

Anthocyanins in the diet of children and adolescents: intake, sources and trends

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Abstract

Purpose Anthocyanin intake estimations in large cohorts include almost exclusively adults. For the purpose of early dietary prevention, however, it is of great interest to estimate anthocyanin intake of children and adolescents.

Methods Anthocyanidin content values from the USDA Database (excluding the values for bananas and nuts) were assigned to foods consumed by 4–18-year-old participants of the DONALD Study. Between 1990 and 2009, 920 participants provided 6,707 3-day weighed dietary records. Intake of anthocyanins (expressed as their aglycones anthocyanidins) and their food sources were determined. For investigating age and time trends in anthocyanidin density (mg/MJ), a polynomial mixed regression model was built.

Results We found the estimated median anthocyanidin intake to be around 6 mg/day, strawberries representing the main source. Anthocyanidin density of the diet was about 0.2 mg/MJ higher in girls than in boys, decreased with age, decreased over time in the first half of the study period and increased over time thereafter.

Conclusions Anthocyanin intake in the young is characterised by differences in anthocyanidin density of the diet between girls and boys and by decreasing density from young childhood to adolescence. Observations in this

German study population should be extended by further studies in other countries.

Keywords Anthocyanin intake · Children · Adolescents · Age and time trends · Banana

Introduction

Anthocyanins build a class of flavonoids within the group of polyphenols and give many flowers, fruits and vegetables their orange to blue colours [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]. Results from human studies suggest beneficial effects of anthocyanin intake on risk of cardiovascular disease [3, 4] and some types of cancer, for example, colorectal cancer [5], even though still inconclusive [2, 6, 7]. Observed associations of plant food consumption and, for example, reduced risk of ischaemic heart disease mortality [8] and overall cancer [9] may in part be due to anthocyanins and other secondary plant metabolites [10]. In this context, anthocyanin intake estimations provide important information on the common dietary practice, which is required in the developmental process of practical dietary recommendations.

For adults, there are several published intake estimations, mostly on the basis of a single dietary assessment of a survey or within the scope of epidemiological studies. They cover a wide intake range between 0 and 215 mg/day expressed as anthocyanins [11–15], and between 0 and 69 mg/day expressed as anthocyanidins (=aglycones of anthocyanins) [7, 15–27]. Recently new intake estimations were conducted within the large EPIC cohort and national

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subcohorts, allowing a comparison of anthocyanin intake in different European countries [28–30]. Nevertheless, to our knowledge, there is only one publication, which provides anthocyanidin intake data of children and adolescents, presenting values between 0.00 and 0.71 mg/day for young Australians aged between 2 and 18 [19]. Therefore, more data on anthocyanin intake in children and adolescents are needed, because they are a special target group for implementing potentially health-promoting substances in the diet, as food choices established during childhood and adolescence to some extent track into adulthood [31–33]. Changes of intake over age and time are therefore also a point of interest. We can contribute to both these research questions with estimations from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study as it is an open cohort study from infancy to young adulthood conducted since 1985.

As a first step, in a recent work, we provided anthocyanidin intake and food sources as well as age and time trends in anthocyanidin density of infants and toddlers from the DONALD Study [34] using anthocyanidin content data from the USDA Database [35] (excluding the values for bananas and nuts) [36]. As age and time were found to have significant influences on the anthocyanidin density of the diet, we were interested in following up these trends in children and adolescents in the same study period from 1990 to 2009.

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 of the diet in a sample of healthy children and adolescents of the DONALD study.

Methods

Study sample

The ongoing, open cohort study DONALD has collected detailed data on dietary behaviour, growth, development, metabolism and health status at regular intervals between infancy and young adulthood since 1985. Since 1989, infants have been recruited and systematically followed up until young adulthood. Details on the study protocol have been published elsewhere [37].

The DONALD study is exclusively observational and non-invasive till the age of 18 and has been approved by the Ethics 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, 3-day weighed dietary records, which are part of the regular DONALD assessments, were used to determine food intake. Participants

aged 4–18 years, who are considered here, provide one record around their birthday every year. Therefore, in a single study year, dietary records cover all seasons and age groups on the sample level. For a single participant, a maximum of 15 records can be collected over time from age 4 to 18. The present analysis included the 3-day weighed dietary records of participants aged 4–18 years in the 20-year study period from 1990 to 2009. As a result, 6,707 records from 920 participants (461 boys) were available. The number of records available per study year ranged from 197 to 390 (mean = 335.4). The number of records available per participant ranged from one ($n = 92$; 10.0 % of the total sample) to 15 ($n = 80$; 8.7 %; mean = 7.3).

