120, 180, and 360 min after consumption of the study drink using tubes (S-Monovette, Sarstedt, Nümbrecht, Germany) coated with EDTA [analyses of ascorbic acid, fat-soluble pro-/vitamins, antioxidant capacity, 8-isoprostaglandine $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) and phenolic acids in plasma], heparin (determination of DNA strand breaks in leukocytes), and tubes without anticoagulant (analysis of cholesterol, triglycerides, and uric acid).

Preparation of Plasma Samples. After blood withdrawal, EDTA tubes were placed on ice immediately. Then, blood was centrifuged at 3000*g* for 20 min at 4 °C. For the determination of ascorbic acid, 500 μL of a cold 6% perchloric acid/2% metaphosphoric acid solution (v/v) was added to 500 μL of fresh EDTA plasma to precipitate proteins and to stabilize ascorbic acid. After centrifugation (3000*g*, 10 min, 4 °C), the supernatant was aliquoted and stored at −80 °C until analysis. To avoid oxidation, 10 μL of butylhydroxytoluol (0.5 wt %/v in ethanol) was added to 1000 μL of fresh EDTA plasma for later analysis of 8-iso-$PGF_{2\alpha}$ and fat-soluble pro-/vitamins. Ten microliters of a solution of 0.4 M NaH_2PO_4 with 20% ascorbic acid and 0.1% EDTA

Table 2. Plasma Antioxidant Capacity and 8-Isoprostaglandine $F_{2\alpha}$ before and after Consumption of Juice or Control Beverage[a]

	TEAC (mmol/L TE)		FCR (mg CE/L)		TOSC[b]		8-iso-$PGF_{2\alpha}$ (pg/mL)	
	juice	control	juice	control	juice	control	juice	control
0 h	1.26 ± 0.13	1.21 ± 0.14	21.4 ± 4.0	22.3 ± 5.3	43.7 ± 3.2	44.2 ± 2.4	17.1 ± 8.2	18.7 ± 8.5
0.5 h	1.25 ± 0.11	1.21 ± 0.14	21.0 ± 3.6	21.3 ± 4.4	43.6 ± 3.0	41.3 ± 3.5**	17.1 ± 5.3	15.4 ± 7.7
1.0 h	1.29 ± 0.14	1.23 ± 0.14	20.8 ± 2.6	20.3 ± 3.5	44.9 ± 3.7	41.7 ± 3.1*	16.1 ± 10.0	18.3 ± 10.5
1.5 h	1.25 ± 0.13	1.23 ± 0.14	21.5 ± 3.9	19.5 ± 2.8	46.0 ± 4.1	41.6 ± 2.6	19.0 ± 14.3	22.2 ± 12.8
2.0 h	1.25 ± 0.15	1.23 ± 0.16	21.1 ± 2.9	21.3 ± 3.2	44.1 ± 4.7	41.6 ± 2.3*	15.2 ± 6.5	22.5 ± 15.5
3.0 h	1.24 ± 0.12	1.22 ± 0.15	21.4 ± 3.1	21.8 ± 3.2	43.4 ± 3.0	41.6 ± 2.2**	17.7 ± 8.1	16.8 ± 10.3
6.0 h	1.24 ± 0.13	1.24 ± 0.14	21.7 ± 3.1	22.1 ± 2.9	46.6 ± 4.2	41.6 ± 3.0**	17.7 ± 7.9	19.0 ± 12.1

[a]Data are the mean ± SD, based on $n = 12$, except for TOSC ($n = 9$). CE, catechin equivalents; FCR, Folin−Ciocalteu reducing capacity; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandine $F_{2\alpha}$; TE, trolox equivalents; TEAC, trolox equivalent antioxidant capacity; TOSC, total oxidant scavenging capacity. [b]Repeated-measures ANOVA showed significant interactions by time × beverage ($P = 0.049$) for TOSC. Separate analysis of the changes for juice and sugar solution indicated that changes were significant only after ingestion of sugar solution. Significant differences versus baseline were analyzed with a paired t test: *, $P < 0.05$; **, $P < 0.01$. Relative changes compared to baseline were not different for both treatments for TEAC and 8-iso-$PGF_{2\alpha}$, whereas treatment differences could be detected for FCR and TOSC after 0.5, 1.5, and 6 h postconsumption and for TOSC additionally after 2 h. Differences in the AUC of TEAC, FCR, TOSC, and 8-iso-$PGF_{2\alpha}$ between the consumption of juice and sugar solution did not occur.

(pH 3.6) was added to 500 μL of plasma in which phenolic acids should be detected. Heparinized blood was used immediately for the determination of DNA strand breaks in leukocytes.

Dietary Intake of Energy and Nutrients. The intake of energy, macronutrients, dietary fiber, and antioxidant pro-/vitamins on the day before the study was documented in a prospective 24 h dietary record and calculated using Ebis Pro 4.0 software (University of Hohenheim, Stuttgart, Germany) based on German Nutrient database, version II.3. The flavonoid intake was calculated by using the USDA database.[13]

Analytical Methods. *Plasma Antioxidant Capacity.* Because no single assay truly reflects overall antioxidant capacity, multiple assays with different radicals and mechanisms (hydrogen or electron transfer, which reflect radical quenching and radical reduction, respectively) should be used.[14] Thus, TEAC[15] (CV 1.2%) was measured and expressed as TE. Furthermore, the Folin−Ciocalteu reducing capacity (FCR)[14] was assessed with the modifications of Arendt et al.[16] to avoid interferences with plasma proteins (CV 2.0%). The FCR of plasma was expressed as catechin equivalents. Additionally, total oxidant scavenging capacity (TOSC) in plasma [diluted 1:20 (v/v) with aqua dest.] was determined against peroxyl radicals (CV 3.6%) according to the method of Lichtenthäler et al.[17]

