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SHORT COMMUNICATION

Anthocyanins in the diet of infants and toddlers: intake, sources and trends

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Abstract
Purpose Anthocyanins, a colourful group of flavonoids in many fruits and vegetables, are proposed to provide positive impact on human health. However, intake estimations have almost exclusively been conducted in adult populations. As infants and toddlers are a promising age group for health promotion, we examined their anthocyanin intake (as anthocyanidins), food sources and trends of age and time in anthocyanidin density.
Methods Anthocyanidin content values from the USDA Database were assigned to foods consumed in 4,617 3-day weighed dietary records from 1990 to 2009 by 942 3–36-month-old subjects of the DONALD Study. As we assume that anthocyanidins found in bananas do not originate from anthocyanins, the anthocyanidin value for bananas was excluded from our analysis. To investigate age and time trends in anthocyanidin density, polynomial mixed regression models were used.
Results Median anthocyanidin intake was zero in young infants and around 4 mg/day in older infants and toddlers, strawberries and pomaceous fruit representing the main sources. Anthocyanidin density increased from 6 to 18 months of age, followed by a slight decrease till 36 months of age. During the 20-year study period, a decrease in density in infants was observed, but a slight increase in toddlers.
Conclusions Anthocyanidin density in the diet seems to increase notably from infancy to toddlerhood and to have decreased in the youngest over the last 20 years. These first observations in a German population of infants and toddlers need to be extended by further studies examining anthocyanin intake in these age groups in other countries.

Keywords Anthocyanin intake · Infants · Toddlers · Age and time trends · Banana

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Introduction

Anthocyanins, phenolic plant metabolites belonging to the group of flavonoids, are responsible for the orange to blue colours of many flowers, fruits and vegetables [1]. Evidence from cell and animal studies indicates that they or their metabolites might exert positive health effects resulting from their anti-oxidative, anti-carcinogenic and anti-inflammatory properties [2]. However, results from epidemiological studies are inconclusive [2, 3]. Besides physiological reasons like low bioavailability of anthocyanins [4] and interindividual differences of gut microbiota metabolites [5], one reason for these inconsistencies may be the complexity of estimating anthocyanin intake due to biological variances in anthocyanin content in foods, analytical variances in quantifying anthocyanins in food, shortage of suitable food composition databases and imprecise methods of dietary assessment. For adults, the estimated intake of anthocyanins ranges between 2.7 and 215 mg/day [6–10], of anthocyanidins (= aglycones of anthocyanins) between 2.9 and 47 mg/day [11–17].

Up to now, to our knowledge, there is only one paper, which provides anthocyanidin intake data of children, presenting values around zero for Australian children and adolescents [11]. But particularly young children could be a specific target group for implementing potentially health-promoting substances in the diet, because in early childhood food patterns and preferences are formed [18] and nutrition in the first years of life is proposed to be determining for health in later life [19].

Therefore the objective of this investigation was to estimate usual intake and food sources of anthocyanins, expressed as anthocyanidins, as well as age and time trends in anthocyanidin density in the diet in a sample of healthy infants and toddlers of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study. Anthocyanidin intakes were estimated on the basis of detailed 3-day weighed dietary records, and the anthocyanidin values were taken from the widely used USDA database for the flavonoid content of selected foods, version 2.1, released in 2007 [20].

Methods

Study sample

Food consumption data of infants and toddlers were taken from the DONALD Study, which is an ongoing, open cohort study. In the DONALD study, detailed data on diet, growth, development and metabolism between infancy and adulthood are collected since 1985. Since 1989, infants are recruited and systematically followed up at least until the age of 18 years. The regular DONALD assessments include 3-day weighed dietary records every 3 months in the first year of life, twice a year in the second year of life and once a year from 2 years of age on. Details have been published elsewhere [21].

The DONALD study, which is exclusively observational and non-invasive till the age of 18, has been approved by the International Scientific Committee of the Research Institute of Child Nutrition and the Ethic Commission of the University of Bonn. All examinations and assessments are performed with parental, and later on with the children's written consent.