Dietary assessment

All foods and beverages consumed were recorded on three consecutive days. Until the age of 8, parents were primarily responsible for recording, but the proportion of records carried out with the assistance of the child increased from less than 1 % at age 4 to more than 50 % at age 8. From the age of 9 onwards responsibility was transferred stepwise from the parents to the participants, and with age 15 more than 50 % of the records were completed by the participant either alone or with assistance of the parents. Foods and beverages (including leftovers) were weighed using electronic food scales (± 1 g), but household measures (e.g. number of spoons, scoops) were allowed when weighing was not possible. Despite the changes in responsibility for recording over the course of age, amount of weighed foods in the records was constantly high. In each age group, more than 60 % of the dietary records had an amount of weighed foods of more than 90 %. Additionally, more than 25 % of the records in each age group had an amount of weighed foods of 50–90 %. The complete food collection details have been published previously [38].

Food database LEBTAB

Any food consumed by the DONALD participants is stored in the in-house food composition and nutrient database LEBTAB. It is based on standard nutrient tables, primarily from Germany [39], but compositions of composite foods are estimated by recipe simulation using labelled nutrient contents and ingredients [40]. 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 [40]. 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 [35]. This database contains condensed data from the international 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.

Assignment of anthocyanidin data to food items

Corresponding to our recent estimation of anthocyanin intake in infants and toddlers of the DONALD study [34], all commercial food products consumed by the subjects

were first broken down into their ingredients, for example, a commercial fruit yoghurt into fruits, plain yoghurt and sugar. This procedure resulted in 997 different food items (ingredients and recorded staple foods) stemming from a total of 7,096 foods (Fig. 1). Of the 997 consumed food items, 105 (11 %) were considered as anthocyanin-containing, that is, fruits, vegetables and juices, based on the USDA database. To these, the respective anthocyanidin values as sum of all analysed anthocyanidins were assigned; 892 (89 %) were supposed to be anthocyanin-free, respectively, either because they were no plant foods or according to a zero value in the USDA database. Additionally, the anthocyanidin contents of bananas and nuts were supposed to be zero for the present assessment, despite non-zero values are provided by the USDA database. These anthocyanidin values, however, may have been derived from proanthocyanidins rather than from anthocyanins, since the extraction method used in the original reference had not been appropriate [36, 41].