Concentrations of Antioxidants in Plasma/Serum. Ascorbic acid in plasma was measured by HPLC with UV/vis detection at 243 nm (CV 1.8%), which is a standard procedure for the analysis of plasma samples. Analysis was done according to the method of Steffan.[18]

α-Tocopherol and β-carotene were also determined by HPLC. The protocol of Erhardt et al.[19] was modified by using apocarotenal as internal standard, Nucleosil 100-5 CN (Macherey-Nagel, Düren, Germany) as column, and a solution of 98% hexane and 2% isopropanol as mobile phase. α-Tocopherol was detected at 292 nm (CV 4.1%) and β-carotene at 450 nm (CV 3.5%).

Uric acid in serum was determined photometrically within routine analysis (Urea Flex reagents cassette, Dimension Vista 1500, Siemens Healthcare Diagnostics, Eschborn, Germany) (CV 1% according to the manufacturer).

Phenolic Acids in Plasma. A solid phase extraction using Supel-Select HLB SPE tubes (bed wt, 60 mg; volume, 3 mL) (Supelco, Steinheim, Germany) was performed to eliminate plasma proteins. After conditioning with 6 mL methanol and 6 mL 0.1% formic acid, the cartridge was loaded with plasma (450 μL). After washing with 6 mL aqua dest., 6 mL of a mixture of methanol, acetonitrile, and formic acid (50:49.9:0.1, v/v/v) was used for elution. The eluate was evaporated under nitrogen to dryness and reconstituted with a solution (50 μL) of methanol, water, and trifluoroacetic acid (20:79.9:0.1, v/v/v).

Thereafter, single compounds with reducing capacity were determined in the samples by HPLC-CEAD detection at 100, 200, 300, and 400 mV using the conditions (instrument settings, elution) previously described by Zimmermann et al.[20] and Ritter et al.[21] The analytical column was an Aqua 3 μm C18, 150 mm, 4.6 mm i.d., with a guard column (Security Guard, Aqua RP-18, 4 mm, 3 mm i.d.) (both from Phenomenex, Aschaffenburg, Germany). For analysis, 20 μL of the sample (plasma, juice, and protocatechuic acid, gallic acid, vanillic acid, ferulic acid, and caffeic acid as standards) was injected. The limits of detection were 2 μg/L for gallic acid, 2.5 μg/L for protocatechuic acid, 7.5 μg/L for caffeic acid, 25 μg/L for vanillic acid, and 2.5 μg/L for ferulic acid, estimated using a signal-to-noise ratio of 3.

Lipid Peroxidation. Total 8-iso-$PGF_{2\alpha}$ concentration was determined as sum of free plus esterified 8-iso-$PGF_{2\alpha}$ in EDTA-plasma by an ELISA kit (Cayman Chemical, Ann Arbor, MI, USA; CV 10%) as described previously.[22]

DNA Strand Breaks. DNA single-strand breaks (SB) were measured in leukocytes in vivo and after 20 min of incubation at 4 °C with 300 μM H_2O_2 ex vivo using the single-cell gel electrophoresis assay (also called Comet assay). The protocol of Arendt et al.[16] was used; however, the electrophoresis period was extended to 20 min. Fifty nuclei per slide were evaluated for DNA damage by calculating tail moment with Comet Assay III software (Perceptive Instruments, Suffolk, UK). The difference in tail moment between untreated cells (SB in vivo) and cells challenged with H_2O_2 was calculated to determine the resistance of DNA versus H_2O_2 ex vivo (CV 22%).

Triglycerides and Cholesterol. Triglycerides in serum as well as total cholesterol were measured within routine procedures by using Flex reagent cartridges and the Dimension Vista System (Siemens Healthcare Diagnostics; CV 3% for triglycerides and 5% for cholesterol according to the manufacturer). Cholesterol concentration was determined to calculate the α-tocopherol to cholesterol ratio as an indicator for the α-tocopherol status.

Statistics. Because data were normally distributed according to the Kolmogorov−Smirnow test, parametrical tests were used. The effect of beverage, time, interactions of beverage and time (beverage × time), and the order of beverage intake on laboratory parameters was investigated with repeated-measures ANOVA. In the case of significant effects, a paired t test was performed subsequently. In addition, relative changes from baseline to each postconsumption data point were determined to compare the changes within each subject between both treatments by using the paired t test. The area under the curve (AUC) was calculated for all parameters by the trapezoidal rule for nonuniform intervals. AUC obtained for each subject after consumption of juice and sugar solution was compared for both treatments by a paired t test. Results are shown as the mean and standard deviation. Statistical evaluation was performed with PASW Statistics, version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Six males and six females with a mean age of 33 ± 7 years and a body mass index of 23.0 ± 3.1 kg/m^2 participated in our study.

Their usual intake of fruits plus vegetables was 3.3 ± 0.8 portions per day. The intake of energy (8.54 ± 2.27 MJ), protein (86 ± 28 g), fat (95 ± 38 g), carbohydrates (205 ± 65 g), dietary fiber (12 ± 5 g), ascorbic acid (18 ± 16 mg), β-carotene (0.4 ± 0.2 mg), vitamin E (5 ± 3 mg α-tocopherol equivalents), and flavonoids (0.5 ± 0.7 mg) was not significantly different between the days before each study day. This indicates an excellent compliance to dietary restrictions.