For the present evaluation, 4,617 3-day weighed dietary records of 942 subjects (466 boys, 476 girls) aged 3 months to 3 years in the study period of 1990–2009 were analysed. Per participant, between one ($n = 106$; 11.3% of the total sample) and seven ($n = 257$, 27.3%) 3-day records were available (mean = 4.9). Per study year between 184 and 312 records were available (mean = 230.9).

Dietary assessment

Parents weighed and recorded all foods and beverages consumed using electronic food scales (±1 g) on 3 consecutive days. Semi-quantitative recording (e.g. number of spoons, scoops) was allowed when weighing was not possible. The complete food collection details have been described previously [22].

Food database LEBTAB

Any food consumed by the DONALD participants is stored in the in-house food composition and nutrient database LEBTAB. Compositions of composite foods are estimated by recipe simulation using labelled nutrient contents and ingredients. For longitudinal analysis, LEBTAB is updated continuously by new foods recorded by the participants. A new food or a commercial food product that already exists in the database but has undergone a change in composition (i.e. new ingredients, change of fortification) evokes a new entry [23]. For this evaluation, dietary supplements and pharmaceuticals were excluded.

Flavonoid database

Anthocyanidin contents of foods were taken from the USDA database for the flavonoid content of selected foods [20]. This database contains condensed data from the literature expressed as anthocyanidins. Where literature gave values for individual glycosides, USDA scientists converted the glycoside values into aglycone forms using conversion factors based on molecular weight to make data consistent across the database. Values for the following 6 main anthocyanidins in fruits and vegetables are presented as mg per 100 g edible portion: cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin.

Matching of food and anthocyanidin content data

First, all commercial food products consumed by the subjects were broken down into ingredients, for example, a commercial complementary food product into fruits, cereals and water. This procedure resulted in 890 different food items (ingredients and recorded staple foods) stemming from a total of 6,190 foods. About 796 food items (89.4%) like animal products, breast milk and mineral waters were defined as anthocyanin-free, 94 food items were considered as containing anthocyanins, i.e., fruits, vegetables and fruit juices, and the respective anthocyanidin values from the USDA database as sum of all analysed anthocyanidins were assigned. The unexpected anthocyanidin values for bananas and nuts were excluded from this analysis. An analysis made by Geisenheim Research Center showed no anthocyanins in fresh bananas (pulp) using a methanolic extraction method and HPLC–MS analysis for detection of anthocyanins [24].

Statistical analysis

SAS® procedures (Version 9.1.3; Statistical Analysis System, Cary, NC, USA) were used for data analysis. Energy intake (MJ) and anthocyanidin intake (mg) were calculated as individual sums of 3 recorded days using LEBTAB. Subsequently, anthocyanidin intake was calculated (1) as individual means of 3 recorded days in absolute values (mg/day) and (2) as anthocyanidin density relative to energy intake (mg/MJ) to adjust for increasing energy intake with age.

To investigate age and time trends in anthocyanidin density, a polynomial mixed effects regression model (Proc Mixed) including both fixed and random effects was used. Anthocyanidin density in the diet (mg/MJ) was the independent variable. The age variables (age, age^2, age^3) and the time variables (time, time^2) as well as the combination of the age variables with the time variables (age*time, age^2*time) were the principal fixed effects. Random effects were considered to allow variation between individuals with respect to the initial level (intercept) and to quadratic trends of anthocyanidin density over age and time. A repeated statement was used to account for the lack of independence that exists between repeated observations on the same person. This final model was selected by comparing several models based on the Akaike information criterion (AIC). The variable sex did not lead to an improvement of the AIC and there was no significant impact of sex on the intake of anthocyanidins, so that the variable sex was not included in the model.

Results

In 719 of 4,617 dietary records (15.57%), no anthocyanin containing foods were consumed, most of these records ($n = 690$, 96%) being from 3 to 6-month-old infants. Accordingly, anthocyanidin intake in 50% of infants aged 3 and 6 months was near zero (Table 1). In contrast, in only 16 of 702 records (2.28%) from 9-month-old infants and in 13 of 2,831 records (0.46%) from 1 to 3-year-old toddlers, no anthocyanin containing foods were present. For these older infants and toddlers, the median of anthocyanidin intake ranged from 3.47 to 4.25 mg/day and anthocyanidin density from 0.89 to 1.15 mg/MJ, the values of the 90th percentile being 3.3–5.3-fold higher than the median (Table 1).

