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Anthocyanins in the diet of children
- Intake estimations and sensory evaluation of relevant foods -

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"There are two schools of thought about food tables. One tends to regard the figures in them as having the accuracy of atomic weight determinations; the other dismisses them as valueless on the ground that a foodstuff may be so modified by the soil, the season or its rate of growth that no figure can be a reliable guide to its composition. The truth, of course, lies somewhere between these points of view."

Widdowson, EM and McCance, RA. Food tables Their scope and limitations. Lancet (1943) 241(6234):230-232

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ABBREVIATIONS

ANTHONIA Project	ANTHOcyanins - Nutritional Investigations in Alliance Project
BMBF	German Federal Ministry of Education and Research (BundesMinisterium für Bildung und Forschung)
CHD	Coronary Heart Disease
CVD	CardioVascular Disease
DONALD Study	DOrtmund Nutritional and Anthropometric Longitudinally Designed Study
eBASIS	BioActive Substances in foods Information System
EPIC	European Prospective Investigation into Cancer and Nutrition
EsKiMo Study	Eating study as a KiGGS Module (Ernährungsstudie als KiGGS Modul); KiGGS: German Health Interview and Examination Survey for Children and Adolescents (Kinder- und Jugendgesundheitssurvey)
EuroFIR	European Food Information Resource
FAO	Food and Agricultural Organisation
FFF DB	Functional Food Factors DataBase
FFQ	Food Frequency Questionnaire
FKE	Research Institute of Child Nutrition (Forschungsinstitut für KinderErnährung)
HPFS	Health Professionals Follow-up Study
KIHD Study	Kuopio Ischaemic Heart Disease risk factor Study
LEBTAB	In-house food and nutrient database (LEBensmittelTABelle) of the FKE
NHANES	National Health And Nutrition Examination Survey
NHS	Nurses Health Study
PAL	Phenylalanine Ammonia-Lyase
Q	Research Question
USDA DB	US Department of Agriculture DataBase for the flavonoid content of selected foods
SU.VI.MAX Study	SUpplementation en VItamines et Mineraux Antioxydants Study
4DFR	4-Day Food Record
24HR	24 Hour Recall
48HR	48 Hour Recall

ABSTRACT

Anthocyanins are orange to blue coloured secondary plant metabolites that are present in plant foods and might contribute to the prevention of chronic diseases, such as cancer and cardiovascular disease. From a public health point of view, a health-promoting diet is particularly important in childhood, as food preferences and dietary habits may be established during this period and maintained into adulthood.

The aims of this thesis were to assess anthocyanin intake (as aglycones, called anthocyanidins), sources of anthocyanins, and trends with age and time of anthocyanidin density of the diet of infants and toddlers (0-3 years), and children and adolescents (4-18 years) from 1990-2009, and to examine the liking of anthocyanin-rich juices and smoothies (whole fruit drinks) in 4-17-year-olds as potential products to increase anthocyanin intake.

Within the scope of the BMBF joint research project ANTHONIA, this thesis used the existing data and structure of the longitudinal DONALD Study - specifically the regular 3-day weighed dietary records for anthocyanin intake estimations and the participants' regular visits to the Research Institute of Child Nutrition for the purpose of conducting hedonic sensory testings. Anthocyanin content data were taken from the USDA Database for the Flavonoid Content of Selected Foods. However, the anthocyanidin content values for bananas and nuts provided by the USDA have been excluded, because they base on an unsuitable analytical method. The anthocyanin-rich products were produced by the Geisenheim Research Center.

Anthocyanins were widely present in the diet from late infancy onwards. Pomaceous fruit (pears, apples) were the main source in infancy and strawberries in the older age groups. Anthocyanidin density increased during infancy and decreased thereafter. Over the 20 years, density decreased in infants, slightly increased in toddlers (18-36 months) and decreased in the first half, but increased in the second half of the study period in children and adolescents. Density was higher in girls than in boys during childhood and adolescence.

Anthocyanin-rich products, in particular grape-bilberry juice, were well-liked. Juices were liked better than smoothies and grape-bilberry better than apple-bilberry mixtures. A subgroup of participants rated smoothies higher than juices and/or apple-bilberry higher than grape-bilberry mixtures.

In conclusion, there is scope to increase the anthocyanidin density of children's diets, particularly with increasing age and in boys, and grape-bilberry juice is a promising product with which to achieve this. To finally deduce and implement evidence-based dietary recommendations, research firstly on the efficacy of anthocyanins, and secondly on food development and marketing strategies to reach children and adolescents, is fundamental.

ZUSAMMENFASSUNG

Anthocyane sind orange- bis blau-farbige sekundäre Pflanzenstoffe in pflanzlichen Lebensmitteln die zur Prävention chronischer Krankheiten wie Krebs und Herzkreislauferkrankungen beitragen könnten. Aus Sicht der Volksgesundheit (Public Health) ist eine gesundheitsfördernde Ernährungsweise besonders in der Kindheit wichtig, da Lebensmittelvorlieben und Ernährungsgewohnheiten in dieser Zeit geprägt und bis ins Erwachsenenalter beibehalten werden könnten.

Ziele dieser Doktorarbeit waren daher, Anthocyanaufnahme (als Aglykone, Anthocyanidine genannt), -quellen sowie Alters- und Zeitverläufe der Anthocyanidindichte der Nahrung bei Säuglingen und Kleinkindern (0-3 Jahre) sowie bei Kindern und Jugendlichen (4-18 Jahre) von 1990-2009 zu ermitteln und die Beliebtheit anthocyanreicher Säfte und Smoothies (Ganzfruchtgetränke) bei 4-17jährigen zu untersuchen, als mögliche Produkte zur Erhöhung der Anthocyanzufuhr in dieser Altersgruppe.

Im Rahmen des BMBF-Verbundprojekts ANTHONIA wurden für die vorliegende Doktorarbeit vorhandene Daten und Struktur der Langzeitstudie DONALD genutzt, im Besonderen die regelmäßigen 3-Tage-Wiege-Ernährungsprotokolle für die Anthocyanaufnahmeschätzung, und die regelmäßigen Besuche der Teilnehmer am Forschungsinstitut für Kinderernährung zur Durchführung hedonischer sensorischer Tests. Daten zum Anthocyangehalt von Lebensmitteln wurden der „USDA Database for the Flavonoid Content of Selected Foods” entnommen, wobei die darin enthaltenen Anthocyanidinwerte für Bananen und Nüsse ausgeschlossen wurden, da sie auf einer ungeeigneten Analysemethode beruhen. Die anthocyanreichen Produkte wurden von der Forschungsanstalt Geisenheim produziert.

Anthocyane waren vom Säuglingsalter an in der Nahrung weit verbreitet. Kernobst (Äpfel, Birnen) war die Hauptquelle bei Säuglingen, Erdbeeren in den älteren Altersgruppen. Die Anthocyanidindichte der Nahrung stieg im Säuglingsalter an und fiel danach ab. Über die 20 Jahre sank die Anthocyanidindichte bei Säuglingen, stieg bei Kleinkindern (18-36 Monate) leicht und sank in der ersten, aber stieg in der zweiten Hälfte der Studienperiode bei Kindern und Jugendlichen. Die Dichte war in Kindheit und Jugend bei Mädchen höher als bei Jungen.

Anthocyanreiche Produkte, besonders Traube-Heidelbeersaft, waren sehr beliebt, dabei Säfte beliebter als Smoothies und Traube-Heidelbeer- beliebter als Apfel-Heidelbeermischungen. Eine Untergruppe von Teilnehmern bewertete Smoothies besser als Säfte und/oder Apfel-Heidelbeer- besser als Traube-Heidelbeermischungen.

Schlussfolgernd lässt sich sagen, dass es Spielraum zur Erhöhung der Anthocyanidindichte der Nahrung bei Kindern gibt, insbesondere mit steigendem Alter und bei Jungen, und

dass Traube-Heidelbeersaft ein vielversprechendes Produkt hierfür ist. Um letztendlich evidenzbasierte Ernährungsempfehlungen abzuleiten und umzusetzen, ist Forschung zum Einen zur Wirksamkeit der Anthocyane und zum Anderen zu Produktentwicklung und Marketingstrategien zur Erreichung von Kindern und Jugendlichen grundlegend.

1. INTRODUCTION

1.1 ANTHOCYANINS - A BRIEF OVERVIEW

Anthocyanins are water-soluble orange to blue coloured secondary plant metabolites [1], belonging to the flavonoid group, which makes up the most common subgroup of polyphenols in the human diet [2]. Anthocyanins are widely distributed in seed plants and occur in their flowers, leaves, fruits, seeds and other tissues [1] and are ingested by humans with fruit, vegetables and products thereof, such as jam, juice and wine [3]. Their name derives from the Greek words anthos (flower) and kyanos (blue) and was coined in 1835 by the German pharmacist Ludwig Clamor Marquart [4].

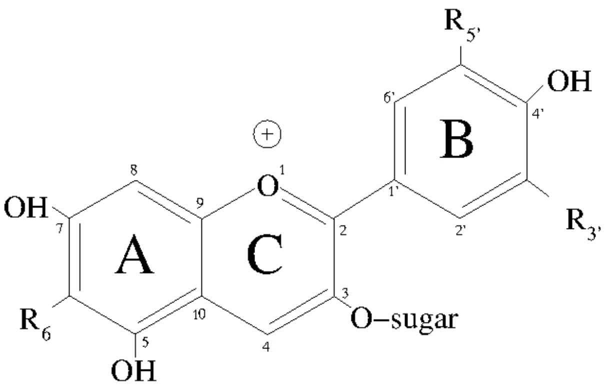
More than a century and a half later, scientific attention started to focus on dietary anthocyanins in the context of prevention of chronic diseases, such as cardiovascular disease (CVD) [5, 6] and cancer [7-9], because it was speculated that antioxidant and anti-inflammatory properties studied *in vitro* and in animals [7, 10] might in part explain the reduction of risk of these diseases through fruit and vegetable consumption that is described in epidemiological studies [11]. Indeed, recent epidemiological studies suggest several potential health-promoting effects of anthocyanins. For instance, case-control studies showed a protective effect of anthocyanins on colorectal cancer [9] and acute myocardial infarction [12]. In a cross-sectional study, higher anthocyanin intake was positively associated with bone mineral density [13]. Large cohort studies revealed associations between anthocyanin intake and CVD [5], diabetes [14] and Parkinson's Disease [15]. The *in vitro* antioxidant activity of anthocyanins [16] is not approved as a crucial mechanism for the potential protective effects in humans [17], due to low bioavailability. It therefore remains to be elucidated, which molecules or combinations of molecules function via which pathway.

1.2 ANTHOCYANINS - BIOCHEMISTRY

Structure and biosynthesis

Until now, more than 635 different anthocyanins have been identified in nature [3], and about 70 different varieties are present in food plants [18]. Anthocyanins are glycosides - the aglycones (called anthocyanidins) consist of three carbon rings with two aromatic (A and B) and one O-heterocyclic (C) ring (Figure 1), which is characteristic of flavonoids [2].

Among the 31 different anthocyanidins described, cyanidin, delphinidin, malvidin, peonidin, petunidin and pelargonidin are the aglycones of about 90% of all anthocyanins (Figure 1) [19]. The attachment of sugars, most commonly glucose, galactose, rhamnose and arabinose, leads to 3-glycosides or 3,5-diglycosides [20].



R5'	R3'	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OCH_3	H	Peonidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

Figure 1 Anthocyanin structure (created according to [21])

Anthocyanin colour depends on pH, co-existing compounds and metal ions [1]. PH influences the rearrangement of different molecular forms of anthocyanins present in a dynamic equilibrium (Figure 2) [22]. The red flavylium cation is the most stable form and predominates at pH 1-3 [23]. In flowers, the colour of the anthocyanins attracts pollinators [24] and in fruits, it attracts animals for seed dispersal [25]. In photosynthetic tissues, anthocyanins protect plant cells against damage due to sunlight [26].

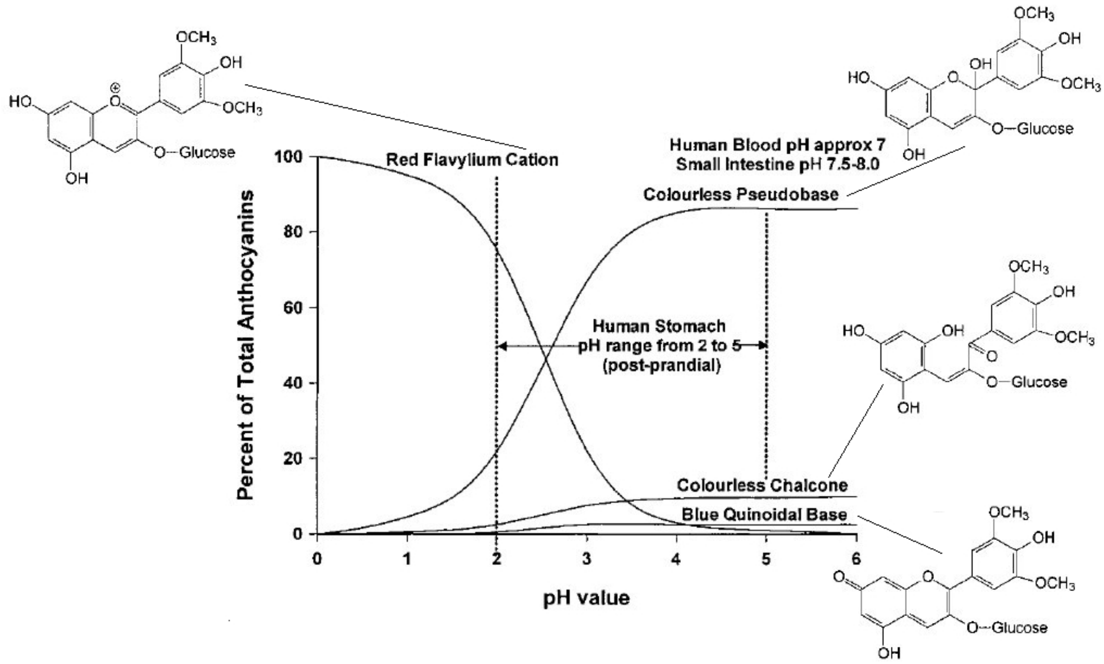


Figure 2 Generalised effect of pH value on anthocyanin equilibria (modified according to [20])

Anthocyanidins are synthesised in the cytoplasm of plant cells and stored in vacuoles as glycosides [1]. In particular, phenylalanine produced in the shikimic acid pathway (which is present in plants, bacteria and fungi, but lacking in animals [26]) is channelled from primary into secondary metabolism by phenylalanine ammonia-lyase (PAL) [27]. Here, in the phenylpropanoid pathway, p-coumaroyl-CoA, the starting molecule for the synthesis of flavonoids, is built [26]. Combining this with three malonyl-CoA molecules (also derived from the primary metabolism), the enzyme chalcone synthase provides the precursor for a large number of flavonoids - a tetrahydroxy-chalcone, containing a second aromatic ring. The steps that follow include ring closure, introduction of an OH group and of two double bonds, and finally glycosylation of the OH group in the C ring and further modifications of the A and B rings [26]. Mutations in enzymes of this biosynthetic pathway [28] determine variation in anthocyanin profile and content between cultivars [29]. Anthocyanin content of a specific food depends also on growing conditions [30], because key enzymes, such as PAL, are inducible by environmental factors, such as pathogen attack or UV light [27].