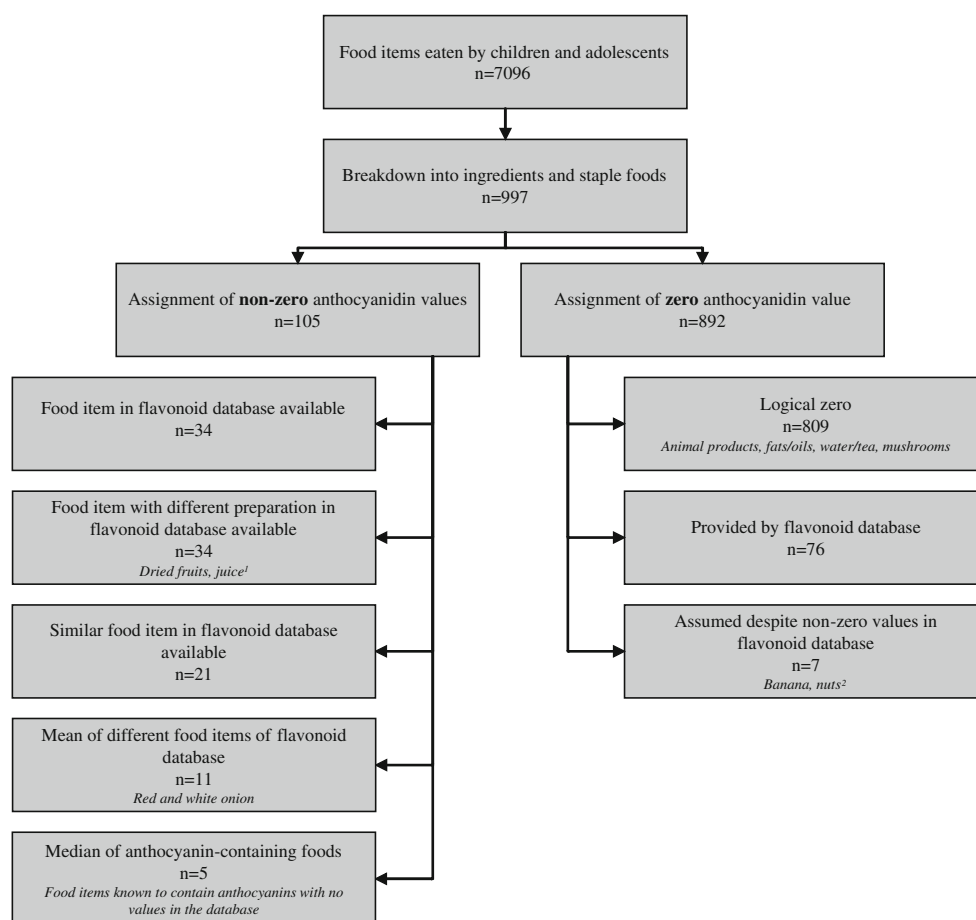


Fig. 1 Number of consumed food items and type of anthocyanidin values assigned using the USDA flavonoid database [35]. ¹For dried and stewed fruit, values from fresh fruit were used. Anthocyanidin contents of juices without a fitting item in the flavonoid database were

estimated as anthocyanidin content of fresh fruits *0.47 using the mean of the recovery rates of anthocyanins in juice compared to fresh fruit described by Kłopotek et al. [64] (strawberries, 67 %) and Hager et al. [63] (black raspberries, 27 %). ²For details, see [36]

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 LEPTAB. Subsequently, anthocyanidin intake was calculated as individual means of 3 recorded days in absolute values (mg/day) and as anthocyanidin density relative to energy intake (mg/MJ).

To investigate age and time trends in anthocyanidin density of the children's and adolescent's diet, a polynomial mixed effects regression model (PROC MIXED) including both fixed and random effects was used. Anthocyanidin density of the diet (mg/MJ) was the independent variable. The principal fixed effects were the linear and the quadratic time trend (time and time²), the linear age trend and sex. Two random statements were used to allow for variation in the initial level (intercept), in the linear age trend and in the quadratic time trend of anthocyanidin density between families and between persons in families. This final model was selected by comparing several models based on the Akaike Information Criterion (AIC) [42].

Results

In only 19 of 6,707 dietary records (0.3 %) from 17 different participants, no anthocyanin-containing foods were consumed. These 'anthocyanin-free' records were found in all ages. However, the estimated mean anthocyanidin intake was twice the median anthocyanidin intake,

representing a skewed distribution with a high number of 'low consumers'. For girls aged 4–18 years, the median of absolute anthocyanidin intake ranged from 5.65 to 6.44 mg/day and for boys from 5.35 to 6.36 mg/day (Table 1). Median anthocyanidin density ranged between 0.82 and 1.06 mg/MJ in girls and boys aged 4 to 9 years, and between 0.60 and 0.83 in the older age groups. The 90th percentile of anthocyanidin density was between 4.5 and 5.5-fold higher than the median in girls and 4.3 to 6.3-fold higher than the median in boys (Table 1).

Main sources for anthocyanins in this study population were fruits. Strawberries were the most important source in every age group contributing to anthocyanidin intake with 23–26 % (Table 2). Anthocyanin-supplying fruits beneath strawberries were apples, sweet cherries, black currants and pears. The latter, however, only was found among the five main sources in the youngest age group of 4–6 years. Only one juice (elderberry) and one vegetable (red cabbage) were represented in the top five sources. The five main sources contributed to anthocyanidin intake between 60 and 62 %.