TEAC and FCR as parameters of plasma antioxidative capacity were not affected by beverage, time, and interactions between beverage and time (Table 2), and AUCs were comparable. Within-subject changes in TEAC relative to baseline were not different between the treatments (data not shown), but differed in FCR at 0.5, 1.5, and 6 h postconsumption (all P values < 0.05). TOSC values decreased significantly over time only after the ingestion of sugar solution (P = 0.02) and reached lower values after 0.5, 1, 2, 3, and 6 h compared to baseline (all P values < 0.05) (Table 2). Relative changes versus baseline were different between the treatments after 0.5, 1.0, 1.5, and 6 h (all P values < 0.05). However, the AUC of TOSC was not different after ingestion of juice compared to control beverage (266 ± 16 vs 250 ± 11%·h; P = 0.054).

As shown in Figure 1, ascorbic acid concentration in plasma was dependent on interactions between beverage and time.

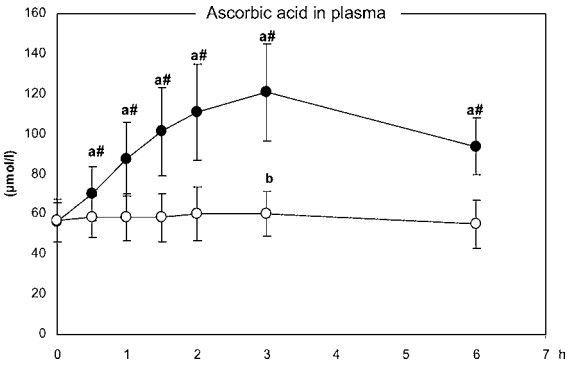

Figure 1. Concentration of ascorbic acid in plasma before and after ingestion of fruit juice and control beverage: (●) after ingestion of juice; (○) after ingestion of control beverage. Data are the mean ± SD, based on n = 12. Letters indicate significant differences compared to baseline (a, P < 0.001; b, P < 0.011, paired t test). Symbols indicate significant changes relative to baseline after ingestion of juice compared to sugar solution (#, P < 0.001, paired t test).

Subsequent statistical analysis showed an impact of time on ascorbic acid for the ingestion of juice (P < 0.001) and sugar solution (P = 0.008): After juice intake, ascorbic acid concentration increased up to 117% (3 vs 0 h) and was significantly higher anytime (P < 0.001) compared to baseline. Three hours after ingestion of the sugar solution, ascorbic acid concentration was slightly (6.5%) higher compared with the initial value (P = 0.011) (Figure 1). However, relative changes to baseline were always higher after consumption of juice compared to sugar solution (all P values < 0.001). As expected, the AUC of the ascorbic acid concentration in plasma was higher after consumption of the test juice compared to control beverage (607 ± 115 vs 351 ± 68 µmol·h/L; P < 0.001).

The concentrations of β-carotene and uric acid as well as the α-tocopherol to cholesterol ratio were influenced neither by time and beverage alone nor by interactions with each other (Table 3). With regard to AUC and relative changes versus baseline, differences between the treatments did not occur (data not shown).

In plasma, additional substances with reducing capacity not present in the juice blend were detected after consumption of fruit juice (Figure 2b) compared to baseline (Figure 2a). These metabolites could not be measured before (Figure 2c) or after consumption of sugar solution (Figure 2d). Interestingly, retention times of these substances observed after juice consumption were different from those of known metabolites such as protocatechuic acid, gallic acid, vanillic acid, ferulic acid, and caffeic acid.

The beverage affected neither 8-iso-$PGF_{2\alpha}$ (Table 2) nor SB in vivo and ex vivo (Table 4). Time had an impact on SB in vivo (Table 4), but did not modulate 8-iso-$PGF_{2\alpha}$ (Table 2) and SB ex vivo (Table 4). Changes relative to baseline and in the AUC of 8-iso-$PGF_{2\alpha}$ or SB in vivo and ex vivo obtained after consumption of juice and sugar solution did not occur (data not shown).

Triglyceride concentration in serum increased after both interventions (P < 0.001) with a significant increase 6 h after ingestion of juice (1.30 ± 0.60 vs 0.86 ± 0.38 mmol/L; P = 0.002) and sugar solution (1.56 ± 0.67 vs 0.94 ± 0.21 mmol/L; P = 0.004) compared to baseline. However, differences in relative changes compared to baseline and the AUC were not detectable.

Because the order in which the study beverages were consumed did not affect any of the parameters investigated, a period effect that might overlap with treatment effect can be excluded.

Table 3. Status of Antioxidants in Plasma before and after Ingestion of Juice or Control Beverage[a]

	β-carotene (µmol/L)		α-tocopherol/cholesterol ratio (µmol/mmol)		uric acid (µmol/L)	
	juice	control	juice	control	juice	control
0 h	0.85 ± 0.52	0.84 ± 0.43	5.8 ± 1.7	5.3 ± 0.8	267 ± 51	265 ± 52
0.5 h	0.80 ± 0.45	0.82 ± 0.46	5.6 ± 1.3	5.2 ± 0.7	272 ± 56	265 ± 53
1.0 h	0.81 ± 0.48	0.83 ± 0.47	5.6 ± 0.8	5.3 ± 0.7	271 ± 53	266 ± 52
1.5 h	0.81 ± 0.48	0.84 ± 0.49	5.4 ± 0.7	5.3 ± 0.8	268 ± 56	267 ± 53
2.0 h	0.83 ± 0.46	0.82 ± 0.44	5.4 ± 0.9	5.3 ± 0.7	264 ± 57	262 ± 50
3.0 h	0.82 ± 0.46	0.88 ± 0.48	5.5 ± 0.8	5.5 ± 0.7	265 ± 56	266 ± 56
6.0 h	0.78 ± 0.46	0.83 ± 0.42	5.7 ± 0.9	5.3 ± 0.5	270 ± 55	258 ± 56

[a]Data are the mean ± SD, based on n = 12. Repeated-measures ANOVA did not show any effects by time and beverage alone and not by interactions of time and beverage. Differences between both treatments were not observed with regard to AUC and relative changes versus baseline.