Main sources for anthocyanidins in infants and toddlers of this study population were fruits (Table 2). While the contribution of pomaceous fruit to anthocyanidin intake decreased with age, the contribution of berry fruit, especially strawberries, increased considerably. As a consequence, from the age of 18 months on berry fruit provided the main amount of anthocyanidins. At all ages, also berry juices and stone fruit (peach, cherries) contributed to anthocyanidin intake. Vegetables (red cabbage) were represented in the top ten sources with 5–6% of total anthocyanidin intake only from the age of 18 months on (data not shown).

Age as well as time showed a significant impact on the anthocyanidin density in the diet (Fig. 1). The fitted polynomial mixed effects regression model describes anthocyanidin density over the course of age and time as a function including a steep positive linear, a negative quadratic and a positive cubic age trend, a negative linear and a slight positive quadratic time trend, as well as a positive combined linear trend of age and time and a slight negative combined effect of quadratic age and linear time.

The stated three age effect estimates describe a steep increase in anthocyanidin density at the beginning of the age curve and a subsequent modest decrease (Fig. 1). The two time effect estimates characterize a decrease in average anthocyanidin density during the 20-year study period since 1990, which is weakened over time. The combined effect estimates of age and time as well as age^2 and time characterize steeper and slightly wider becoming age curves over time.

Table 1 Estimated anthocyanidin intake and anthocyanidin density in the diet of infants and toddlers from the DONALD Study (4,617 dietary records, 942 participants) between 1990 and 2009 (repeated measurements)

Age (months)	mg/d						mg/MJ					
	P10	P25	Median	Mean	P75	P90	P10	P25	Median	Mean	P75	P90
3 ($n = 418$)	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
6 ($n = 666$)	0.00	0.00	0.06	1.51	1.42	4.86	0.00	0.00	0.02	0.57	0.52	1.76
9 ($n = 702$)	0.62	1.60	3.47	5.27	6.95	11.47	0.19	0.56	1.15	1.77	2.27	3.78
12 ($n = 738$)	0.74	1.69	3.64	6.24	7.42	13.45	0.23	0.54	1.10	1.90	2.40	4.28
18 ($n = 699$)	0.78	1.93	4.25	6.99	9.32	16.43	0.22	0.52	1.12	1.90	2.46	4.37
24 ($n = 693$)	0.77	1.79	4.05	8.11	9.17	20.45	0.21	0.49	1.05	2.02	2.26	5.00
36 ($n = 701$)	1.02	2.03	4.09	8.69	9.94	21.53	0.24	0.47	0.89	1.91	2.12	4.66

Table 2 The top five food sources of anthocyanidins in the diet of infants and toddlers from the DONALD study (4,617 dietary records, 942 participants) from 1990 to 2009 (repeated measurements)

	% of total anthocyanidin intake per age group													
Age (months)	3		6		9		12		18		24		36	
Records (*n*)	418		666		702		738		699		693		701	
1	Pear	39.5	Pear	43.4	Pear	40.4	Pear	28.2	Strawberry	19.8	Strawberry	24.5	Strawberry	29.7
2	Apple, with peel	36.5	Apple, with peel	21.6	Apple, with peel	19.3	Strawberry	15.0	Pear	17.7	Pear	12.6	Apple, with peel	9.3
3	Blueberry	4.6	Blueberry	13.0	Blueberry	9.9	Apple, with peel	14.2	Apple, with peel	10.0	Pear	9.3	Pear	9.2
4	Raspberry juice	3.9	Elderberry juice	7.5	Strawberry	8.5	Apple, with peel	13.2	Black currant	8.4	Black currant	8.4	Black currant	8.1
5	Black currant juice	3.6	Strawberry	4.0	Elderberry juice	5.6	Black currant	4.2	Blackberry	6.4	Elderberry juice	7.6	Blueberry	6.1
Sum		88.1		89.5		83.7		74.8		62.3		62.4		62.4