Age as well as time had a significant impact on anthocyanidin density of the diet (Fig. 2). Over the course of age, anthocyanidin density predicted by the fitted mixed polynomial regression model decreased linearly. During the 20-year study period, average anthocyanidin density is predicted by a linear downward time trend as well as a slightly positive quadratic time trend. These two time effect estimates characterise, independent from age and sex, a change in trend with a slightly decreasing anthocyanidin density till about the year 2001 and a slightly increasing density from thereon till today (Fig. 2). Sex had

Table 1 Estimated anthocyanidin intake and anthocyanidin density in the diet of children and adolescents from the DONALD study (6,707 dietary records, 920 participants) between 1990 and 2009 (repeated measurements)

Age [years] (records, participants) [n, n]	mg/d						mg/MJ					
	P10	P25	Median	Mean	P75	P90	P10	P25	Median	Mean	P75	P90
Boys												
4–6 (950, 385)	1.19	2.53	5.35	10.69	11.40	23.21	0.22	0.45	0.96	1.86	2.01	4.09
7–9 (816, 329)	0.90	2.43	5.67	11.55	13.41	28.76	0.13	0.35	0.82	1.66	1.92	4.07
10–12 (668, 270)	0.70	2.26	5.38	12.56	15.33	34.43	0.09	0.28	0.68	1.58	1.86	4.24
13–15 (520, 208)	0.98	2.56	6.31	14.13	16.81	36.86	0.11	0.30	0.70	1.47	1.66	3.72
16–18 (403, 160)	0.38	2.11	6.36	16.33	18.14	39.66	0.03	0.21	0.60	1.55	1.64	3.76
Girls												
4–6 (968, 390)	1.20	2.62	5.66	11.69	12.69	26.40	0.26	0.52	1.10	2.28	2.51	5.22
7–9 (813, 320)	1.28	2.88	6.44	12.62	14.56	30.98	0.21	0.47	1.06	2.00	2.33	4.76
10–12 (665, 262)	1.04	2.57	5.65	12.21	14.39	32.44	0.16	0.38	0.82	1.71	2.05	4.54
13–15 (507, 198)	0.74	2.36	6.19	12.47	15.75	30.98	0.11	0.32	0.83	1.66	2.05	4.03
16–18 (397, 157)	0.86	2.44	5.73	11.91	13.33	28.67	0.12	0.36	0.83	1.63	1.80	4.10

Table 2 Top five food sources of anthocyanidins in the diet of children and adolescents from the DONALD study (6,707 dietary records, 920 participants) from 1990 to 2009 (repeated measurements)

Age [years]	% Of total anthocyanidin intake per age group				
	4–6	7–9	10–12	13–15	16–18
Records (participants) [n]	1,918 (775)	1,629 (649)	1,333 (532)	1,027 (406)	800 (317)
1.	Strawberry	Strawberry	Strawberry	Strawberry	Strawberry
2.	Elderberry juice	Apple with peel	Elderberry juice	Red cabbage	Elderberry juice
3.	Apple with peel	Red cabbage	Cherries, sweet	Black currant	Red cabbage
4.	Pear	Elderberry juice	Red cabbage	Apple with peel	Black currant
5.	Cherries, sweet	Cherries, sweet	Apple with peel	Elderberry juice	Apple with peel
Sum		60.9	59.5	60.3	61.4

a significant effect on anthocyanidin density of the diet in this model: the diets of girls were about 0.2 mg/MJ richer in anthocyanidins than the diets of boys.

Discussion

Anthocyanins were widely distributed in the diet of German children and adolescents of the DONALD study. The estimated median anthocyanidin intake was around 6 mg/day, the estimated mean intake was twice as high and strawberries represented the major source. Anthocyanidin density of the diet was higher in girls than in boys, decreased with age, decreased slightly with time in the first and increased slightly with time in the last half of the 20-year study period.

Due to the occasional consumption of foods very rich in anthocyanins, the distribution of anthocyanin intake in our sample was found to be skewed to the right. This is true for most dietary components [43] and was also shown in other anthocyanin intake estimations reporting mean values much higher than the median values and 10–36 % ‘non-consumers’ [16, 25, 29]. Because of our detailed dietary assessment and the anthocyanidin assignment on the ingredient level, in our sample the left part of the distribution represents ‘low consumers’ instead of ‘non-consumers’ in other studies.

Comparisons between anthocyanin intake in samples of different age and regions are possible only to a very limited extent, because for estimations diverse dietary assessment methods, study designs as well as anthocyanin content data have been used. For example, mean intake in the UK estimated by the use of Food Balance Sheets was 69 mg/day, so more than twice as high as the mean intake estimated using a 24-h dietary recall (24HR) in the two female British EPIC centres (24 and 31 mg/day) [26, 30]. In Finnish studies, intake estimated on the basis of the average berry consumption also resulted in much higher values (82 mg/day) [14] than estimations using a 48-h dietary interview in a cross-sectional study (40 mg/day) [22] or using a 4-day food record in a prospective cohort study (6 mg/day) [7].