Metabolism and bioavailability

After the release of the glycosides from the food matrix as a result of chewing [3], hydrolysis may already start in the oral cavity: some aglycones may be generated by β-glucosidase in saliva, which is derived from the oral microbiota as well as from the human oral epithelial cells shed [31]. Anthocyanins are likely to be in the flavylium cation form in the low pH of the stomach [23] and are absorbed from the stomach as glycosides in rats [32, 33] and humans [34], probably via bilitranslocase [35]. In the small intestine, pH is higher and it is therefore likely that the carbinol pseudobase form predominates [23]. Anthocyanins are able to pass through the small intestinal wall, as was shown in rats, where uptake reached 7.5% of the ingested anthocyanins [36], the absorption rate being presumably highest in the jejunum [37]. However, intact anthocyanins may not be effectively transported into the circulation after uptake by intestinal tissue [36], which is indicated by their very low bioavailability measured in plasma and urine: In most studies total urinary excretion was < 1% of anthocyanins ingested with foods and maximum plasma concentrations were only in the order of magnitude of nanomole or nanogram/L [38]. However, metabolites can be found in blood, particularly protocatechuic acid (3,4-dihydrobenzoic acid), which is the main metabolite of cyanidin-based anthocyanins and was found to account for 44% of the ingested dose of cyanidin-glucoside after blood orange juice consumption [39]. As revealed in studies with ileostoma patients, depending on the sugar moieties up to 85% of ingested anthocyanins reach the colon as glycosides [40, 41]. Therefore, there is a high concentration of intact anthocyanins during the passage through the gastrointestinal tract [42] and they are available for the colonic microbiota. These bacteria can cleave the glycosidic linkage, and the released aglycones, which are unstable in the neutral pH prevailing in the colon, degrade to aldehydes and phenolic acids [43], such as protocatechuic acid [39]. Anthocyanins were detected in the eye tissue of pigs [44] and shown to pass the blood-brain-barrier to reach brain tissues of rats [45] and pigs [44]. To deepen knowledge on human anthocyanin metabolites and their tissue distribution, studies with isotopically labelled anthocyanins will be needed [17].

1.3 ANTHOCYANINS IN THE HUMAN DIET

Sources

Anthocyanins enter the human body by the intake of food plants, such as fruits, vegetables and products thereof, such as juices, wine, jam and again composite foods such as cakes, yoghurts and complementary baby food. During processing and storage changes in anthocyanin content can occur [46]. Mean anthocyanin content (± SD) in anthocyanin-containing foods was calculated as 115 ± 259 mg/100 g and highest concentrations of anthocyanins (up to > 1000 mg/100 g) were found in dark-coloured fruits such as berries and cherry [47]. But also fruits with lower anthocyanin concentrations, such as apples (< 5 mg/100 g) [18, 48], can be important sources when they are consumed frequently. Main sources identified in European adults' diets are wine and berries [49]. Anthocyanins are permitted as food colourant at "quantum satis" in the European Union [50, 51]. An acceptable daily intake (ADI) value was not specified, because "on the basis of the available toxicological, biochemical and clinical data, the total intake of the substance, arising from its natural occurrence and/or its present use or uses in food at the levels necessary to achieve the desired technological effect, will not represent a hazard to health" [52].

Estimation of intake

To estimate dietary anthocyanin intake, both food consumption data and food composition data are required. Food consumption data can either be obtained from food balance sheets at an aggregate population level or from questionnaires or records at an individual level [53]. Dietary assessment methods on the individual level, which are applied in surveys or epidemiological studies, include 24-hour recalls (24HR), estimated or weighed food records, diet histories and food frequency questionnaires (FFQ) (called "semi-quantitative" when subjects are asked to quantify usual portion sizes of food items) [54]. Food composition data for phytochemicals are widely available in tens of thousands of publications, but as a new and important type of scientific archive, databases are being established [55]. Among several databases covering different aspects of phytochemicals [55], food composition data for anthocyanins are available in three databases at present:

The **USDA Database for the Flavonoid Content of Selected Foods** (USDA DB) was published online in March 2003 [56], updated in 2006, 2007 and 2011 [57] and is the most widely used flavonoid content database in epidemiological studies today (Table 1). The version that was the latest during this work (Release 2.1, 2007) includes 3,154 mean values for 385 foods and 27 compounds extracted from 199 papers [48]. It provides mean content values condensed from the literature and anthocyanins are expressed as anthocyanidins.

Where literature gave values for individual glycosides, the glycoside values were converted into aglycone equivalents using conversion factors based on molecular weight. Fivehundred and sixty values in 125 foods are presented for the following six main anthocyanidins as mg per 100 g edible portion: cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin [48]. Each mean content value provided for a given compound and food is accompanied by a quality code (A-D) to indicate the relative quality of the data [56]. The database is downloadable as a pdf file and as a Microsoft Access database [48].

Phenol-Explorer is a newer database, containing information on phenolic compounds in foods and their metabolites in animals and humans, which has been compiled by the French National Institute for Agricultural Research [58]. The first version was published online in August 2009, version 2.0 was launched in October 2011 and included a metabolism section (J. Pérez-Jiménez, personal communication, February 2012). After exclusion of composition data that met exclusion criteria such as the use of an inappropriate method of polyphenol extraction, 37,634 composition data extracted from 638 publications were included in the database [58]. It covers more than 500 polyphenol glycosides, esters and aglycones in more than 450 foods [58], among these 71 different anthocyanins and 38 anthocyanin-containing foods [18]. The values provided are weighted means of the original content values according to the number of samples used to generate them, and were aggregated separately for five categories of analytical methods [58]. They are available expressed both as individual polyphenols and as aglycone equivalents [18]. Phenol-Explorer is accessible and can be queried online. The complete food composition database is downloadable as a Microsoft Access file and users can download results of their queries as Microsoft Excel files [18, 47].

EuroFIR eBASIS (BioActive Substances in Foods Information System) has been built within the framework of the European Food Information Resource (EuroFir) Network and has its origins in earlier British and Danish databases [59]. It covers compositional and biological data on bioactive compounds as well as a list of major food plants consumed in Europe [59]. For information on potentially health-promoting compounds eBASIS provides composition data for 256 compounds from 17 compound classes in 199 foods, among these are 817 data points from 38 peer reviewed publications covering 40 different anthocyanins and 31 anthocyanin-containing foods (J. Plumb, personal communication, using information from eBASIS [60], May 2012). Compositional information is only extracted from peer-reviewed journal articles. Based on answers given by the evaluators within a “Critical Evaluation Scoring System“, a quality code is calculated and provided for each data point [61]. The database is accessible online, can be queried and user-driven reports can be created and downloaded, e.g. as a spreadsheet [61]. However, in contrast to the USDA DB and Phenol-Explorer, access to eBASIS requires paid membership of the EuroFIR AISBL (Association Internationale Sans But Lucratif) [60].

One of the first estimations of the intake of flavonoids, which is widely quoted, is that of the German physiologist Joachim Kühnau from 1976 [62]. The mean daily intake of flavonoids in the USA was estimated to be 1 g and the daily intake of anthocyanins 215 mg in summer and 180 mg in winter respectively, calculated as quercetin equivalents. Intake estimations increased rapidly since the mid 2000s, when the USDA DB was released. Table 1 summarises estimations of anthocyanin intake (including the two estimates derived from the research detailed in this thesis), published between 1976 and 2012. Twenty-one of the 50 studies had the purpose of estimating anthocyanin or flavonoid intake, while the rest were epidemiological studies, mainly case-control studies (n = 14). Twenty-nine estimations are from Europe (5 of these from Germany), 16 from the USA and 5 from Oceania and Japan. Three publications regarded children (including the two detailed here). Overall, results vary from 0 to 215 mg/day for anthocyanins and from 0 to 69 mg/day for anthocyanidins. This large variation results not only from an actual difference in intake, but is also due to the use of different dietary assessment methods and different anthocyanin content data. Therefore, a reasonable comparison between the intake in different countries or populations is rather difficult.

Table 1 Studies providing anthocyanin intake estimations

Author	Year	Country	Data sources			Main food sources	Estimated intake				
								[mg/day]		[mg/MJ]	
			Sample[2]	Food consumption	Anthocyani(di)n content		Compounds[1]	Mean ± SD	Median	Mean ± SD	Median
Kühnau [62]	1976	USA	US population	OECD Food Consumption Statistics 1955-1971 (Average American diet)	Available data from the literature until 1974	Fruit, fruit juices (winter: 44%, summer: 53%)	Anthocyanins expressed in terms of quercetin	180 (winter) 215 (summer)			
Linseisen et al. [63]	1997	Germany	Subcohort of the German National Food Consumption Survey (1985-1988) Age: 30.4 ± 1.2 ♀, 29.6 ± 1.3 ♂ n = 63 + 56 (♀ + ♂)	5-day- to 7-day dietary records	Available data from the literature until 1995	Berries	Anthocyanidins (cyanidin, delphinidin, peonidin, petunidin, malvidin)	6.57 ± 0.96 (all) 6.51 ± 1.17 ♀ 6.64 ± 1.58 ♂	2.72 (all) 2.47 ♀ 3.37 ♂		0.29 ♀ 0.26 ♂
Heinonen 2002, cited in Heinonen 2007 [64]	2002	Finland	Finish population	National Food Consumption Data (Average Finish diet)	Not specified	Berries	Anthocyanins	82.5			
Peterson et al. [65]	2003	Greece	Case-control study on breast cancer (1989-1991) Age: 56.4 ± 0.43 (cases), 54.4 ± 0.32 (controls) n = 820 + 1,548 ♀ (cases + controls)	FFQ (115 food items or beverage categories)	USDA DB 2003		Anthocyanidins		[quintile 3 median]: 20.9		
Lagiou et al. [66]	2004	Greece	Case-control study on stomach adenocarcinoma (1981-1984) Age: 64.5 ± 1.2 (cases), 59.8 ± 1.2 (controls) n = 110 (53 ♀) + 100 (51 ♀) (cases + controls)	FFQ (80 food items or beverage categories)	USDA DB 2003		Anthocyanidins		[quintile 3 median]: 20.4		
Lagiou et al. [67]	2004	Greece	Case-control study on CHD (1990-1991) Age: 57.9 ± 0.5 (cases), 56.5 ± 0.5 (controls) n = 329 (45 ♀) + 570 (246 ♀) (cases + controls)	FFQ (110 food items or beverage categories)	USDA DB 2003		Anthocyanidins		[quintile 3 median]: 22.9		
Melby et al. [68]	2004	Japan	Cross-sectional study on phytochemical intake and health history Age: 49.3 ± 2.8 n = 67 ♀	One-day dietary records weekly for 6 months	Japanese database for functional food factors (FFF) containing 57 FFF in 278 foods		Anthocyanidin	11.3 ± 19.2 µmol/day	1.5 µmol/day		
Kita et al. [69]	2004	Japan	Healthy volunteers (2002) Age: 63 ± 10 n = 79 (63 ♀)	One-day dietary record	FFF database		Anthocyanidin	20.5 µmol/day	7.0 µmol/day		
Bosetti et al. [70]	2005	Italy	Case-control study on breast-cancer (1991-1994) Age [median (range)]: 55 (23-74) (cases), 56 (20-74) (controls) n = 2,569 + 2,588 ♀ (cases + controls)	FFQ (78 foods or food groups)	USDA DB 2003	Onion, garlic	Anthocyanidins		10.4 (controls)		
Rossi et al. [71]	2006	Italy	Case-control study on colorectal cancer (1992-1996) Age [median (range)]: 62 (19-74) (cases), 58 (19-74) (controls) n = 1,953 (828 ♀) + 4,154 (2081 ♀) (cases + controls)	FFQ (78 foods or food groups)	USDA DB 2003	Wine, red fruits	Anthocyanidins	20.0 ± 18.7 (controls)			
Tavani et al. [12]	2006	Italy	Case-control study on acute myocardial infarction (1995-2003) Age [median (range)]: 61 (19-79) (cases), 59 (16-79) (controls) n = 760 (180 ♀) + 682 (243 ♀) (cases + controls)	FFQ (78 foods or food groups)	USDA DB 2003	Red wine, red fruits, onion, garlic	Anthocyanidins	19.2 (controls)			
Lagiou et al. [72]	2006	Greece	Case-control study on peripheral artery occlusive disease (1982-1984) Age [n in the 4 age groups: ≤ 49/50-59/60-69/≥70]: 3/18/32/47 (cases), 5/31/31/33 (controls) n = 100 (12 ♀) + 100 (13 ♀) (cases + controls)	FFQ (110 items)	USDA DB 2003		Anthocyanidins	10.8 ± 1.1 (cases) 13.5 ± 1.5 (controls)			
Wu et al. [73]	2006	USA	National Health and Nutrition Examination Survey (NHANES) (2001−2002) Age: not specified; ≥ 0 according to [74] n: not specified	Not specified; 24HR according to [74]	HPLC analysis of > 100 foods on the US market + data for grape juices by Welch's + published data for wine	Blueberry (27%)	Anthocyanins	12.53			