Discussion

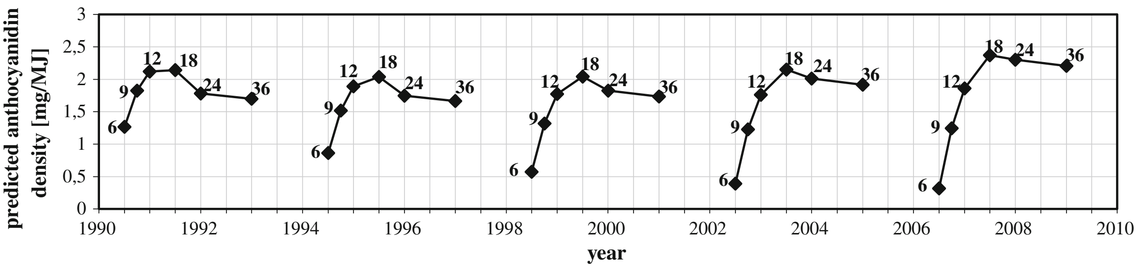
Anthocyanins were widely distributed in the diet of older infants and toddlers in this study population: the estimated median anthocyanidin intake was about 4 mg/day, pomaceous fruit and later on berry fruit, representing the main sources. Anthocyanidin density in the diet increased from 6 to 18 months of age, followed by a slight decrease till the age of 36 months. During the 20-year study period, a decrease in anthocyanidin density in infants and a slight increase in toddlers were observed.

Despite the discussed health effects of anthocyanins, only one intake estimation in children [11] and not any in toddlers under 2 years have been published. We found an estimated median anthocyanidin intake of 4.1 mg/day and a mean anthocyanidin intake being more than twice as high (8.7 mg/day) for 3-year-old toddlers, taking records of a time span of the last 20 years into account.

Johannot et al. [11] estimated anthocyanidin intake in the Australian population and reported for toddlers aged 2–3 years a mean intake of 0.00 mg/day. This may be due to the use of a single 24HR in a quite small number of subjects ($n = 357$, [25]).

Based on the USDA database (version of 2003), the estimated mean anthocyanidin intake in 45 female Flemish dietitians assessed with two FFQ was 7.6 and 7.7 mg/day, whereas the mean intake using a 4-day food record (4DFR) was only 5.5 mg/day [13]. Our somewhat higher values (8.7 mg/day for 3-year olds) than from the Flemish study may be due to our detailed dietary assessment, to an actual higher consumption of fruits in our study population than in Belgium adults or to the use of different versions of the USDA database. Also applying the USDA database (version of 2003), mean anthocyanidin intake in US adults was estimated as 3.1 mg/day [15], but mean intake in Italian and median intake in Greek adults were estimated as 20.1 and 20.9 mg/day [14, 16]. Using the latest version of the USDA database (2007) in another Mediterranean population, the Spanish cohort of EPIC resulted in a similar mean intake of 18.9 mg/day. Interestingly, when subtracting the anthocyanidin intake from red wine (46% of total anthocyanidin intake) and the wrong intake values from bananas (2.4%), the mean intake in the adult EPIC sample would be about 9 mg/day, which is very close to our findings [17]. Based mainly on own food analysis, anthocyanidin intake in Finish adults was estimated as 47 mg/day most likely due to a high berry consumption (mean: 52 g/day in Finish adults) [12].

Anthocyanidin density in the diet was calculated as 7 mg/MJ in Finish and 1.5 mg/1,000 kcal (=0.36 mg/MJ) in US adults [12, 15]. Anthocyanidin density in the diet of our study population (around 1 mg/MJ in toddlers) lies in between these values and seems to be plausible having in



	β (SE)	p
linear trend of anthocyanidin intake over the course of age (age)	6.0155 (0.7446)	<0.0001
quadratic trend of anthocyanidin intake over the course of age (age^2)	-3.5732 (0.4562)	<0.0001
cubic trend of anthocyanidin intake over the course of age (age^3)	0.6215 (0.0848)	<0.0001
linear trend of anthocyanidin intake over the course of time (time)	-0.1690 (0.0344)	<0.0001
quadratic trend of anthocyanidin intake over the course of time ($time^2$)	0.0034 (0.0012)	0.0041
combined effect of linear trends of age and time (age*time)	0.1162 (0.0306)	0.0001
combined effect of quadratic trend of age and linear trend of time (age^2*time)	-0.0247 (0.0087)	0.0044