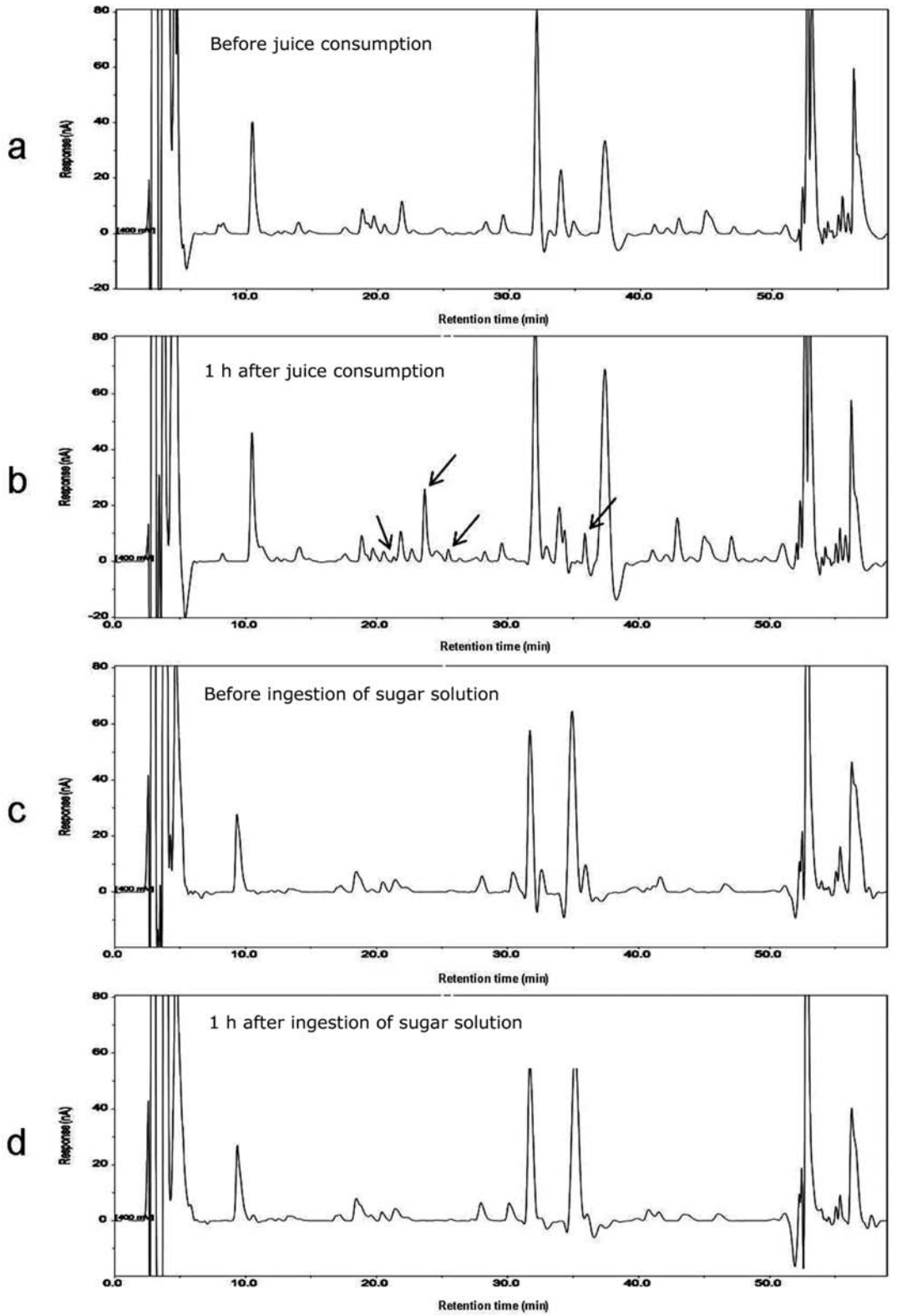

Figure 2. Representative chromatograms of plasma samples obtained from one participant analyzed by HPLC-CEAD. The plasma samples were obtained after an overnight fast, just before juice consumption (a), 1 h after juice consumption (b), after an overnight fast, just before ingestion of sugar solution (c), and 1 h after consumption of sugar solution (d).