Author	Year	Country	Data sources			Main food sources	Estimated intake				
								[mg/day]		[mg/MJ]	
			Sample[2]	Food consumption	Anthocyani(di)n content		Compounds[1]	Mean ± SD	Median	Mean ± SD	Median
Johannot & Somerset [75]	2006	Australia	Representative sample of the Australian population (Australian National Nutrition Survey 1995) Age [min]: 2 n = 13,858	24HR	USDA DB 2003	Wine	Anthocyanidins	until age 18: 0.00 (2-3) - 0.71 (4-7); 19+: 2.90			
Lako et al. [76]	2006	Fiji	5 nutritional surveys: Naduri Longitudinal Survey (1952-1994); Fiji National Nutrition Surveys (1982, 1993); Suva-Nausori Corridor cross-sectional study (1996); Verata cross-sectional study (1999); Fiji Food Choice Study (2001) Age [range]: not specified; not specified; 30-39; 18-27; not specified n = 142-220; 4,964, 4,606; 200 ♀; 20 ♀; 140 households	Weighing household edible raw food consumption over a one- or three-week period; 24HR; three 24HR; two seven-day food records; seven-day food record	Analysis of 72 foods + data for 10 foods from the literature		Anthocyanins	0.04 (average over a 49-year period)			
Rossi et al. [77]	2007	Italy	Case-control study on esophageal cancer (1992-1997) Age [median (range)]: 60 (39-77) (cases), 60 (36-77) n = 304 (29 ♀) + 743 (150 ♀) (cases + controls)	FFQ (78 foods or food groups)	USDA DB 2003	Wine, red fruits	Anthocyanidins		23.7 (controls)		
Rossi et al. [78]	2007	Italy	Case-control study on oral and pharyngeal cancer (1992-2005) Age [median (range)]: 58 (22-78) (cases), 58 (19-79) (controls) n = 805 (146 ♀) + 2,081 (779 ♀) (cases + controls)	FFQ (78 foods and beverages + recipes)	USDA DB 2003	Wine, red fruits	Anthocyanidins	21.9 ± 21.0			
Garavello et al.[79]	2007	Italy	Case-control study on laryngeal cancer (1992-2000) Age [median (range)]: 61 (30-80) (cases), 61 (31-79) (controls) n = 460 (45 ♀) + 1,088 (225 ♀) (cases + controls)	FFQ (78 foods and beverages + recipes)	USDA DB 2003	Wine, red fruits	Anthocyanidins	28.0 ± 21.4 (controls)			
Bosetti et al. [80]	2007	Italy	Case-control study on renal cancer (1992-2004) Age [median (range)]: 62 (24-79) (cases), 62 (22-79) (controls) n = 767 (273 ♀) + 1,534 (546 ♀) (cases + controls)	FFQ (78 foods and beverages + recipes)	USDA DB 2003		Anthocyanidins	20.1 ± 17.8 (controls)			
Fink et al. [81]	2007	USA	Case-control study on breast cancer (1996-1997) Age [range]: 20-98, according to [82] n = 1,434 + 1,440 ♀ (cases + controls)	FFQ (blueberries and raspberries not included)	USDA DB 2003		Anthocyanidins	3.15 (cases) 3.51 (controls)			
Fink et al. [83]	2007	USA	Study on breast cancer survival (baseline interview: 1996-1997, follow-up interview: 2002-2004) Age [range]: 25-98 n = 1,210 ♀	FFQ (100 items, blueberries and raspberries not included)	USDA DB 2003		Anthocyanidins		[3rd quintile]: 0.41-1.60		
Chun et al. [84]	2007	USA	Nutrition Examination Survey (NHANES) (1999−2002) Age [min]: 19 n = 8,809 (4,348 ♀)	24HR	USDA DB 2003	Wine (49%)	Anthocyanidins	3.1 ± 0.5 (all) 3.4 ± 0.6 ♀ 2.9 ± 0.4 ♂		0.36 ± 0.05 (all) 0.26 ± 0.05 ♂ 0.45 ± 0.07 ♀	
Mink et al. [5]	2007	USA	Analysis of the prospective Iowa Women's Health Study (IWHS) on CVD mortality (baseline questionnaire: 1986, end of study: 2002) Age [range]: 55-69 n = 34,489 ♀	FFQ (127 items)	USDA DB 2003		Anthocyanidins		0.2 (participants with intake > 0)		
Ovaskainen et al. [85]	2008	Finland	Subsample of a cross-sectional population survey (FINDIET 2002) Age [range]: 25-64 n = 2,007 (1095 ♀)	48HR	Analyses of foods common in Finland + USDA DB 2007	Bilberry	Anthocyanidins	47 ± 79 (all) 53 ± 76 ♀ 43 ± 82 ♂		7 ± 12 (all) 8 ± 12 ♀ 5 ± 12 ♂	
Mursu et al. [86]	2008	Finland	Prospective cohort study on the risk of cancer within the scope of the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study (Enrolment 1984-1989) Age [range]: 42-60 n = 2,590 ♂	4-day food record (4DFR) using household measures	USDA DB 2006		Anthocyanidins	5.9			
Mursu et al. [87]	2008	Finland	Prospective cohort study on the risk of ischaemic stroke and CVD mortality within the scope of the KIHD Study (Enrolment 1984-1989) Age [range]: 42-60 n = 1,950 ♂	4DFR using household measures	USDA DB 2003		Anthocyanidins	6.2			
Mullie et al. [88]	2008	Belgium	Healthy dietitians (2004) Age [mean (range)]: 40 (22-69)	FFQ designed to assess flavonoid intake (2 times), non-	USDA DB 2003		Anthocyanidins	7.6 ± 8.5 (FFQ1) 7.7 ± 8.1 (FFQ2)			

Author	Year	Country	Data sources			Main food sources	Estimated intake				
								[mg/day]		[mg/MJ]	
			Sample[2]	Food consumption	Anthocyani(di)n content		Compounds[1]	Mean ± SD	Median	Mean ± SD	Median
			n = 45 ♀	consecutive 4DFR				5.5 ± 10.0 (4DFR)			
Cutler et al. [89]	2008	USA	Analysis of the prospective IWHS on risk of cancer (baseline questionnaire: 1986, end of study: 2004) Age [range]: 55-69 n = 34,708 ♀	FFQ (127 items)	USDA DB 2003		Anthocyanidins		0.1		
Frankenfeld et al. [8]	2008	USA	Case-control study on non-Hodgkin lymphoma risk (1998-2000) Age [range]: 20-74 n = 466 (215 ♀) + 390 (195 ♀) (cases + controls)	FFQ (117 items)	USDA DB 2003		Anthocyanidins		[4th quartile]: 0.001-0.152		
Bobe et al. [90]	2008	USA	Analysis of the Polyp Prevention Trial (4-year randomised, multicenter, nutritional intervention trial) on colorectal adenoma recurrence (1991-1994) Age: 61.1 ± 9.9 n = 1,905 (686 ♀)	FFQ (119 items)	USDA DB 2006	"Other fruit" (grape, plum, pineapple) (45%), banana[3] (19%), "other fruit juices" (apple, cranberry, grape), fresh strawberry, apple	Anthocyanidins		10.1		
Chun et al. [6]	2008	USA	Cross-sectional study on serum c-reactive protein concentrations using data from NHANES (1999-2002) Age [min]: 19 n = 8,335 (4,110 ♀)	24HR	USDA DB 2003		Anthocyanidins		[2nd tertile]: 4.7-22.6		
Somerset & Johannot [91]	2008	Australia	Australian National Nutrition Survey (1995-1996) Age [min]: 19 n = 10,851	24HR	USDA DB 2003	Wine (84%)	Anthocyanidins	2.9			
Bobe et al. [92]	2009	USA	Case-control study on esophageal cancer (1986-1989) Age [range]: 30-79 n = 493 + 1235 ♂ (cases + controls)	FFQ (57 foods or food groups,18 beverages)	USDA DB 2007	Banana[3] (10-34%), red wine, apple/pear, peas non green	Anthocyanidins		3.78-6.84 (different case and control groups)		
Dilis & Trichopoulou [93]	2010	Greece	European Prospective Investigation into Cancer and Nutrition (EPIC) (Greek cohort) (enrolment 1994-1999) Age [median]: 54 ♀, 51 ♂ n = 28,572 (16,618 ♀)	FFQ (≈ 200 foods and recipes, 15 types of beverages)	USDA DB 2007	Cherry, apple, peach (cyanidin), eggplant, banana[3], wine (delphinidin), wine (malvidin), strawberry, cherry, apple (pelargonidin), cherry, wine (peonidin), wine, vinegar (petunidin)	Anthocyanidins		10 (all) 9 ♀ 12 ♂		
Zamora-Ros et al. [94]	2010	Spain	Spanish EPIC cohort (enrolment 1992-1996) Age [range]: 35-64 n = 40,683 (25,237 ♀)	Diet history questionnaire (> 600 foods and beverages, ≈ 150 recipes)	USDA DB 2007 + data from the literature	Wine (46%), "fruit, not specified", apple, pear, grape, strawberry, cherry, banana[3] (2.4%), plum, peach	Anthocyanidins	18.88 ± 21.5 (all) 11.38 ± 10.93 ♀ 26.34 ± 26.29 ♂	11.27 (all) 8.27 ♀ 17.4 ♂		
Beking & Vieira [95]	2010	UK, Ireland	Populations of the UK and the Republic of Ireland	FAO Food Balance Sheets	USDA DB 2007 + data from the literature		Anthocyanidins	69.2 (UK) 60.3 (Ireland)			
Drossard et al. [96]	2011	Germany	DONALD Subcohort (1990-2009) Age [range]: 3-36 months n = 942 (476 ♀)	Repeated 3-day weighed dietary records	USDA DB 2007, excl. banana[3] and nuts	Pear, apple, strawberry	Anthocyanidins	0.05 (3 months) - 8.69 (36 months)	0.00 (3 months) - 4.25 (18 months)	0.02 (3 months) - 2.02 (24 months)	0.00 (3 months) - 1.15 (9 months)
Lesser et al. [97] (supplemented by S. Lesser, personal communication, June 2012)	2011	Germany	Residents in nursing homes (2006) Age: 86 ± 7 ♀, 81 ± 8 ♂ n = 712 (80% ♀)	3-day weighed food record (kept by researchers)	USDA DB 2007		Anthocyanidins		15.8		
Lesser et al. [97] (supplemented by S. Lesser, personal communication, June 2012)	Un-pub-lished	Germany	Care-dependent elderly living at home (2010) Age [range]: 65-96 n = 338 (64% ♀)	3-day estimated food records (kept by participants/care staff)	USDA DB 2007		Anthocyanidins		7.8		

Author	Year	Country	Data sources: Sample[2]	Data sources: Food consumption	Data sources: Anthocyani(di)n content	Main food sources	Estimated intake: Compounds[1]	[mg/day] Mean ± SD	[mg/day] Median	[mg/MJ] Mean ± SD	[mg/MJ] Median
Perquier et al. [98]	2011	France	French E3N-EPIC cohort (dietary assessment: 1993-1995) Age [range of year of birth]: 1925-1950 n = 73,034 ♀	Self-administered diet history questionnaire	Phenol-Explorer	Cherry (31%), wine, strawberry, raspberry	Anthocyanins	71.15			
Pérez-Jiménez et al. [98]	2011	France	SU.VI.MAX study (randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals) (enrolment: 1994, 7.5 y of follow-up) Age: 51.5 ± 4.4 n = 4,942 (2,346 ♀)	Six 24H dietary records	Phenol-Explorer	Red wine (41%), cherry, strawberry, black grape	Anthocyanidins Anthocyanins	35 ± 29 57 ± 47			
Zamora-Ros et al. [49]	2011	10 Western European Countries	EPIC cohort (enrolment 1992-2000) Age [range]: 35-74 n = 36,037 (23,009 ♀)	24HDR	USDA DB 2007 + Phenol-Explorer	Southern Europe: Wine (25%), grape; Central: Berries (17%), wine; Northern: Wine (25%), non-alcoholic beverages	Anthocyanidins	29.44 ± 0.53 ♂ 33.52 ± 0.39 ♀ south to north gradient			
Cassidy et al. [99]	2011	USA	Study on hypertension using data from NHS I (1990); NHS II (1991); HPFS (1990) Age [mean (range)]: 55 (30-55); 36 (25-42); 56 (40-75) n = 46,672 ♀; 87,242 ♀; 23,043 ♂	FFQ every 4 years	USDA DB 2007 + EuroFIR eBASIS + data from the literature	Blueberry (about 30-40%), banana (about 14-20%)[3], strawberry	Anthocyanidins	12.5 (NHS I ♀) 14.0 (NHS II ♀) 15.2 (HPFS ♂)			
Landberg et al. [100]	2011	USA	Cross-sectional study on inflammation markers and endothelial dysfunction using data from NHS (1990) Age [range]: 43-70 n = 2012 ♀	FFQ (131 items)	USDA DB 2007 + EuroFIR eBASIS + data from the literature	Berries (62%)	Anthocyanidins		10		
Drossard et al. [101]	2012	Germany	DONALD Subcohort (1990-2009) Age [range]: 4-18 n = 920 (459 ♀)	Repeated 3-day weighed dietary records	USDA DB 2007, excl. banana[3] and nuts	Strawberry	Anthocyanidins	11.69 (4-6 ♀) - 12.62 (7-9 ♀) ; 10.69 (4-6 ♂) - 16.33 (16-18 ♂)	5.66 (4-6 ♀) - 6.44 (7-9 ♀) ; 5.35 (4-6 ♂) - 6.36 (16-18 ♂)	1.63 (16-18 ♀) - 2.28 (4-6 ♀) ; 1.47 (13-15 ♂) - 1.86 (4-6 ♂)	0.82 (10-12 ♀) - 1.10 (4-6 ♀) ; 0.60 (16-18 ♂) - 0.96 (4-6 ♂)
Welch et al. [13]	2012	UK	Cross-sectional study on bone mineral density using data from the TwinsUK adult twin registry (1996-2000) Age [mean (range)]: 48 (18-79) n = 3,160 ♀	FFQ	USDA DB 2007 + Phenol-Explorer	Grape (about 20%), pear, wine, berries and fruit yogurts	Anthocyanidins		13.7		
Kesse-Guyot et al. [102]	2012	France	Prospective study on cognitive function using data from the SU.VI.MAX study (baseline: 1994-1996, follow-up: 2007-2009) Age [range]: 45-60 n = 2,574 (1,161 ♀)	Six 24H dietary records	Phenol-Explorer		Anthocyanins	71 ± 52 ♂ 45 ± 37 ♀			
Wedick et al. [14]	2012	USA	Prospective study on type 2 diabetes using data from NHS I (1984-2008); NHS II (1991-2007); HPFS (1986-2006) Age [mean (range)]: 50 (37–65); 36 (26-45); 53 (40-75) n = 70,359 ♀; 89,201 ♀; 40,420 ♂	FFQ every 2 to 4 years (118 - 166 items)	USDA DB 2007 + EuroFIR eBASIS + data from the literature		Anthocyanidins		8.1 (NHS I ♀) 8.0 (NHS II ♀) 8.3 (HPFS ♂)		
Cassidy et al. [103]	2012	USA	Prospective study on stroke using data from NHS (1990-2006) Age [range]: 30-55 at time of recruitment (1976) n = 69,622 ♀	FFQ every 4 years	USDA DB 2007 + EuroFIR eBASIS + data from the literature	Blueberry (40%), strawberry, apple, red wine, raisin	Anthocyanidins		[3rd quintile]: 9.20-13.56		
McCullough et al. [104]	2012	USA	Prospective study on CVD mortality using data from the Cancer Prevention Study II Nutrition Cohort (1999-2006) Age: 70 ♂, 69 ♀ n = 98,469 (60,289 ♀)	FFQ (152 items)	USDA DB 2007		Anthocyanidins		[quintile 3 median]: 9.8		