Fig. 1 Predicted anthocyanidin density in the diet [mg/MJ] of infants and toddlers over the courses of age and time, resulting from the tabulated polynomial mixed effects regression model. The model is based on dietary anthocyanidin intake data of infants and toddlers from the DONALD Study (4199 records, 934 participants) between 1990 and 2009 (repeated measurements). Numbers at data points indicate months of age

mind different intake patterns in these countries and age groups.

However, only the use of the same flavonoid database as well as same dietary assessment methods in different cohorts would allow comparing different dietary patterns reasonably. In this regard, it is of current interest to compare the USDA database with other recent databases such as Phenol-Explorer [26] or the BioActive Substances in Food Information System (eBasis) provided by EuroFIR [27].

Pears, strawberries and apples were the main anthocyanidin sources in our study population in spite of their relatively low anthocyanidin content, because they were consumed frequently. Contribution to total anthocyanidin intake of the two most important sources of anthocyanidins (pears and apples) was 76% at the age of 3 months and only 39% at the age of 3 years (strawberries and apples), indicating an increased diversification of food sources. The high amount of anthocyanidins from strawberries in toddlers may be due to a change in food choices from baby food based on apples and pears to more sugary products like jam or fruit yoghurt, which often contain strawberries in Germany. Anthocyanin-rich vegetables are red cabbage and eggplant, but only the first is a common vegetable in German children's diet. Assuming that the intake of anthocyanins exerts positive health effects, it may be appreciated to increase intake of anthocyanin-rich foods already in infancy and early childhood when food preferences are beginning to be formed. An appropriate possibility to increase anthocyanin intake in toddlers and children may be a juice or smoothie made out of anthocyanin-rich varieties of popular fruits or vegetables, for example a purple variety of carrots.

Our study is the first, which has analysed age and time trends in anthocyanidin density in the diet and identified five age curves for different time periods. Obviously, density first dramatically increases from 6 to 18 months and thereafter decreases slightly till the age of 36 months. The infant diet in Germany includes more generous amounts of fruits than the usually less 'fruitful' family diet. This assumption is confirmed by a recent analysis of DONALD, which found a less healthy diet in preschool and school children than in toddlers [28]. Over the 20-year study period, anthocyanidin density in the diet of 6-month-old infants decreased towards zero. This is likely to be explained by a longer time of exclusive breastfeeding and therefore a later introduction of complementary food in recent years, corroborated by a regression analysis (data not shown). Favourable time trends were found in toddlers, where the decrease in density from 18 to 36 months of age got less distinct over time and density increased with time.

A weakness of our data analysis was the common inevitable difficulty in estimating anthocyanin or anthocyanidin intake due to inaccuracy of dietary assessment and variation in anthocyanin content in food resulting from biological variances [29] as well as from influences of food processing [30].

A strength of our analysis is that we used the worldwide mostly applied US flavonoid database in combination with 3-day weighed dietary records, which are more detailed than 24HR or FFQ. But one may question whether it is reasonable to apply a US database to foods consumed by a German population. However, values presented in the US table cover a wide range of varieties of different regions including Europe, and foods are nowadays sold worldwide. One may also question whether a single record of 3 consecutive days can reflect an individual's true variability in diet [31]. However, our great number of subjects and records should result in reliable mean intake values of age groups. In addition, our nutrient database LEBTAB allows the exact breakdown of recipes of foods, such that small amounts of anthocyanin-containing ingredients eaten can be identified.

Another strength of our analysis is that we can provide data of a long time period and in an age group that has not been analysed yet and where probably dietary patterns are established.

In conclusion, with this work, we could contribute to the understanding of anthocyanin intake and its trends in German infants and toddlers. More data on anthocyanin intake in early childhood are necessary to examine whether our observations in a German population are comparable to that in other countries.

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Conflict of interest The authors declare that they have no conflict of interest.

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