DISCUSSION

The primary goal of this study was to investigate if bolus consumption of a specifically designed fruit juice rich in anthocyanins and ascorbic acid increases plasma antioxidant capacity and reduces markers of oxidative stress in healthy nonsmokers. To answer these questions, a randomized controlled study with crossover design was performed to avoid between-subject effects. A sugar solution with equimolar amounts of monosaccharides served as control drink to exclude

Table 4. DNA Single-Strand Breaks in Vivo and ex Vivo in Peripheral Leukocytes before and after Ingestion of Juice or Control Beverage[a]

	DNA strand breaks in vivo[b] (TM)		DNA strand breaks ex vivo (TM)	
	juice	control	juice	control
0 h	14.4 ± 5.0	15.4 ± 5.7	−1.7 ± 4.7	−0.6 ± 3.8
0.5 h	15.2 ± 5.5	14.4 ± 5.8	−0.3 ± 3.9	−0.6 ± 5.7
6.0 h	22.4 ± 6.4	18.2 ± 7.3	−0.8 ± 3.5	1.4 ± 5.8

[a]Data are the mean ± SD, based on $n = 12$. TM, tail moment. DNA strand breaks ex vivo were calculated as difference between strand breaks obtained by exposure to 300 μM H_2O_2 and those without H_2O_2 challenge. [b]Effects by time were observed only for DNA strand breaks in vivo ($P < 0.001$, repeated-measures ANOVA). Interactions by time and beverage did not occur. Relative changes compared to baseline and the 6 h AUC were not different between treatments.

antioxidant effects, which may result from a fructose-mediated increase of uric acid. This study design should allow a reliable evaluation of fruit juice effects on the pro-/antioxidative balance.

Despite an obvious increase of ascorbic acid (Figure 1) and other substances with reducing capacity in plasma (Figure 2b), TEAC and FCR did not increase after juice ingestion (Table 2). However, within-subject changes in FCR relative to baseline were higher after ingestion of sugar solution compared to juice, suggesting that juice consumption may contribute to stabilize FCR. However, this effect is less clear as significant effects were not detectable for all time points and AUC was not different between both treatments. TEAC and FCR are based on measurements of electron transfer.[14] Ascorbic acid and, probably, the unknown substances function as hydrogen donators; this effect cannot be detected by these assays. Interestingly, TOSC, an assay detecting hydrogen transfer, decreased absolutely and relatively to baseline after the consumption of the control beverage (Table 2). This phenomenon may be due to the polyphenol-poor diet the day before the study.[23] Maintenance of TOSC levels after juice intake (Table 2) may be explained by the enhanced extracellular ascorbic acid levels.

At baseline and after consumption of the control beverage, ascorbic acid levels were below the desirable steady-state concentrations in healthy adults (70−85 μmol/L),[24] probably due to dietary restrictions. As expected, plasma concentrations of ascorbic acid temporarily increased after juice consumption (Figure 1); the extent, however, was, relatively low considering the supraphysiological dose (>900 mg/day) ingested. Reduced bioavailability at supraphysiological compared to physiological doses, but also exceeding the threshold plasma concentration for urinary excretion (55−60 μmol/L),[25] may explain this observation. The slow, but significant, increase of plasma ascorbic acid in the control group 3 h postconsumption may be due to circadian rhythms, which were observed by Loh and Wilson,[26] achieving maximum values at noon and minimum values at midnight.

The concentration of further exogenous antioxidants in plasma (β-carotene and α-tocopherol), which also contribute to plasma antioxidant capacity,[27] did not change significantly (Table 3). β-Carotene is generally ingested with açai,[28] camu-camu,[29] and blackberries,[30] but the dose ingested in our study by juice consumption was obviously too low to increase the β-carotene level in plasma.

It is known that anthocyanins consumed as food ingredients cannot be detected in plasma at all or at least in marginal concentrations. One explanation may be the low stability of the flavylium cation under physiological pH conditions.[31] Bolus ingestion of açai juice providing 110 mg of anthocyanins (sum of cyanidin-3-glucoside and cyandin-3-rutinoside) led to maximum plasma concentrations of 4.8 nmol/L cyanidin-3-glucoside in the study of Mertens-Talcott et al.[9] Because our juice provided only 27.9 mg of anthocyanins (Table 1), only traces of anthocyanins might occur in plasma. Anthocyanins are degraded to low molecular weight phenolic acids by the microflora of the gut as shown in vitro and in vivo.[32] After ingestion of blood orange juice rich in cyanidin-3-glucoside, only traces of cyanidin-3-glucoside were found in plasma and feces within 6 h postconsumption, but relatively high amounts of protocatechuic acid were found, which accounted in total for 72% of ingested cyanidin-3-glucoside.[33] Because the major part of protocatechuic acid appeared in plasma, with maximum concentrations obtained at 2 h postconsumption, deglycosylation of cyanidin-3-glucoside by small intestine β-glucosidases and the subsequent degradation into phenolic acids in the intestinal lumen or in the blood seem to explain primarily the metabolic fate of cyanidin-3-glucoside, rather than the microbial degradation and absorption of protocatechuic acid in the colon.[33] To evaluate mucosal uptake of fruit juice polyphenol ingredients, we thus evaluated plasma appearance of known low molecular weight anthocyanin metabolites such as protocatechuic acid, gallic acid, vanillic acid, caffeic acid, and ferulic acid by using a HPLC technology that is highly sensitive for the detection of reducing substances in plasma. Most surprisingly, these metabolites could not be detected in plasma. Instead, several unknown metabolites with antioxidative properties occurred in plasma after juice consumption (Figure 2b). Because we were not able to isolate these metabolites in quantitative amounts from plasma samples, the chemical structures are still unknown. It cannot be excluded that two peaks reflect the occurrence of parental cyanidin-3-glucoside and cyanidin-3-rutinoside, for which retention times were, unfortunately, not determined. Probably, phenolic acids are further degraded already in the gut and/or after mucosal uptake.