This compilation of studies raises no claim to completeness.

[1] Anthocyandins in the USDA DB include the 6 main anthocyanidins cyanidin, delphinidin, malvidin, peonidin, petunidin and pelargonidin.

[2] Age is given in years and as mean (and ± SD if provided) unless stated otherwise.

[3] Bananas and nuts were falsely identified as anthocyanin sources due to the non-zero values provided by the USDA DB 2007 [105, 106].

1.4 CHILDREN AS CONSUMERS OF ANTHOCYANIN-RICH FOODS

Anthocyanin intake and sources

Prior to this thesis, the only available study on anthocyanin intake in children was based on the Australian National Nutrition Survey 1995 and was published in 2006 [75]. Estimated intake was 0 mg/day in 2-3-year-olds and not above 0.7 mg/day until age 18. As the main sources of anthocyanins for children were not presented here, information on these is still lacking.

In Germany, pomaceous fruit (e.g., apples, pears) are the fruits most commonly consumed by children and adolescents [107]. Berries, as an anthocyanin-rich group, are consumed in the same amounts as tropical (e.g., bananas) and citrus fruits (e.g., oranges). Cherries are also a source of anthocyanins and follow next as part of the stone fruit group [107]. The sorts of vegetables consumed the most are fruity vegetables (cucumbers, tomatoes), followed by root and tuber vegetables (e.g., carrots), leafy vegetables, and cabbage [107]. The most popular juices among Germans including children are anthocyanin-free apple and orange juice [108, 109].

Therefore, it seems that anthocyanin-rich fruits and vegetables do not play a major role in plant food intake. In addition, like adults, children and adolescents do not meet the recommended daily intake of fruits and vegetables [110-113]. To increase intake, products popular among this age group, such as juices [114, 115] or "smoothies" [116], may be a good possibility. The latter are made of whole fruits or vegetables and in most cases are blended with juices to create a smooth texture [117].

Sensory properties of anthocyanin-rich foods

Anthocyanin-rich fruits and vegetables are characterised by red colour, for example strawberries and raspberries, or dark colour at very high concentrations, for example in black elderberry or black currant [47]. Whereas polyphenol-rich fruits and vegetables may taste bitter and evoke the tactile sensation of astringency, which decreases acceptance among consumers and especially children [118], pure anthocyanins do not contribute to bitterness and astringency themselves [119]. To develop tasty anthocyanin-rich products, either polyphenol-rich products may be blended with juices familiar to children, such as apple juice [120], or anthocyanin-rich varieties of well-liked fruits need to be chosen or developed. For example, Accent and Dakapo are grape sorts that contain anthocyanins not only in the skin, but also in the flesh [121]. Accent is characterised by a vanilla aroma, Dakapo has a rather neutral taste [121].

Development of food preferences

Food preferences in humans develop as an interaction between genetic predispositions on the one hand - such as neophobia, innate preference for sweet or aversion to sour and bitter - and environmental factors on the other hand [122]. The early environment provides flavour experiences in utero, through breast or formula feeding, and through complementary feeding, which lay the foundation of later food choices and are important in establishing life-long food habits [123]. Food acceptance by children is influenced by learning processes such as “mere exposure” to novel food, social influences (parents, peers, media) and association of physiological consequences of food intake with its taste cues [124]. Food preferences seem to already be built in the first years of life [125] and may be maintained into adulthood [126, 127]. Nevertheless, factors such as mere exposure effect, associative conditioning and sensory-specific satiety influence these preferences and therefore food choice throughout the life-span [128]. Younger children might have less stable preferences than older children [129] and preferences might still be more open to influence in childhood than in adulthood, as suggested by a short repeated exposure [130].

Sensory testing with children

There are three main purposes for sensory testing with children: basic research that aims to understand sensory perceptions of infants and children, sensory evaluation with children as trained judges (descriptive analyses) and consumer testing with children as untrained users [131]. Descriptive analyses may more reasonably be carried out by adults [131]. In contrast, valuable data in basic research and product development can be obtained by sensory testing with children [131, 132]. Consumer tests are done routinely by food manufacturers [131], because there is a large market for children’s foods and beverages [133].

Sensory tests with untrained consumers are called hedonic tests and they are chosen to examine which products are preferred and how well products are liked [134]. The term “preference” is used when one product is preferred over another, whereas the term “liking” refers to a hedonic evaluation of a food without direct comparison to other products [128]. Sensory testing methods used to study preference and liking of products are paired comparisons, hedonic scaling and preference ranking, and all of these methods can be suitable from as early as age 4 [131]. However, there is a large variation in cognitive abilities among children of the same age [131, 132]. Children, in contrast to adults, may need more support with conducting a sensory test and under age 8 usually one-on-one interviews are required [133], because reading and writing skills are not sufficient for self-administered tasks [131].

1.5 OBJECTIVE

The overarching aim of this thesis was to estimate and describe anthocyanin intake in children and at the same time provide an approach to increase anthocyanin intake in this age group via the development of popular anthocyanin-rich food products. Figure 3 summarises the research context and highlights the topics of this thesis, which are formulated as research questions 1 to 4 (Q1 - Q4) as follows.

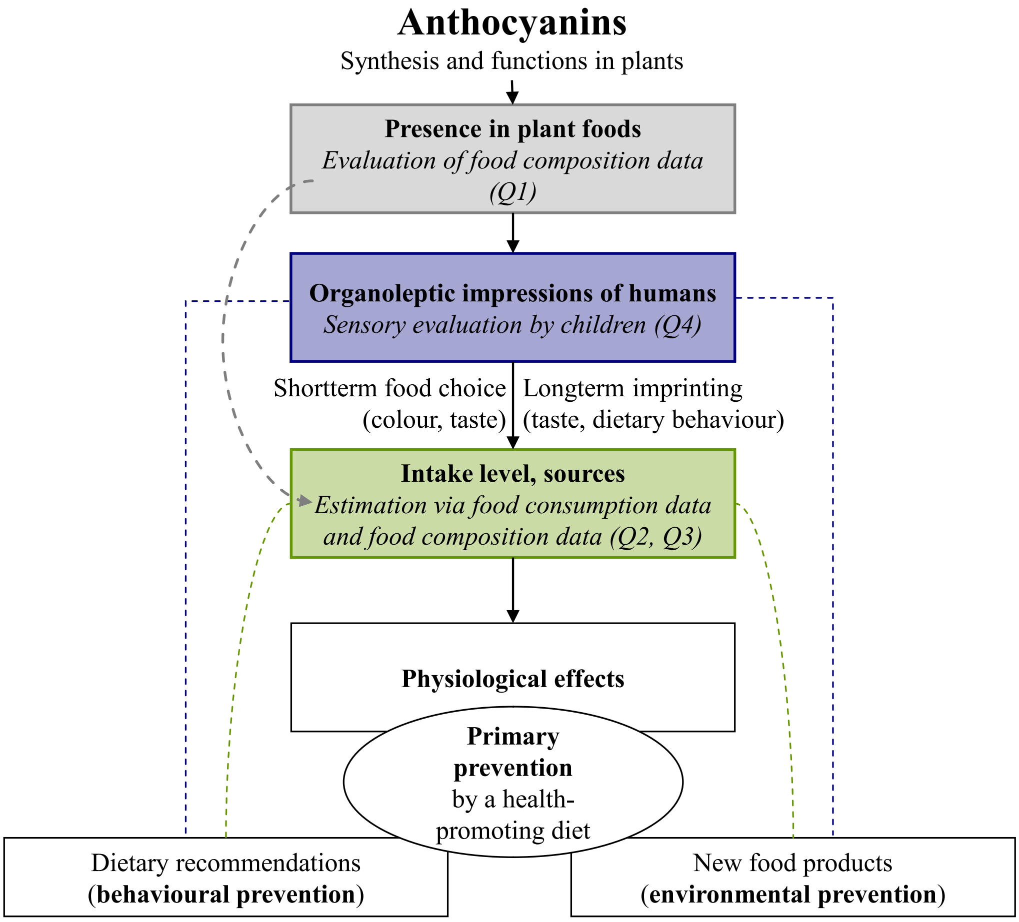


Figure 3 Integration of the specific research topics of this thesis into the general research context

Question 1: Evaluation of food composition data

For anthocyanin intake estimations, anthocyanin content data (expressed as anthocyanidins) were taken from the USDA DB 2007 [48], which was the most comprehensive database on flavonoid content of foods at the beginning of this thesis. However, the question arose as to whether or not bananas contain anthocyanins and the non-zero value given in the database was considered. The questions to be answered were:

- Do bananas contain anthocyanins?
- What may be the effect of false values on results of epidemiological studies?

Question 2: Anthocyanins in the diet of infants and toddlers (0-3 years): intake, sources, age and time trends

Anthocyanin intake estimations for young age groups are lacking. However, as their food preferences may determine food choices and health in later life, it is of special interest to have an insight into their anthocyanin intake. Therefore, with the use of existing dietary data, the questions to be answered were:

- What is the anthocyanidin intake of infants and toddlers in absolute values (mg/day) and in relative values (anthocyanidin density of the diet in mg/MJ)?
- What are the main sources of anthocyanins in the diet of infants and toddlers?
- Does the anthocyanidin density of the diet of infants and toddlers change with age and time?

Question 3: Anthocyanins in the diet of children and adolescents (4-17 years): intake, sources, age and time trends

In parallel with question 2, the questions to be answered were:

- What is the anthocyanidin intake of children and adolescents in absolute values (mg/day) and in relative values (anthocyanidin density of the diet in mg/MJ)?
- What are the main sources of anthocyanins in the diet of children and adolescents?
- Does the anthocyanidin density of the diet of children and adolescents change with age and time?

Question 4: Liking of anthocyanin-rich juices and smoothies by children and adolescents

As intake of fruits and vegetables is lower than recommended in children and adolescents and intake of anthocyanins might be health-promoting, it is of interest to develop anthocyanin-rich products especially liked by this age group. Therefore, a sensory evaluation of anthocyanin-rich fruit juices as well as fruit smoothies was conducted. The questions to be answered were:

- Do children and adolescents accept anthocyanin-rich fruit products, and are fruit composition and viscosity decisive?
- Does blending anthocyanin-rich juice with popular apple juice influence the acceptance of an anthocyanin-rich grape-bilberry juice?
- Can individual preferences be distinguished?

1.6 RESEARCH STRUCTURE

ANTHONIA *(ANTHOcyanins - Nutritional Investigations in Alliance)* is a joint research project focussed on “Anthocyanins in berry fruit juices - *In vivo*-studies on bioavailability and effects on microbiota" (Figure 4) [135]. The project was funded by the German Federal Ministry of Education and Research (BMBF; Research Fund No. 0315379) from 2009 to 2012 and coordinated by Prof. Dr. Clemens Kunz, Institute of Nutritional Science, Justus Liebig University Giessen. The Research Institute of Child Nutrition (FKE) in Dortmund was responsible for one of the seven subprojects and used the existing structure and data of the DONALD Study.