Despite these known difficulties in comparing estimated intake, at least a vague classification of our data in relation to other studies should be made. The only published intake estimation in children and adolescents reported a mean anthocyanidin intake of 0.0–0.7 mg/day in 2–18-year-old Australians [19]. This is considerably less than our findings of around 6 mg/day as median anthocyanidin intake and 11–16 mg/day as mean intake. The reason for the low estimation might be an underestimation due to the use of a single 24HR in a quite small number of subjects ($n = 357$) [44] or different food intake patterns in Australia and Germany. In German, adults’ mean anthocyanidin intake

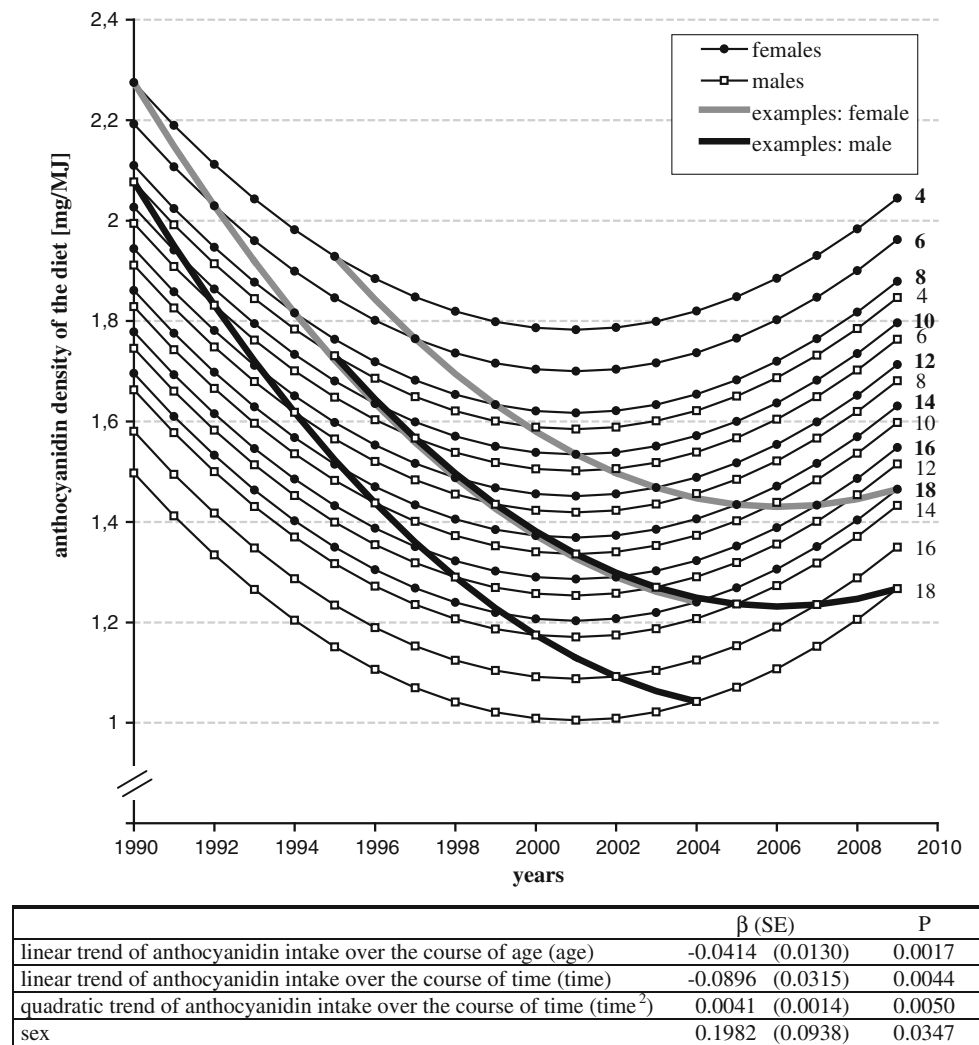


Fig. 2 Age and time trends of estimated anthocyanidin density (mg/MJ) of the diet of children and adolescents from the DONALD study (6,707 records, 920 participants) between 1990 and 2009 (repeated measurements), predicted by a polynomial mixed effects regression model. Numbers at data points indicate years of age. The pure time trend is represented by the course of the sixteen *parallel curves* for eight age groups, stratified by sex. The pure age trend is represented by the constant difference on the *vertical axis* between the *curves*. The combination of these trends results in the bold curves, which therefore are example *curves* for girls aged 4 years in 1990 and girls aged 4 years in 1995 (grey bold lines), as well as for boys aged 4 years in 1990 and boys aged 4 years in 1995 (black bold lines). The two *parallel curves* starting in 1990 representing children aged

4 years in 1990 are mainly characterised by a decreasing anthocyanidin density with increasing age, enhanced by the decrease in anthocyanidin density over the course of time. Only a slight attenuation of this decrease gets effective through the quadratic time trend. The constant difference between the *two curves* represents the higher anthocyanidin density in girls' diets than in boys', independent from age and time. The two *parallel curves* starting in 1995 represent children who were born 4 years later than those previously described. Their trend of anthocyanidin density is also mainly characterised by the linear decrease in anthocyanidin density with growing age. However, here the quadratic time trend leads not only to attenuation, but even to a reversion of the trend in adolescence

was 7 mg in an early estimation [16] and 30–41 mg/day in a recent estimation in the EPIC cohort [30]. Our estimation lies in between, probably due to less available anthocyanin content data in the former and due to wine consumption as well as less detailed dietary assessment in adults in the latter estimation. Only a few of the recent estimations in subgroups of European adults are lower [7, 25, 29], whereas most of them are higher than in our young sample [7, 14, 15, 17, 18, 21, 22, 26, 30]. In contrast, estimated