Lipid peroxidation in vivo assessed by plasma 8-iso-PGF_{2a} did not change in our study. This observation is in contrast to the results of a recent bolus study: 2 h after ingestion of a cyanidin-rich juice blend with açai as predominant ingredient, lipid peroxidation measured by thiobarbituric acid reactive substances (TBARS) decreased in healthy nonsmokers.[10] However, TBARS are less specific for lipid peroxidation than the isoprostanes[34] analyzed in our study. On the other hand, ELISA kits are less specific for the determination of 8-iso-$PGF_{2\alpha}$ than gas chromatography−mass spectrometry.[35] Hence, cross-reactivity with metabolites such as 2,3-dinor-8-iso-$PGF_{2\alpha}$ (4% according to the manufacturer) may have masked potential changes in 8-iso-$PGF_{2\alpha}$ in our study. As usual formation of 8-iso-$PGF_{2\alpha}$ is suggested to be low in healthy subjects, and parts of 8-iso $PGF_{2\alpha}$ are metabolized and excreted in the urine,[35] the changes in the plasma concentration of 8-iso-$PGF_{2\alpha}$ might have been low, probably too low to be detected within 6 h after treatment.

SB in vivo were only affected by time ($P < 0.001$) and not by beverage (Table 4). This fits the results of a previous study by our group in which only effects by time occurred after bolus ingestion of white tea, green tea, and water.[23] Time-dependent

effects may simply reflect circadian rhythms. Contrary to SB in vivo, SB ex vivo were not modulated by time or beverage (Table 4). Comparable bolus studies with juices investigating SB ex vivo are not available, but white and green tea did not show any changes by time or beverage either.[23] Cell-based antioxidant capacity, determined in erythrocytes, increased after bolus consumption of an açai-rich juice, probably due to an intracellular accumulation of antioxidants,[10] but did not change leukocytes' resistance against challenge with reactive oxygen species ex vivo after single intake of açai pulp or juice.[9] Primarily, endogenous glutathione determines the protection against oxidative cell injury.[36] It is assumed that the consumption of the exotic fruit juice did not influence glutathione levels. It should be, however, mentioned that the broad interindividual variation of SB in vivo and against oxidative challenge ex vivo limits the power of the study considering these markers of DNA damage.

In the present study, the time-dependent increase of triglycerides in serum may reflect postprandial changes induced by the standardized meals because the fructose intake from our study beverages (7.2 g) was below the dose of 50 g that has been shown to increase serum triglycerides after a bolus intake.[37] Even if postprandial hypertriglyceridemia is suggested to trigger the increase in biomarkers of oxidative stress such as malondialdehyde in healthy subjects,[38] 8-iso-$PGF_{2\alpha}$ did not change (Table 3). Thus, confounding effects on lipid peroxidation in vivo by triglycerides in the present study are unlikely.

At first glance, the lack of clear changes in markers of antioxidant capacity and oxidative stress investigated by a good panel of tests after bolus intake of the juice blend is surprising. Our study design and the panel of tests were well-chosen with respect to the aim of the present study. It remains unknown if the lack of changes is due to analytical limitations (e.g., ELISA kits lack specificity in 8-iso-$PGF_{2\alpha}$) or due to the choice of methods and subjects. Because the challenge with reactive oxygen species is usually low in healthy nonsmokers, changes in markers of antioxidant capacity and oxidative stress are hard to detect as they would be subtle anyway. Perhaps changes are much likely to occur under conditions of acute or chronic oxidative stress. Functional effects may afford an accumulation of selected substances that may be achieved only by prolonged consumption of the juice blend. Because anthocyanins' bioavailability depends on the food matrix,[39] juice consumption within a meal would have led to other results. This is also important for fat-soluble antioxidants such as carotenoids, the bioavailability of which would be much higher if the juice was consumed together with a fat-containing meal. However, juice ingestion with a meal would be another setting to investigate if it is a measure to cope with postprandial oxidative stress. As dietary anthocyanins are thought to prevent pathophysiological changes involved in obesity, diabetes, and cardiovascular diseases by mechanisms beyond antioxidant capacity,[40] the juice blend used in our study may promote human health even if antioxidant effects were not detectable. Microbial degeneration of anthocyanins by the gut microflora may contribute to human health via prebiotic effects as recently shown after ingestion of procyanidins-rich cocoa.[41]

In conclusion, bolus ingestion of a blended juice of açai, Andean blackberries, and camu-camu rich in ascorbic acid and anthocyanins only increased the concentration of plasma ascorbic acid and several unknown substances with reducing properties. It seems to stabilize the pro-/antioxidant balance in healthy nonsmokers, but did not reduce markers of oxidative stress. Even if product-specific preventive effects by consumption of these novel drinks cannot be expected with regard to the parameters determined in systemic circulation, health-promoting effects by further mechanisms beyond antioxidant effects may occur. It cannot be excluded that beneficial effects by this juice blend may rather occur in situations with increased oxidative challenge, for example, smoking and physical activity, and after food intake.

AUTHOR INFORMATION

Corresponding Author

*Phone: ++49 228 73 3814. Fax: ++49 228 73 3217. E-mail: ellinger@uni-bonn.de.

Funding

This study was part of the PAVUC (Producing Added Value from Underutilized Tropical Fruit Crops) project (0015279) and, thus, partly supported by a grant of the European Union. The results of this study were presented in parts at the 47th Scientific Conference of the German Nutrition Society in Jena, Germany (March 2010), and at the Workshop of the Regional Association North-Rhine Westphalia of the Society of Food Chemistry in Münster, Germany (March 2010).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Brazilian Agricultural Research Corporation (Embrapa, Brazil) and the Centro National de Investigacion en Tecnologia de Alimentos (CITA, Costa Rica) for providing the camu-camu pulp and the blackberry juice, respectively.