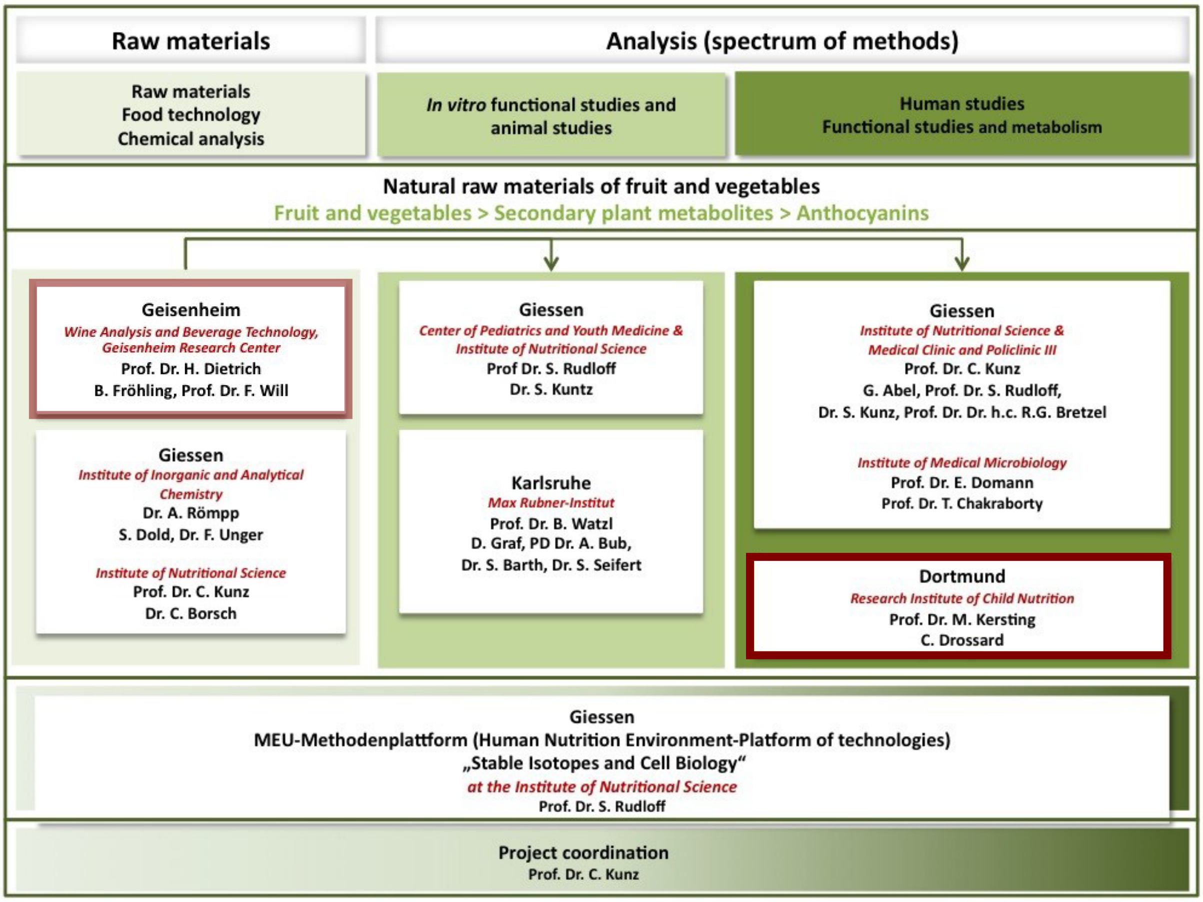


Figure 4 Joint research structure of collaborating institutes in the ANTHONIA Project [136]. Collaborators relevant for this thesis are indicated by the red boxes.

The **DONALD** (*DOrtmund Nutritional and Anthropometric Longitudinally Designed*) Study is a longitudinal open cohort study conducted at the FKE in Dortmund since 1985. Detailed data on diet, growth, development and metabolism between infancy and adulthood have been collected and used for analysis of different research questions. Since 1989, infants have been recruited and systematically followed up at least until the age of 18. The regular visits to the FKE take place every 3 months in the first year of life, twice a year in the second year of life and once a year from the age of 2 onwards (Figure 5). The assessments always include a medical examination, anthropometric measurements, questionnaires and, relevant for this thesis, detailed 3-day weighed dietary records [137].

For these records, the parents of the younger participants or the older participants themselves weigh and record all foods and beverages consumed using electronic food scales (± 1 g) on three consecutive days. Recording house-hold measures, such as number of spoons or scoops, is allowed when weighing is not possible [138]. Any food consumed by the participants is stored in the in-house food 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 with 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 [139].

Within the scope of this thesis, all participants aged 4-17 were invited to take part in an additional tasting session, subsequent to their regular examinations, between February and December 2010.

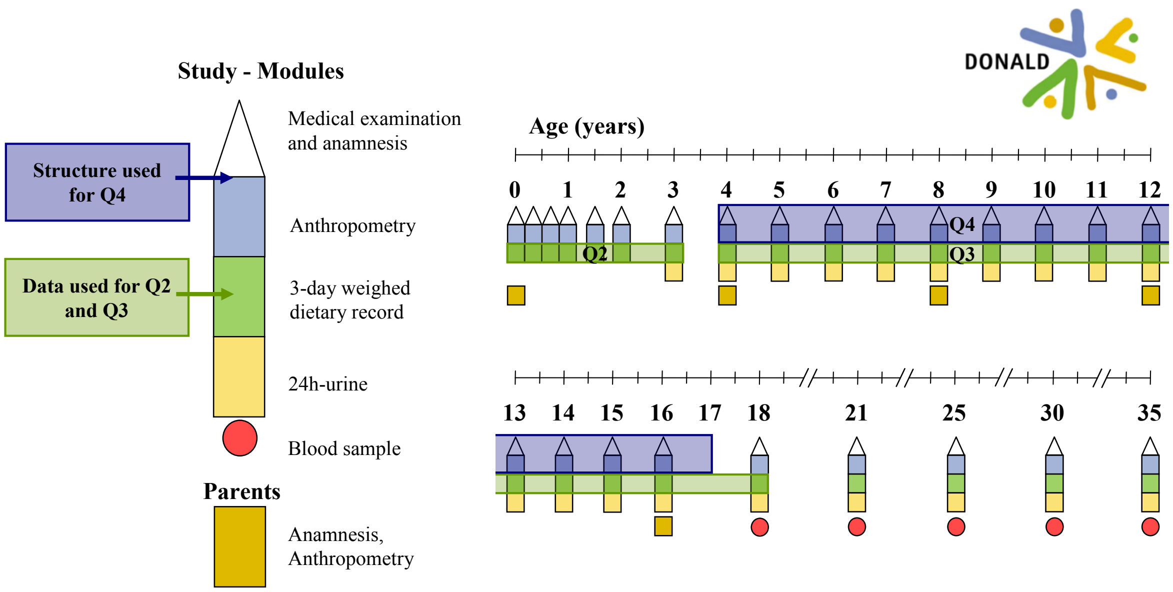


Figure 5 DONALD study schedule. Data and structure used for the present work are highlighted.

Ethical considerations

The DONALD Study is exclusively observational throughout and non-invasive until the age of 18. The study as a whole as well as the additional tasting sessions have been approved by the Ethics Committee of the University of Bonn. All examinations and assessments, including tasting sessions, are performed with parental, and later on the participants', informed consent.

The following table provides overviews of the data and methods used for this work. Precise descriptions can be found in the published manuscripts.

All calculations and statistical analyses were carried out using SAS (Statistical Analysis System, versions 9.1.3 and 9.2, Cary, NC, USA).

Table 2 Data and methods used for this work

	DONALD data and structure	Additional data sources and methods
Q1:	**Evaluation of food composition data**	
Data		USDA DB 2007 [48] Anthocyanin analysis of banana fruit by the Geisenheim Research Center
Q2:	**Anthocyanins in the diet of infants and toddlers: intake, sources, age and time trends**	
Q3:	**Anthocyanins in the diet of children and adolescents: intake, sources, age and time trends**	
Data and sample	Food and nutrient database LEBTAB Q2: 3-day weighed dietary records from participants aged 3-36 months between 1990 and 2009 (942 participants, 934 for mixed model after exclusion of 3-month-olds; 4617 records, 4199 for mixed model) Q3: 3-day weighed dietary records from participants aged 4-18 years between 1990 and 2009 (920 participants; 6707 records)	USDA DB 2007 [48]
Methods	Dietary assessment (3-day weighed dietary records)	
Special statistical methods	Polynomial mixed effects regression model (SAS PROC MIXED) including both fixed and random effects to model age and time trends in anthocyanidin density of the diet and to take into account repeated measurements [140]	
Q4:	**Liking of anthocyanin-rich juices and smoothies by children and adolescents**	
Data	Characteristics of DONALD participants	Production of anthocyanin-rich juices and smoothies by the Geisenheim Research Center Anthocyanin and further analysis of the products by the Geisenheim Research Center
Structure	Regular visits at the FKE	Sensory Panel at the Geisenheim Research Center to study aging processes of products
Sample	DONALD participants aged 4-17 years taking part in the annual visit and providing full data sets (n = 326)	
Methods		Hedonic tests: Pairwise comparisons, pairwise comparisons with forced choice to derive a rank order, age-adapted hedonic scales Questionnaire
Special statistical methods	Mapping technique “Internal Preference Mapping” for applying principal component analysis on preference data, in this case using fruit juice samples as objects and judgements of the participants as variables (SAS PROC PRINQUAL) [141].	

2. PUBLICATIONS

Question 1:

Drossard C, Fröhling B, Dietrich H, Kersting M.

Anthocyanin analysis in banana fruit–a mistake.

American Journal of Clinical Nutrition (2011) 93(4):865-6; author reply 866-7.

First published online 2011 Feb 2.

DOI: 10.3945/ajcn.110.010454

Drossard C, Kersting M.

Banana as a relevant source of anthocyani(di)ns in European populations?

British Journal of Nutrition (2012) 107(5):765-766; author reply 767.

First published online 2011 Sept 28.

DOI: 10.1017/S0007114511004430

Question 2:

Drossard C, Alexy U, Bolzenius K, Kunz C, Kersting M.

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

European Journal of Nutrition (2011) 50(8):705-711.

First published online 2011 Mar 22.

DOI: 10.1007/s00394-011-0184-5

Question 3:

Drossard C, Bolzenius K, Kunz C, Kersting M.

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

European Journal of Nutrition.

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Question 4:

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Letters to the Editor

Anthocyanin analysis in banana fruit—a mistake

Dear Sir:

Recently, Cassidy et al (1) reported that "participants in the highest quintile of anthocyanin intake (predominantly from blueberries and strawberries) had an 8% reduction in risk of hypertension [. . .] compared with that for participants in the lowest quintile of anthocyanin intake." In Figure 1 of their article, bananas are shown to be the second or third important source of anthocyanins. However, this finding has not been addressed by the authors, neither in the "Results" nor in the "Discussion" sections, probably because it cannot be easily explained that bananas are a relevant source of anthocyanins.

The US Department of Agriculture (USDA) database for flavonoids used by the authors (2) provides a delphinidin value for bananas of 7.39 mg/100 g, which is taken from Harnly et al (3), who used acidified methanol (1.2 N HCl) at 75°C for extraction of anthocyanins. This extraction method is patented as a method for assaying anthocyanidins, obtained by hydrolysis of oligomeric proanthocyanidins (4). We analyzed fresh bananas (pulp) by using a methanolic extraction method (80% MeOH) and HPLC–mass spectrometry analysis for detection of anthocyanins. We did not find any anthocyanins in banana pulp. Also, Kevers et al (5) found no anthocyanins in bananas using the pH differential method. We treated banana pulp with HCl (pH 1) at 37°C to examine if anthocyanins are present in a colorless form and which become colorful in acidic medium. No color appeared. However, at 85°C, a red color appeared, indicating the breakdown of proanthocyanidins to anthocyanidins.

Therefore, we assume that the reported anthocyanidin content in bananas did not derive from anthocyanins but rather from proanthocyanidins. This is also the case for some of the values for nuts in the USDA database, and which refer to the same publication as for bananas (3).

To get an idea of the potential effect this analytic artifact would have on the estimated anthocyanidin density in the diet of German infants and toddlers, which might result from the usually high banana consumption in these age groups, we used 4617 3-d weighed dietary records of 942 participants of the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study in combination with the USDA database to calculate 3 anthocyanidin intake scenarios: one including the values for bananas and nuts, one without the value for bananas, and one without the values for bananas and nuts.

Including the delphinidin value for bananas given by the USDA database falsely doubled the estimated median anthocyanidin density in the diet of infants and toddlers. Therefore, bananas would be mistaken as the main source of anthocyanins in this population (**Figure 1**).

Because of our results, Cassidy et al may be interested in recalculating the effect of anthocyanin intake on the risk of hypertension by excluding the anthocyanidin value for bananas from their intake estimation. Risk reduction attributable to anthocyanin intake may thereby even be enhanced.

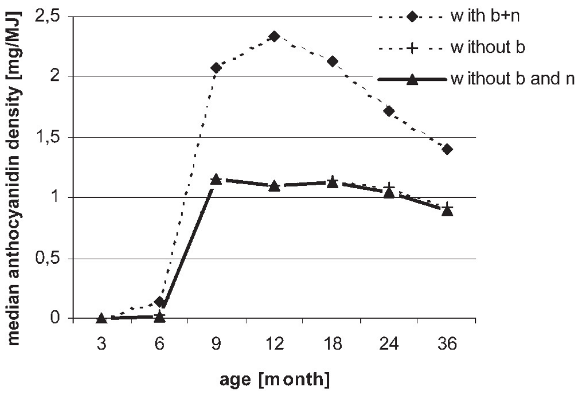

FIGURE 1 Estimated anthocyanidin density (mg/MJ) in the diet of infants and toddlers of the DONALD (DOrtmund Nutritional and Anthropometric Longitudinally Designed) Study with the US Department of Agriculture database (2) anthocyanidin values for bananas and nuts (with b+n), without the value for bananas (without b), and without the values for bananas and nuts (without b and n).