intake in adults beyond Europe, that is USA, Oceania and Japan, is in the range of or below our estimated intake [12, 13, 19, 20, 23, 24, 27, 45, 46], indicating that differences are due to different food patterns rather than age.

In our sample aged 4–18 years, the top five sources of anthocyanins contributed to intake with 60–62 % in each age group. This is similar to toddlers of the DONALD study, but in infants contribution of the five main sources was higher (84–90 %) [34]. This finding indicates that the

diversification of food sources levelled off after infancy and kept stable until adolescence. Taken together, strawberries were the major source for anthocyanins from the age of 18 months throughout childhood and adolescence till the age of 18 years [34]. Although they are a seasonal fruit, they are consumed frequently as a popular ingredient in jam and fruit yogurts throughout the year. As in infants and toddlers also in children and adolescents, red cabbage was the only common anthocyanin-containing vegetable.

In EPIC, fruits were the main source for anthocyanins, too [30]. However, not strawberries, but grapes, stone fruits, apples and pears were major sources before berry fruit. Such differences may be due to the more detailed dietary record data in DONALD including ingredients and types of jam and yogurts in comparison with the 24HR in EPIC. This methodological explanation is supported by the findings in infants and toddlers from the DONALD study, whose main sources of anthocyanins were apples and pears like in EPIC [34]. This young age group hardly consumes products such as jam or yogurt, but rather consumes fruits as part of porridges or commercial weaning food. Additionally, data presented from EPIC are aggregated data for central Europe (Germany, The Netherlands, UK) and not specific German intake data. In an earlier estimation, berry fruit was the main source in a German adult population [16].

Our study is the first that has analysed age and time trends in anthocyanidin density in the diet of children and adolescents. Following an anthocyanidin density of 0.9 mg/MJ in 3-year-old DONALD participants [34], anthocyanidin density was in the same range in the youngest age group of 4–6-year-old children of the present work, but decreased subsequently with age and was higher in girls than in boys.

The decrease in anthocyanidin density of the diet with age in childhood is plausible, as fruit intake relative to energy intake was found to be higher in younger than in older children and adolescents in Germany and other European countries [47–49]. Fruit and vegetable intake is considerably below the recommended amount in childhood and adolescence, and it is therefore desirable to increase intake [49]. As shifts in dietary choices during transition from childhood to adolescence are influenced by environmental and social factors [50], it is desirable to develop multidisciplinary strategies for increasing consumption.