ABBREVIATIONS USED

AUC, area under the curve; FCR, Folin−Ciocalteu reducing capacity; 8-iso-$PGF_{2\alpha}$, 8-isoprostaglandine $F_{2\alpha}$; SB, DNA single-strand breaks; TEAC, trolox equivalent antioxidant capacity; TOSC, total oxidant scavenging capacity; TBARS, thiobarbituric acid reactive substances.

REFERENCES

(1) Nikolic, M.; Nikic, D.; Petrovic, B. Fruit and vegetable intake and the risk for developing coronary heart disease. *Cent. Eur. J. Public Health* **2008**, *16*, 17−20.

(2) *Food, Nutrition, Physical Activity, and the Prevention of Cancer*; World Cancer Research Fund/American Institute for Cancer Research: Washington, DC, 2007.

(3) Liu, R. H. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* **2003**, *78* (3 Suppl.), 517S−520S.

(4) Rodrigues, R. B.; Marx, F. Camu camu [*Myrciaria dubia* (H.B.K.) McVaugh]: a promising fruit from the Amazon Basin. *Ernaehrung/Nutrition* **2006**, *30*, 376−381.

(5) Lichtenthaler, R.; Rodrigues, R. B.; Maia, J. G.; Papagiannopoulos, M.; Fabricius, H.; Marx, F. Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (acai) fruits. *Int. J. Food Sci. Nutr.* **2005**, *56*, 53−64.

(6) Schauss, A. G.; Wu, X.; Prior, R. L.; Ou, B.; Huang, D.; Owens, J.; Agarwal, A.; Jensen, G. S.; Hart, A. N.; Shanbrom, E. Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, *Euterpe oleraceae* Mart. (acai). *J. Agric. Food Chem.* **2006**, *54*, 8604−8610.

(7) Acosta-Montoya, O.; Vaillant, F.; Cozzano, S.; Mertz, C.; Pérez, A. M.; Castro, M. V. Phenolic content and antioxidant capacity of