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REFERENCES

1. Cassidy A, O'Reilly EJ, Kay C, et al. Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nutr 2010; 93(2):338–47.
2. Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture. USDA database for the flavonoid content of selected foods release 2.1. 2007. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=6231 (cited 9 January 2009).
3. Harnly JM, Doherty RF, Beecher GR, et al. Flavonoid content of U.S. fruits, vegetables, and nuts. J Agric Food Chem 2006;54:9966–77.
4. Fukui Y, Nakahara K. Method for analyzing oligomeric proanthocyanidin (Opc). 2008. Available from: http://www.faqs.org/patents/app/20080311662 (cited 6 March 2010).
5. Kevers C, Falkowski M, Tabart J, Defraigne JO, Dommes J, Pincemail J. Evolution of antioxidant capacity during storage of selected fruits and vegetables. J Agric Food Chem 2007;55:8596–603.

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Letter to the Editor

Banana as a relevant source of anthocyani(di)ns in European populations?

(First published online 28 September 2011)

Recently, Zamora-Ros *et al.*[1] published interesting results using the large European Prospective Investigation into Cancer and Nutrition (EPIC) dietary data set in combination with anthocyanidin content data from two databases (USDA database for the flavonoid content of selected foods[2] and Phenol-Explorer[3]) for an estimation of anthocyanidin intake in ten European countries. They revealed differences in anthocyanidin intake between northern, central and southern Europe. We agree with the authors that 'these descriptive data will be valuable for future aetiological research focused on the relationships between anthocyanidins and chronic diseases'. We also agree to the authors indicating that it is important to take into account differences among individual anthocyanidins regarding their effects against cancer and cardiovascular as well as neurodegenerative diseases.

However, we do not agree with the statement 'there are no large differences in the anthocyanidin data between the two databases', at least on one point: one noticeable difference is the relatively high delphinidin value (7·39 mg/100 g) given for bananas by the USDA database[2] in contrast to no anthocyanidin values given for bananas by Phenol-Explorer[3]. As bananas are stated as the major source of delphinidin in EPIC subjects from central and northern Europe and the second main source in those from southern Europe in the results section of Zamora-Ros *et al.*[1], we assume that the authors used the value from the USDA database. However, in the supplementary table provided by Zamora-Ros *et al.*[4] bananas are not listed, despite the fact that their delphinidin content listed in the USDA database is higher than that of other fruits provided in the supplementary table and the fact that banana is mentioned as a relevant source.

In this context, we would like to comment on two points. First, as estimated anthocyanidin intake is supposed to be a measure for anthocyanin intake assuming that they or their metabolites are effective in promoting health-benefits, it is essential to include only those anthocyanidin values in intake estimations supposed to be derived from anthocyanins. It has not been shown yet that bananas contain anthocyanins[5–7], but rather contain proanthocyanidins[8] or not yet specified anthocyanidin-polysaccharides[6]. The analytical methodology used to generate the values for bananas in the USDA database[2,9], which was used for the intake estimation of Zamora-Ros *et al.* and other studies, is probably not suitable to measure anthocyanidins from anthocyanins[7]. Therefore, we suggest to exclude this delphinidin value for bananas from the intake estimations of anthocyanins[7,10]. As a consequence of questioning a specific content value, the derived results and interpretations have to be questioned, too. In the present analysis of EPIC data, the inverse regional gradient found for delphinidin, in contrast to the increasing intake of total anthocyanidins, cyanidin, malvidin and peonidin from north to south, might be affected by the exclusion of the delphinidin value for bananas. One could assume that anthocyanidins derived from molecules other than anthocyanins, e.g. from proanthocyanidins, are also bioaccessible in humans and lead to the same effects as those derived from anthocyanins. But in this case, any anthocyanidin derived from molecules other than anthocyanins in any food should be included in the intake estimations, not only those found in bananas.

Secondly, irrespective of the doubts concerning the delphinidin value for bananas, the estimated contribution of bananas to anthocyanidin intake cannot be retrieved from the data as presented. In Table 4, presenting the contribution of foods to the intake of anthocyanidins it is not apparent to which group bananas were allocated. The only reasonable group among those mentioned in this table would be berries, as botanically bananas are berries. But then, it would not be likely that bananas are mentioned as the main source of delphinidin in northern and central Europe and at the same time the berries group is stated as the third important source after non-alcoholic beverages in the results section. The real contribution of bananas to delphinidin intake in this estimation is therefore not clear.

In conclusion, we are aware of the great possibilities for identifying the effects of anthocyanins on diseases with the EPIC data, but we believe that it is important to first be certain about the underlying content data before using them for aetiological studies on the relationship between anthocyani(di)n intake and chronic diseases.

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References

1. Zamora-Ros R, Knaze V, Lujan-Barroso L, *et al.* (2011) Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Br J Nutr* (epublication ahead of print version 12 April 2011).
2. Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Center, *et al.* (2007) USDA Database for the Flavonoid Content of Selected Foods Release 2.1. http://www.ars.usda.gov/Services/docs.htm?docid=6231 (accessed 2009).
3. Phenol-Explorer. Database on polyphenol content of foods. http://www.phenol-explorer.eu/ (accessed 2011).
4. Zamora-Ros R, Knaze V, Lujan-Barroso L, *et al.* (2011) Annex Table 1. Food composition table on anthocyanidins expressed as aglycones (mg/100 mg fresh weight) of the most abundant raw food sources. http://journals.cambridge.org/action/displaySuppmaterial?cupCode=1&type=4&jid=BJN&volumeId=-1&issueId=-1&aid=8255424&sessionId=68E0D743C5C8BBDDEF795449CADED391.tomcat1 (accessed 2011).
5. Kevers C, Falkowski M, Tabart J, *et al.* (2007) Evolution of antioxidant capacity during storage of selected fruits and vegetables. *J Agric Food Chem* **55**, 8596–8603.
6. Bennett RN, Shiga TM, Hassimotto NM, *et al.* (2010) Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (*Musa acuminata* Juss.) cultivars. *J Agric Food Chem* **58**, 7991–8003.
7. Drossard C, Frohling B, Dietrich H, *et al.* (2011) Anthocyanin analysis in banana fruit – a mistake. *Am J Clin Nutr* **93**, 865–866; author reply 866–867.
8. Pérez-Jiménez J, Arranz S & Saura-Calixto F (2009) Proanthocyanidin content in foods is largely underestimated in the literature data: an approach to quantification of the missing proanthocyanidins. *Food Res Int* **42**, 1381–1388.
9. Harnly JM, Doherty RF, Beecher GR, *et al.* (2006) Flavonoid content of US fruits, vegetables, and nuts. *J Agric Food Chem* **54**, 9966–9977.
10. Drossard C, Alexy U, Bolzenius K, *et al.* (2011) Anthocyanins in the diet of infants and toddlers: intake, sources and trends. *Eur J Nutr* (epublication ahead of print version 22 March 2011).

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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.

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

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].

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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	Blueberry	14.2	Apple, with peel	10.0	Apple, with peel	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

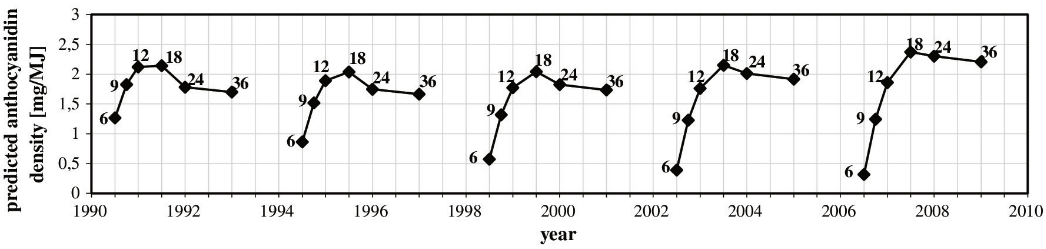
Anthocyanins were widely distributed in the diet of older infants and toddlers in this study population: the estimated median anthocyanidin intake was about 4 mg/day, pomaceous fruit and later on berry fruit, representing the main sources. Anthocyanidin density in the diet increased from 6 to 18 months 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.

References

1. Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733–749
2. Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. Cancer Lett 269:281–290
3. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr (2007) Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 85:895–909
4. Nielsen IL, Dragsted LO, Ravn-Haren G, Freese R, Rasmussen SE (2003) Absorption and excretion of black currant anthocyanins in humans and watanabe heritable hyperlipidemic rabbits. J Agric Food Chem 51:2813–2820
5. Gill CI, McDougall GJ, Glidewell S, Stewart D, Shen Q, Tuohy K, Dobbin A, Boyd A, Brown E, Haldar S, Rowland IR (2010) Profiling of phenols in human fecal water after raspberry supplementation. J Agric Food Chem 58:10389–10395
6. Watzl B, Briviba K, Rechkemmer G (2002) Anthocyane. Ernährungsumschau 49:148–150
7. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem 54:4069–4075
8. Linseisen J (1997) Flavonoidzufuhr Erwachsener in einem bayrischen Teilkollektiv der Nationalen Verzehrsstudie. Zeitschrift für Ernährungswissenschaft 36:403–412
9. Kühnau J (1976) The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev Nutr Diet 24:117–191
10. Heinonen M (2007) Antioxidant activity and antimicrobial effect of berry phenolics–a Finnish perspective. Mol Nutr Food Res 51:684–691
11. Johannot L, Somerset SM (2006) Age-related variations in flavonoid intake and sources in the Australian population. Public Health Nutr 9:1045–1054
12. Ovaskainen ML, Torronen R, Koponen JM, Sinkko H, Hellstrom J, Reinivuo H, Mattila P (2008) Dietary intake and major food sources of polyphenols in Finnish adults. J Nutr 138:562–566
13. Mullie P, Clarys P, Deriemaeker P, Hebbelinck M (2008) Estimation of daily human intake of food flavonoids. Int J Food Sci Nutr 59:291–298
14. Peterson J, Lagiou P, Samoli E, Lagiou A, Katsouyanni K, La Vecchia C, Dwyer J, Trichopoulos D (2003) Flavonoid intake and breast cancer risk: a case–control study in Greece. Br J Cancer 89:1255–1259
15. Chun OK, Chung SJ, Song WO (2007) Estimated dietary flavonoid intake and major food sources of US adults. J Nutr 137:1244–1252
16. Bosetti C, Rossi M, McLaughlin JK, Negri E, Talamini R, Lagiou P, Montella M, Ramazzotti V, Franceschi S, LaVecchia C (2007) Flavonoids and the risk of renal cell carcinoma. Cancer Epidemiol Biomarkers Prev 16:98–101
17. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventos RM, Berenguer T, Jakszyn P, Barricarte A, Ardanaz E, Amiano P, Dorronsoro M, Larranaga N, Martinez C, Sanchez MJ, Navarro C, Chirlaque MD, Tormo MJ, Quiros JR, Gonzalez CA (2010) Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). J Am Diet Assoc 110:390–398
18. Birch LL (1998) Development of food acceptance patterns in the first years of life. Proc Nutr Soc 57:617–624
19. Wu TC, Chen PH (2009) Health consequences of nutrition in childhood and early infancy. Pediatr Neonatol 50:135–142
20. Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture (2007) USDA Database for the Flavonoid Content of Selected Foods Release 2.1. http://www.ars.usda.gov/Services/docs.htm?docid=6231. Accessed 2009
21. Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U, Lentze MJ (2004) The DONALD study. History, current status and future perspectives. Eur J Nutr 43:45–54
22. Kersting M, Sichert-Hellert W, Lausen B, Alexy U, Manz F, Schoch G (1998) Energy intake of 1 to 18 year old German children and adolescents. Z Ernahrungswiss 37:47–55
23. Sichert-Hellert W, Kersting M, Chahda C, Schäfer R, Kroke A (2007) German food composition database for dietary evaluations in children and adolescents. J Food Compos Anal 20:63–70
24. Drossard C, Frohling B, Dietrich H, Kersting M (2011) Anthocyanin analysis in banana fruit–a mistake. Am J Clin Nutr Feb 2. [Epub ahead of print]
25. Magarey A, Daniels LA, Smith A (2001) Fruit and vegetable intakes of Australians aged 2–18 years: an evaluation of the 1995 National Nutrition Survey data. Aust N Z J Public Health 25:155–161

26. Phenol-Explorer (2010) Database on polyphenol content of foods. http://www.phenol-explorer.eu/. Accessed 2010
27. EuroFIR eBasis 2010 BioActive substances in food information system. http://ebasis.eurofir.org/. Accessed 2010
28. Kersting M, Alexy U (2011) Ernährung bei Vorschulkindern—Empfehlungen und Wirklichkeit. Austrian J Clin Endocrinol Metab 4:1–5 (pre-publishing online)
29. Latti AK, Jaakola L, Riihinen KR, Kainulainen PS (2010) Anthocyanin and flavonol variation in bog bilberries (Vaccinium uliginosum L.) in Finland. J Agric Food Chem 58:427–433
30. Amarowicz R, Carle R, Dongowski G, Durazzo A, Galensa R, Kammerer D, Maiani G, Piskula MK (2009) Influence of post-harvest processing and storage on the content of phenolic acids and flavonoids in foods. Mol Nutr Food Res 53(Suppl 2):S151–S183
31. Ziegler P, Briefel R, Clusen N, Devaney B (2006) Feeding Infants and Toddlers Study (FITS): development of the FITS survey in comparison to other dietary survey methods. J Am Diet Assoc 106:S12–S27

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ORIGINAL CONTRIBUTION

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.

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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 antioxidative, 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].