The downward trend in anthocyanidin density of the diet in our study sample from 1990 onwards has reversed since 2001. A similar decreasing and afterwards increasing secular trend was found for fruit consumption in Danish school children between 1988 and 2006 [51]. In German children and adolescents, fruit and vegetable intake was also higher in 2006 than in the 1980s [52]. As fruits were the main sources of anthocyanins in our study sample, such an increase in fruit consumption is likely to contribute to a

big part to the increase in anthocyanidin density. Fruit consumption may have increased in Germany as well as in Denmark due to the campaign ‘Saday’ (or ‘6aday’ in Denmark), which aims to increase fruit and vegetable intake and was established nationwide in Germany in the year 2000 and in Denmark in the year 2001 [51, 53]. To further clarify the reasons for the observed favourable time trend in anthocyanidin density since 2001, further analysis of the DONALD data are desirable.

In line with our results in children and adolescents, studies analysing gender differences in anthocyanin intake in adults mainly indicate a higher anthocyanidin density of women’s diets [16, 20, 22]. Results for absolute anthocyanin intake are varying. For example, in Southern European countries anthocyanidin intake in men was higher, attributable to the higher intake of red wine in men [28–30], whereas in central European countries and in Finland women had a higher absolute intake [22, 30].

The main reason for the higher anthocyanidin density in girls compared to boys in all age groups of our sample may be the higher consumption of fruits and vegetables in girls in Germany in most age groups [52, 54]. Similar results were found in other European children [55] as well as in German adults [56]. Perhaps the selection of fruit products of girls may also be richer in anthocyanins than that of boys, what remains to be studied. A study by Bere et al. [57] found that higher preference for fruit and vegetables among adolescent girls than among adolescent boys was the main reason for the higher intake of fruits and vegetables in girls. The authors therefore suggested that further research should investigate why girls like fruit and vegetables more than boys. Interestingly, in a study in adults not preferences, but rather poorer nutrition knowledge of men mediated the gender differences in intake of fruit and vegetables [58].

Even though it is not known which level of anthocyanin intake may be health-promoting, our data show that there is scope to increase anthocyanidin density of the diet in later childhood and adolescence and in particular in boys. Therefore, suitable approaches to counteract the decrease in intake during youth are needed. Additionally, it is of special interest to study the gender differences in food patterns as well as the reasons for decreasing and increasing time trends to derive practical health-promoting strategies.

Inherent weaknesses in every intake estimation to be named are the inaccuracy of dietary assessment on the one hand and the technological [59] as well as the biological [60] variability in food content data on the other hand. In most studies, both over- and underestimation of the true anthocyanin intake are conceivable. In our study, anthocyanin content in processed foods such as jam may have been overestimated, as losses, for example, due to high temperatures during storage and processing [59], were not

considered. It is appreciated that work is going on to provide retention factors for processed and cooked foods by the database 'Phenol-Explorer' [61]. In contrast, the omission of anthocyanin intake from dietary supplements may have caused underestimation to some extent, however, not quantifiable. A further limitation of our study is that the study sample is non-representative, as participants of the DONALD study have a higher socio-economic status compared to the general German population [37]. However, dietary recommendations derived from DONALD analyses are nearly the same to those derived from the nationwide representative survey 'EsKiMo' [54, 62].

A major strength of our analysis is the detailed dietary assessment method used in the DONALD study, which is based on annual 3-day weighed dietary records and the detailed entering of consumed foods in the in-house food and nutrient database [37]. This allowed us to capture also anthocyanins consumed in composite foods. Another strength is the use of the USDA database, which was widely applied in previous intake estimations as well as epidemiological studies. Therefore, comparison with other intake estimations is facilitated at least on this point. One may question whether it is reasonable to apply an US database to foods consumed by a German population. However, values presented cover a wide range of varieties of different regions including Europe and foods are nowadays sold worldwide. A further strength is the longitudinal design of our study, which made it possible to calculate age and time trends based on individual intake data, assessed with the same methods over 20 years.

In conclusion, this work for the first time provides data on anthocyanin intake and its trends in young Germans. It is characterised by differences in anthocyanidin density of the diet between girls and boys and by decreasing density from young childhood to adolescence. Observations in this German study population should be extended by further studies in other countries to widen knowledge on anthocyanin intake in childhood and adolescence.

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