tropical highland blackberry (*Rubus adenotrichus* Schltdl.) during three edible maturity stages. *Food Chem.* **2010**, *119*, 1497−1501.
(8) Inoue, T.; Komoda, H.; Uchida, T.; Node, K. Tropical fruit camu-camu (*Myrciaria dubia*) has anti-oxidative and anti-inflammatory properties. *J. Cardiol.* **2008**, *52*, 127−132.
(9) Mertens-Talcott, S. U.; Rios, J.; Jilma-Stohlawetz, P.; Pacheco-Palencia, L. A.; Meibohm, B.; Talcott, S. T.; Derendorf, H. Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich acai juice and pulp (*Euterpe oleracea* Mart.) in human healthy volunteers. *J. Agric. Food Chem.* **2008**, *56*, 7796−7802.
(10) Jensen, G. S.; Wu, X.; Patterson, K. M.; Barnes, J.; Carter, S. G.; Scherwitz, L.; Beaman, R.; Endres, J. R.; Schauss, A. G. In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-blinded, placebo-controlled, crossover study. *J. Agric. Food Chem.* **2008**, *56*, 8326−8333.
(11) Hallfrisch, J. Metabolic effects of dietary fructose. *FASEB J.* **1990**, *4*, 2652−2660.
(12) Livesey, G. Fructose ingestion: dose-dependent responses in health research. *J. Nutr.* **2009**, *139*, 1246S−1252S.
(13) U.S. Department of Agriculture (USDA) database for the flavonoid content of selected foods, release 2.1.
(14) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.
(15) Miller, N. J.; Rice-Evans, C.; Davies, M. J. A new method for measuring antioxidant activity. *Biochem. Soc. Trans.* **1993**, *21*, 95S.
(16) Arendt, B. M.; Ellinger, S.; Kekic, K.; Geus, L.; Fimmers, R.; Spengler, U.; Muller, W. U.; Goerlich, R. Single and repeated moderate consumption of native or dealcoholized red wine show different effects on antioxidant parameters in blood and DNA strand breaks in peripheral leukocytes in healthy volunteers: a randomized controlled trial (ISRCTN68505294). *Nutr. J.* **2005**, *4*, 33.
(17) Lichtenthaeler, R.; Marx, F.; Kind, O. M. Determination of antioxidative capacities using an enhanced total oxidant scavenging capacity (TOSC) assay. *Eur. Food Res. Technol.* **2003**, *215*, 166−173.
(18) Steffan, J. *Determination of L-Ascorbic Acid and Dehydro-L-ascorbic acid in Human Blood Plasma by HPLC/UV and HPLC/EC: Method Development, Validation and Application* (in German); University of Paderborn: Paderborn, Germany, 1999.
(19) Erhardt, J. G.; Heinrich, F.; Biesalski, H. K. Determination of retinol, antioxidant vitamins and homocysteine in skin puncture blood. *Int. J. Vitam. Nutr. Res.* **1999**, *69*, 309−314.
(20) Zimmermann, B. F.; Papagiannopoulos, M.; Brachmann, S.; Lorenz, M.; Stangl, V.; Galensa, R. A shortcut from plasma to chromatographic analysis: straightforward and fast sample preparation for analysis of green tea catechins in human plasma. *J. Chromatogr., B: Anal. Technol Biomed. Life Sci.* **2009**, *877*, 823−826.
(21) Ritter, C.; Zimmermann, B. F.; Galensa, R. Chiral separation of (+)/(−)-catechin from sulfated and glucuronidated metabolites in human plasma after cocoa consumption. *Anal. Bioanal. Chem.* **2010**, *397*, 723−730.
(22) Roggenbuck, C.; Lammert, F.; Berthold, H. K.; Giese, T.; Stallmach, A.; Stehle, P.; Ellinger, S. High-dose oral supplementation of antioxidants and glutamine improves the antioxidant status in patients with Crohn's disease: a pilot study. *e-SPEN, Eur. e-J. Clin. Nutr. Metab.* **2008**, *3*, e246−e253.
(23) Müller, N.; Ellinger, S.; Alteheld, B.; Ulrich-Merzenich, G.; Berthold, H. K.; Vetter, H.; Stehle, P. Bolus ingestion of white and green tea increases the concentration of several flavan-3-ols in plasma, but does not affect markers of oxidative stress in healthy non-smokers. *Mol. Nutr. Food Res.* **2010**, *54*, 1636−1645.
(24) Padayatty, S. J.; Sun, H.; Wang, Y.; Riordan, H. D.; Hewitt, S. M.; Katz, A.; Wesley, R. A.; Levine, M. Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann. Intern. Med.* **2004**, *140*, 533−537.
(25) Levine, M.; Rumsey, S. C.; Daruwala, R.; Park, J. B.; Wang, Y. Criteria and recommendations for vitamin C intake. *JAMA, J. Am. Med. Assoc.* **1999**, *281*, 1415−1423.
(26) Loh, H. S.; Wilson, C. W. Vitamin C: plasma and taste threshold circadian rhythms, their relationship to plasma cortisol. *Int. J. Vitam. Nutr. Res.* **1973**, *43*, 355−362.
(27) Cao, G.; Prior, R. L. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin. Chem.* **1998**, *44*, 1309−1315.
(28) Schauss, A. G.; Wu, X.; Prior, R. L.; Ou, B.; Patel, D.; Huang, D.; Kababick, J. P. Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, *Euterpe oleraceae* Mart. (acai). *J. Agric. Food Chem.* **2006**, *54*, 8598−8603.
(29) Zanatta, C. F.; Mercadante, A. Z. Carotenoid composition from the Brazilian tropical fruit camu-camu (*Myrciaria dubia*). *Food Chem.* **2007**, *101*, 1526−1532.
(30) Marinova, D.; Ribarova, F. HPLC determination of carotenoids in Bulgarian berries. *J. Food Compos. Anal.* **2007**, *20*, 370−374.
(31) McGhie, T. K.; Walton, M. C. The bioavailability and absorption of anthocyanins: towards a better understanding. *Mol. Nutr. Food Res.* **2007**, *51*, 702−713.
(32) Williamson, G.; Clifford, M. N. Colonic metabolites of berry polyphenols: the missing link to biological activity? *Br. J. Nutr.* **2010**, *104* (Suppl. 3), S48−S66.
(33) Vitaglione, P.; Donnarumma, G.; Napolitano, A.; Galvano, F.; Gallo, A.; Scalfi, L.; Fogliano, V. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. *J. Nutr.* **2007**, *137*, 2043−2048.
(34) Del Rio, D.; Serafini, M.; Pellegrini, N. Selected methodologies to assess oxidative/antioxidant status in vivo: a critical review. *Nutr. Metab. Cardiovasc. Dis.* **2002**, *12*, 343−351.
(35) Halliwell, B.; Lee, C. Y. Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues. *Antioxid. Redox Signal.* **2010**, *13*, 145−156.
(36) Shan, X. Q.; Aw, T. Y.; Jones, D. P. Glutathione-dependent protection against oxidative injury. *Pharmacol. Ther.* **1990**, *47*, 61−71.
(37) Livesey, G.; Taylor, R. Fructose consumption and consequences for glycation, plasma triacylglycerol, and body weight: meta-analyses and meta-regression models of intervention studies. *Am. J. Clin. Nutr.* **2008**, *88*, 1419−1437.
(38) Bloomer, R. J.; Kabir, M. M.; Marshall, K. E.; Canale, R. E.; Farney, T. M. Postprandial oxidative stress in response to dextrose and lipid meals of differing size. *Lipids Health Dis.* **2010**, *9*, 79.
(39) Yang, M.; Koo, S. I.; Song, W. O.; Chun, O. K. Food matrix affecting anthocyanin bioavailability: review. *Curr. Med. Chem.* **2011**, *18*, 291−300.
(40) Tsuda, T. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. *Mol. Nutr. Food Res.* **2012**, *56*, 159−170.
(41) Tzounis, X.; Rodriguez-Mateos, A.; Vulevic, J.; Gibson, G. R.; Kwik-Uribe, C.; Spencer, J. P. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *Am. J. Clin. Nutr.* **2011**, *93*, 62−72.
(42) Gordon, A.; Jungfer, E.; da Silva, B. A.; Maia, J. G.; Marx, F. Phenolic constituents and antioxidant capacity of four underutilized fruits from the Amazon region. *J. Agric. Food Chem.* **2011**, *59*, 7688−7699.
(43) Singleton, V. L.; Rossi, J. A. J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(44) Prior, R. L.; Fan, E.; Ji, H.; Howell, A.; Nio, C.; Payne, M. J.; Reed, J. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *J. Sci. Food Agric.* **2010**, *90*, 1473−1478.
(45) Chang, C.-C.; Yang, M.-H.; Wen, H.-M.; Chern, J.-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **2002**, *10*, 178−182.

(46) Willis, R. B.; Allen, P. R. Improved method for measuring hydrolyzable tannins using potassium iodate. *Analyst* **1998**, *123*, 435−439.