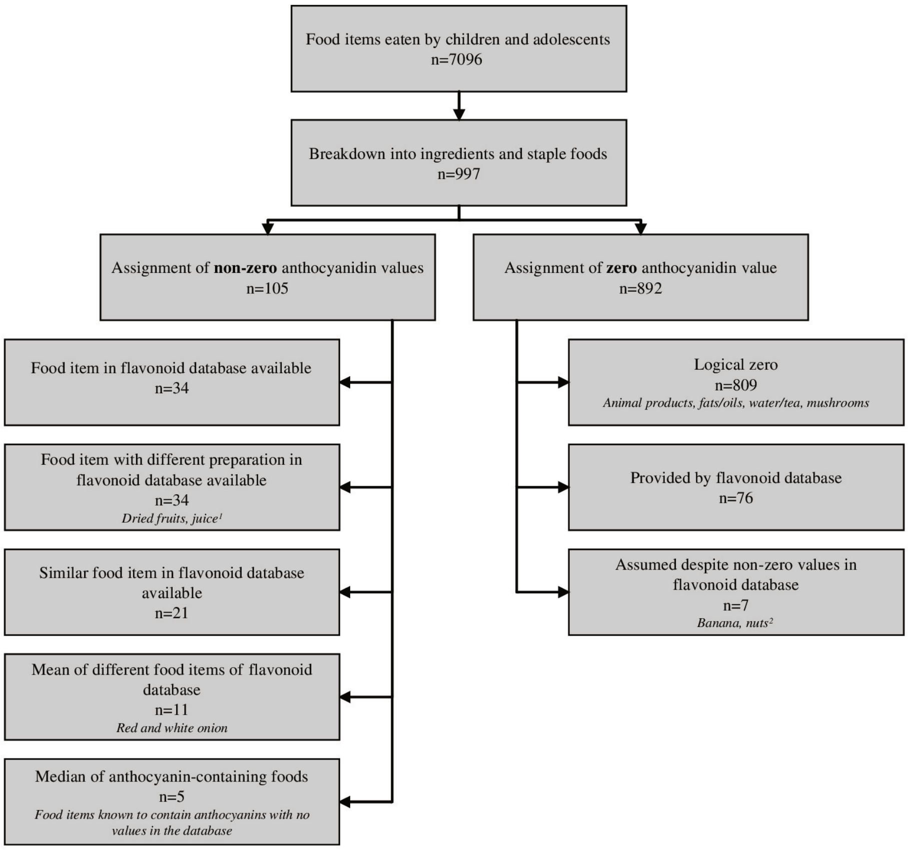


Fig. 1 Number of consumed food items and type of anthocyanidin values assigned using the USDA flavonoid database [35]. [1]For dried and stewed fruit, values from fresh fruit were used. Anthocyanidin contents of juices without a fitting item in the flavonoid database were estimated as anthocyanidin content of fresh fruits *0.47 using the mean of the recovery rates of anthocyanins in juice compared to fresh fruit described by Klopotek et al. [64] (strawberries, 67 %) and Hager et al. [63] (black raspberries, 27 %). [2]For 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 LEBTAB. 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 time^2), 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)

	% Of total anthocyanidin intake per age group									
Age [years]	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	24.7	Strawberry	25.8	Strawberry	23.1	Strawberry	25.0	Strawberry	22.9
2.	Elderberry juice	12.5	Apple with peel	9.2	Elderberry juice	10.5	Red cabbage	11.7	Elderberry juice	14.2
3.	Apple with peel	8.8	Red cabbage	8.6	Cherries, sweet	9.7	Black currant	8.6	Red cabbage	10.4
4.	Pear	7.5	Elderberry juice	8.3	Red cabbage	8.7	Apple with peel	8.1	Black currant	7.5
5.	Cherries, sweet	7.4	Cherries, sweet	7.6	Apple with peel	8.3	Elderberry juice	8.0	Apple with peel	7.1
Sum		60.9		59.5		60.3		61.4		62.1

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.

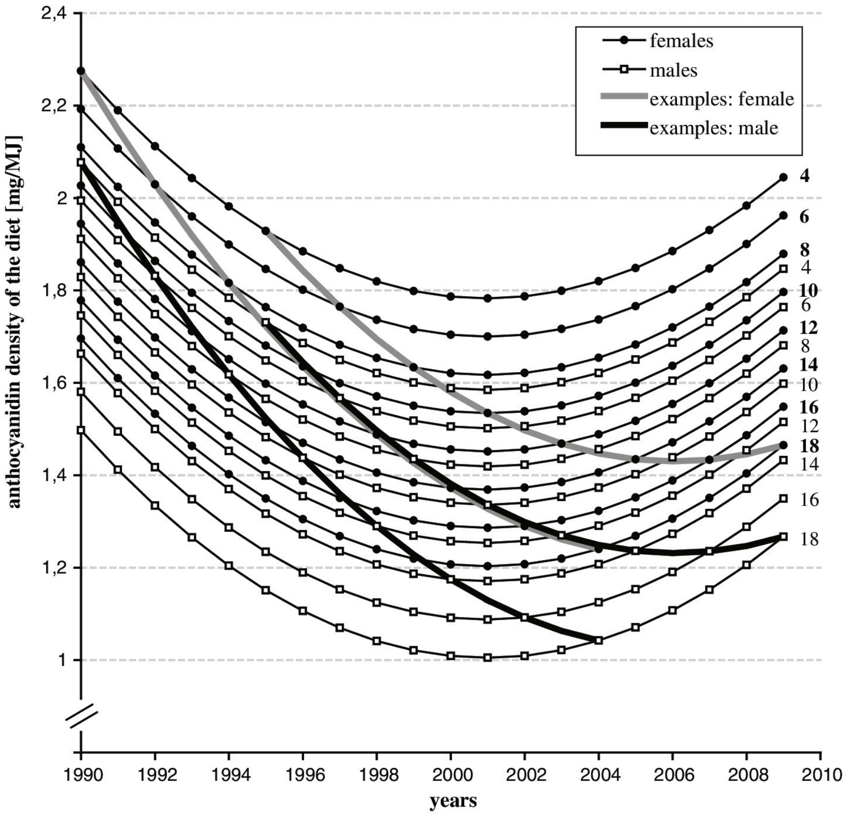
Discussion

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 Finish 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



	β (SE)	P
linear trend of anthocyanidin intake over the course of age (age)	-0.0414 (0.0130)	0.0017
linear trend of anthocyanidin intake over the course of time (time)	-0.0896 (0.0315)	0.0044
quadratic trend of anthocyanidin intake over the course of time (time^2)	0.0041 (0.0014)	0.0050
sex	0.1982 (0.0938)	0.0347

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 '5aday' (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 anthocyanindin 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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References

1. Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733–749
2. Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. Cancer Lett 269:281–290
3. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr (2007) Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 85:895–909
4. Chong MF, Macdonald R, Lovegrove JA (2010) Fruit polyphenols and CVD risk: a review of human intervention studies. Br J Nutr 104(Suppl 3):S28–S39
5. Rossi M, Bosetti C, Negri E, Lagiou P, La Vecchia C (2010) Flavonoids, proanthocyanidins, and cancer risk: a network of case-control studies from Italy. Nutr Cancer 62:871–877
6. Rossi M, Negri E, Talamini R, Bosetti C, Parpinel M, Gnagnarella P, Franceschi S, Dal Maso L, Montella M, Giacosa A, La Vecchia C (2006) Flavonoids and colorectal cancer in Italy. Cancer Epidemiol Biomarkers Prev 15:1555–1558
7. Mursu J, Nurmi T, Tuomainen TP, Salonen JT, Pukkala E, Voutilainen S (2008) Intake of flavonoids and risk of cancer in finnish men: the Kuopio ischaemic heart disease risk factor study. Int J Cancer 123:660–663
8. Crowe FL, Roddam AW, Key TJ, Appleby PN, Overvad K, Jakobsen MU, Tjonneland A, Hansen L, Boeing H, Weikert C, Linseisen J, Kaaks R, Trichopoulou A, Misirli G, Lagiou P, Sacerdote C, Pala V, Palli D, Tumino R, Panico S, Bueno-de-Mesquita HB, Boer J, van Gils CH, Beulens JW, Barricarte A, Rodriguez L, Larranaga N, Sanchez MJ, Tormo MJ, Buckland G, Lund E, Hedblad B, Melander O, Jansson JH, Wennberg P, Wareham NJ, Slimani N, Romieu I, Jenab M, Danesh J, Gallo V, Norat T, Riboli E (2011) Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European prospective investigation into cancer and nutrition (EPIC)-heart study. Eur Heart J 32:1235–1243
9. Boffetta P, Couto E, Wichmann J, Ferrari P, Trichopoulos D, Bueno-de-Mesquita HB, van Duijnhoven FJ, Büchner FL, Key T, Boeing H, Nöthlings U, Linseisen J, Gonzalez CA, Overvad K, Nielsen MR, Tjonneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Lagiou P, Naska A, Benetou V, Kaaks R, Rohrmann S, Panico S, Sieri S, Vineis P, Palli D, van Gils CH, Peeters PH, Lund E, Brustad M, Engeset D, Huerta JM, Rodriguez L, Sanchez MJ, Dorronsoro M, Barricarte A, Hallmans G, Johansson I, Manjer J, Sonestedt E, Allen NE, Bingham S, Khaw KT, Slimani N, Jenab M, Mouw T, Norat T, Riboli E, Trichopoulou A (2010) Fruit and vegetable intake and overall cancer risk in the European prospective investigation into cancer and nutrition (EPIC). J Natl Cancer Inst 102:529–537
10. Watzl B (2008) Anti-inflammatory effects of plant-based foods and of their constituents. Int J Vitam Nutr Res 78:293–298
11. Kühnau J (1976) The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev Nutr Diet 24:117–191
12. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem 54:4069–4075
13. Lako J, Wattanapenpaiboon N, Wahlqvist M, Trenerry C (2006) Phytochemical intakes of the Fijian population. Asia Pac J Clin Nutr 15:275–285
14. Heinonen M (2007) Antioxidant activity and antimicrobial effect of berry phenolics—a finnish perspective. Mol Nutr Food Res 51:684–691
15. Perez-Jimenez J, Fezeu L, Touvier M, Arnault N, Manach C, Hercberg S, Galan P, Scalbert A (2011) Dietary intake of 337 polyphenols in French adults. Am J Clin Nutr 93:1220–1228
16. Linseisen J, Radtke J, Wolfram G (1997) Flavonoidzufuhr Erwachsener in einem bayrischen Teilkollektiv der Nationalen Verzehrsstudie. Z Ernährungswiss 36:403–412

17. Peterson J, Lagiou P, Samoli E, Lagiou A, Katsouyanni K, La Vecchia C, Dwyer J, Trichopoulos D (2003) Flavonoid intake and breast cancer risk: a case–control study in Greece. Br J Cancer 89:1255–1259
18. Bosetti C, Spertini L, Parpinel M, Gnagnarella P, Lagiou P, Negri E, Franceschi S, Montella M, Peterson J, Dwyer J, Giacosa A, La Vecchia C (2005) Flavonoids and breast cancer risk in Italy. Cancer Epidemiol Biomarkers Prev 14:805–808
19. Johannot L, Somerset SM (2006) Age-related variations in flavonoid intake and sources in the Australian population. Public Health Nutr 9:1045–1054
20. Chun OK, Chung SJ, Song WO (2007) Estimated dietary flavonoid intake and major food sources of U.S. adults. J Nutr 137:1244–1252
21. Bosetti C, Rossi M, McLaughlin JK, Negri E, Talamini R, Lagiou P, Montella M, Ramazzotti V, Franceschi S, LaVecchia C (2007) Flavonoids and the risk of renal cell carcinoma. Cancer Epidemiol Biomarkers Prev 16:98–101
22. Ovaskainen ML, Törrönen R, Koponen JM, Sinkko H, Hellström J, Reinivuo H, Mattila P (2008) Dietary intake and major food sources of polyphenols in Finnish adults. J Nutr 138:562–566
23. Frankenfeld CL, Cerhan JR, Cozen W, Davis S, Schenk M, Morton LM, Hartge P, Ward MH (2008) Dietary flavonoid intake and non-Hodgkin lymphoma risk. Am J Clin Nutr 87:1439–1445
24. Cutler GJ, Nettleton JA, Ross JA, Harnack LJ, Jacobs DR Jr, Scrafford CG, Barraj LM, Mink PJ, Robien K (2008) Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. Int J Cancer 123:664–671
25. Mullie P, Clarys P, Deriemaeker P, Hebbelinck M (2008) Estimation of daily human intake of food flavonoids. Int J Food Sci Nutr 59:291–298
26. Beking K, Vieira A (2011) An assessment of dietary flavonoid intake in the UK and Ireland. Int J Food Sci Nutr 62:17–19
27. Cassidy A, O'Reilly EJ, Kay C, Sampson L, Franz M, Forman J, Curhan G, Rimm EB (2011) Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nutr 93:338–347
28. Dilis V, Trichopoulou A (2010) Antioxidant intakes and food sources in Greek adults. J Nutr 140:1274–1279
29. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventos RM, Berenguer T, Jakszyn P, Barricarte A, Ardanaz E, Amiano P, Dorronsoro M, Larranaga N, Martinez C, Sanchez MJ, Navarro C, Chirlaque MD, Tormo MJ, Quiros JR, Gonzalez CA (2010) Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). J Am Diet Assoc 110:390–398
30. Zamora-Ros R, Knaze V, Lujan-Barroso L, Slimani N, Romieu I, Touillaud M, Kaaks R, Teucher B, Mattiello A, Grioni S, Crowe F, Boeing H, Förster J, Quiros JR, Molina E, Huerta JM, Engeset D, Skeie G, Trichopoulou A, Dilis V, Tsiotas K, Peeters PH, Khaw KT, Wareham N, Bueno-de-Mesquita B, Ocke MC, Olsen A, Tjonneland A, Tumino R, Johansson G, Johansson I, Ardanaz E, Sacerdote C, Sonestedt E, Ericson U, Clavel-Chapelon F, Boutron-Ruault MC, Fagherazzi G, Salvini S, Amiano P, Riboli E, Gonzalez CA (2011) Estimation of the intake of anthocyanidins and their food sources in the European prospective investigation into cancer and nutrition (EPIC) study. Br J Nutr 106(7):1090–1099
31. Lien N, Lytle LA, Klepp KI (2001) Stability in consumption of fruit, vegetables, and sugary foods in a cohort from age 14 to age 21. Prev Med 33:217–226
32. Mikkilä V, Räsänen L, Raitakari OT, Pietinen P, Viikari J (2005) Consistent dietary patterns identified from childhood to adulthood: the cardiovascular risk in young Finns study. Br J Nutr 93:923–931
33. Patterson E, Wärnberg J, Kearney J, Sjöström M (2009) The tracking of dietary intakes of children and adolescents in Sweden over six years: the European youth heart study. Int J Behav Nutr Phys Act 6:91
34. Drossard C, Alexy U, Bolzenius K, Kunz C, Kersting M (2011) Anthocyanins in the diet of infants and toddlers: intake, sources and trends. Eur J Nutr 50:705–711
35. Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture (2007) USDA database for the flavonoid content of selected foods release 2.1. http://www.ars.usda.gov/Services/docs.htm?docid=6231. Accessed 2009
36. Drossard C, Fröhling B, Dietrich H, Kersting M (2011) Anthocyanin analysis in banana fruit—a mistake. Am J Clin Nutr 93:865–866; author reply 866–867
37. Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U, Lentze MJ (2004) The DONALD study. History, current status and future perspectives. Eur J Nutr 43:45–54
38. Kersting M, Sichert-Hellert W, Lausen B, Alexy U, Manz F, Schöch G (1998) Energy intake of 1 to 18 year old German children and adolescents. Z Ernährungswiss 37:47–55
39. Souci SW, Fachmann W, Kraut H (2000) Food composition and nutrition tables. Medpharm Scientific Publishers, Stuttgart
40. Sichert-Hellert W, Kersting M, Chahda C, Schäfer R, Kroke A (2007) German food composition database for dietary evaluations in children and adolescents. J Food Compos Anal 20:63–70
41. Drossard C, Kersting M (2012) Banana as a relevant source of anthocyani(di)ns in European populations? Brit J Nutr 107: 765–767
42. Singer D, Willett J (2003) Applied longitudinal data analysis—modeling change and event occurrence. Oxford University Press, New York
43. Tooze JA, Kipnis V, Buckman DW, Carroll RJ, Freedman LS, Guenther PM, Krebs-Smith SM, Subar AF, Dodd KW (2010) A mixed-effects model approach for estimating the distribution of usual intake of nutrients: the NCI method. Stat Med 29:2857–2868
44. Magarey A, Daniels LA, Smith A (2001) Fruit and vegetable intakes of Australians aged 2–18 years: an evaluation of the 1995 national nutrition survey data. Aust N Z J Public Health 25:155–161
45. Melby MK, Murashima M, Watanabe S (2004) Phytochemical intake and relationship to past health history in Japanese women. BioFactors 22:265–269
46. Kita J, Tada J, Ito M, Shirakawa M, Murashima M, Zhuo XG, Watanabe S (2004) Intake of phytochemicals among Japanese, calculated by the new FFF database. BioFactors 22:259–263
47. Kersting M, Alexy U, Kroke A, Lentze MJ (2004) Nutrition of children and adolescents. Results of the DONALD Study. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 47:213–218
48. Rasmussen M, Krolner R, Klepp KI, Lytle L, Brug J, Bere E, Due P (2006) Determinants of fruit and vegetable consumption among children and adolescents: a review of the literature. Part I: quantitative studies. Int J Behav Nutr Phys Act 3:22
49. Richter A, Vohmann C, Stahl A, Heseker H, Mensink GBM (2008) Der aktuelle Lebensmittelverzehr von Kindern und Jugendlichen in Deutschland Teil 2: Ergebnisse aus EsKiMo. Ernährungsumschau 55:28–36
50. Story M, Neumark-Sztainer D, French S (2002) Individual and environmental influences on adolescent eating behaviors. J Am Diet Assoc 102:S40–S51
51. Rasmussen M, Krolner R, Svastisalee CM, Due P, Holstein BE (2008) Secular trends in fruit intake among Danish schoolchildren, 1988 to 2006: changing habits or methodological artefacts? Int J Behav Nutr Phys Act 5:6
52. Stahl A, Vohmann C, Richter A, Heseker H, Mensink GB (2009) Changes in food and nutrient intake of 6- to 17-year-old Germans between the 1980s and 2006. Public Health Nutr 12:1912–1923

53. 5 am Tag e.V. (2011) 5 am Tag—Obst und Gemüse. http://www.5amtag.org/fileadmin/user_upload/Vereinsseite/Mitgliedschaft/1_Kompaktinfos.pdf. Accessed 2012
54. Mensink GBM, Heseker H, Richter A, Stahl A, Vohmann C (2007) Forschungsbericht Ernährungsstudie als KiGGS-Modul (EsKiMo). http://www.bmelv.de/cae/servlet/contentblob/378624/publicationFile/25912/EsKiMoStudie.pdf. Accessed 2012
55. Yngve A, Wolf A, Poortvliet E, Elmadfa I, Brug J, Ehrenblad B, Franchini B, Haraldsdottir J, Krolner R, Maes L, Perez-Rodrigo C, Sjöström M, Thorsdottir I, Klepp KI (2005) Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: the pro children cross-sectional survey. Ann Nutr Metab 49:236–245
56. Max Rubner-Institut Federal Research Institute of Nutrition and Food (2008) Nationale Verzehrsstudie II Ergebnisbericht, Teil 2. http://www.was-esse-ich.de/uploads/media/NVSII_Abschlussbericht_Teil_2.pdf. Accessed 2011
57. Bere E, Brug J, Klepp KI (2008) Why do boys eat less fruit and vegetables than girls? Public Health Nutr 11:321–325
58. Baker AH, Wardle J (2003) Sex differences in fruit and vegetable intake in older adults. Appetite 40:269–275
59. Amarowicz R, Carle R, Dongowski G, Durazzo A, Galensa R, Kammerer D, Maiani G, Piskula MK (2009) Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. Mol Nutr Food Res 53(Suppl 2):S151–S183
60. Lätti AK, Jaakola L, Riihinen KR, Kainulainen PS (2010) Anthocyanin and flavonol variation in bog bilberries (Vaccinium uliginosum L.) in Finland. J Agric Food Chem 58:427–433
61. Perez-Jimenez J, Neveu V, Vos F, Scalbert A (2010) Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: an application of the phenol-explorer database. J Agric Food Chem 58:4959–4969
62. Kersting M, Alexy U (2008) Die DONALD Studie. Ernährungsumschau 55:16–19
63. Hager A, Howard LR, Prior RL, Brownmiller C (2008) Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed black raspberry products. J Food Sci 73:H134–H140
64. Klopotek Y, Otto K, Böhm V (2005) Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. J Agric Food Chem 53:5640–5646

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Research report

Liking of anthocyanin-rich juices by children and adolescents ☆

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ABSTRACT

There is evidence that a diet rich in plant foods is protective against cardiovascular disease and cancer, partly attributable to secondary plant metabolites such as anthocyanins, a colourful group of flavonoids. As at present children and adolescents do not consume the recommended amounts of fruits and vegetables, one possible way of increasing intake, and particularly intake of anthocyanins, may be an anthocyanin-rich juice, since fruit juice is popular with young Germans. We produced eight different fruit products (six juices, two smoothies), and conducted hedonic tests with participants from the DONALD Study. Paired comparisons showed that most subjects preferred apple to apple–bilberry juice, but grape vs. grape–bilberry juice was liked equally frequently. Rated on a hedonic scale the grape–bilberry mixture was preferred to apple–bilberry, both as juice and as smoothie. With regard to viscosity, juices were preferred to smoothies, both as grape–bilberry and as apple–bilberry. Internal Preference Mapping revealed however consumer subgroups with different preferences, raising the question which product should be promoted in order to reach a large target group. The product richest in anthocyanins, grape–bilberry juice, was accepted very well and may therefore be suitable for promotion to children, although the high sugar content of this juice must be taken into account.

Introduction

National and international institutions recommend five portions of fruits and vegetables a day as part of a preventive diet against cancer and cardiovascular disease (Boeing et al., 2007; World Cancer Research Fund/American Institute for Cancer Research, 2007). However, neither German children and adolescents nor adults consume the recommended daily amounts (Max Rubner-Institut Federal Research Institute of Nutrition and Food, 2008; Stahl, Vohmann, Richter, Heseker, & Mensink, 2009). Therefore, possibilities for prevention on the individual, as well as on the population, level are not being exploited. This is also the case in other Western countries (Lorson, Melgar-Quinonez, & Taylor, 2009; Yngve et al., 2005). The positive physiological effects of plant food consumption may particularly be due to the heterogeneous class of secondary plant metabolites they contain, which exhibit anti-oxidative, anti-inflammatory and anti-carcinogenic properties (Watzl, 2008). Within the class of polyphenols, and the subclass of flavonoids, anthocyanins, and therefore anthocyanin-rich foods such as berries and grapes, are thought to have a high preventive potential and may play a role in the prevention of cardiovascular disease and cancer (Basu, Rhone, & Lyons, 2010; Chun, Chung, Claycombe, & Song, 2008; Mink et al., 2007; Wang & Stoner, 2008). Therefore, in addition to the target to increase fruit and vegetable intake as a whole, it may be a special target to improve the supply of anthocyanin-rich fruits and vegetables in the population. As food preferences are established in early childhood, and may be maintained until adulthood (Nicklaus, Boggio, Chabanet, & Issanchou, 2004), it could be promising to accustom young children to anthocyanin-rich foods. However, recommending special healthy foods for children and adolescents can only be fruitful if children and adolescents like and accept these foods. As fruit juice is popular in children and adolescents (Kohler et al., 2007; Sichert-Hellert & Kersting, 2004), juice may be a promising way of increasing anthocyanin intake. However, the most frequently consumed juices in Germany are anthocyanin-free apple and orange juice (Association of the German Fruit Juice Industry, 2011; German Nutrition Society, 2008). As it is still a point of discussion whether consumption of sugar-sweetened beverages and also 100% fruit

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juice is associated with weight gain, at least in subgroups in some studies (Libuda et al., 2008; Malik, Schulze, & Hu, 2006), developing an attractive and healthy fruit juice should also take sugar content into account.

The objective of our study therefore was to use a convenient sample of children and adolescents comprising a broad age range to examine:

- whether blending anthocyanin-rich juice with popular apple juice influences the acceptance of an anthocyanin-rich grape–bilberry juice
- to what extent children and adolescents accept anthocyanin-rich fruit products, and whether fruit composition and viscosity are decisive
- and whether individual preferences can be distinguished.

Methods

Participants

The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study is an open cohort study conducted by the Research Institute of Child Nutrition Dortmund (FKE), which collects detailed data on dietary behaviour, growth and development from participants from the age of 3 months to at least 18 years (Kroke et al., 2004). Its study protocol involves participants visiting the FKE on an annual basis in order to undergo anthropometric and medical examinations, as well as various additional study modules depending on age. Between February and December 2010 all participants aged 4–17 years were invited to take part in an additional tasting session subsequent to their regular examinations. This sensory evaluation study was conducted in the context of the joint research project "Anthocyanins–Nutritional Investigations in Alliance" (ANTHONIA), which aims to prove the effectiveness of anthocyanins in humans and to develop new products based on berry fruit to increase the population's anthocyanin intake. During the study period 346 DONALD participants aged 4–17 years came to their yearly visit. Four participants were excluded due to our exclusion criteria (fruit allergies and intolerance), and 11 refused to take part in the tasting session, because of time limitations or other reasons. After the exclusion of five data sets due to missing or implausible data, the tasting sessions of 326 participants were evaluated. Sample characteristics are shown in Table 1.

The DONALD Study as a whole, and the additional tasting sessions, have been approved by the Ethics Committee of the University of Bonn. All examinations, including tasting sessions, are performed with parental, and later on with the children's, written consent.

Fruit juice and fruit smoothie samples

For this study, six fruit juices and two fruit smoothies were produced by the Department of Wine Analysis and Beverage Technology at Geisenheim Research Center (Table 2). Three different fruits were used as ingredients: grape, bilberry and apple. Anthocyanin-rich varieties of grapes (Dakapo and Accent) were chosen. Apple juice was produced using popular varieties of apples cultivated by the Department of Pomology at the Geisenheim Research Center. Bilberry puree for smoothies was purchased from Bayernwald Früchteverwertung GmbH and bilberry concentrate for juices from Döhlergroup. The six juices were two pure juices, apple (A) and grape (G), a blend of each pure juice with bilberry (80%:20%) (AB and GB), and two mixtures in which apple juice was added to the grape–bilberry blend in different concentrations (A50GB and A60GB) (Table 2). The two smoothies were mixtures of bilberry puree with Juice A or Juice G (20%:80%), respectively. Juices and smoothies were pasteurised, filled into glass bottles and transported to Dortmund, where they were stored at 4 °C at the FKE. To ensure stability of sensory properties a panel at Geisenheim conducted sensory analyses of the products every 3 months. Sensory profiles concerning colour, odour and taste did not change during the testing period (data not shown).

Table 1
Characteristics of participants.

Age categories [years]	Sex distribution [male/female]	Sum
4–6	43/31	74
7–9	44/39	83
10–12	37/35	72
13–15	36/25	61
16–17	17/19	36
Sum	177/149	326

Table 2
Composition and analytic results of tested juices and smoothies.

Sample	Composition (%)	Anthocyanins [mg/100 mL]	Sugar [g/100 mL]
Juice GB	Grape (80) Bilberry (20)	84	17.5
Juice G	Grape (100)	77	20.3
Juice A50GB	Apple (50) Juice GB (50)	38	14.4
Juice A60GB	Apple (60) Juice GB (40)	33	13.8
Juice AB	Apple (80) Bilberry (20)	6	10.5
Juice A	Apple (100)	n.d.	11.2
Smoothie GB	Grape (80) Bilberry Puree (20)	96	17.2
Smoothie AB	Apple (80) Bilberry Puree (20)	23	10.1

Procedure

Evaluations of products were performed individually by each participant, or as a pair in the case of siblings, in a room specifically arranged for the tasting sessions. The procedure was standardised in terms of sample preparation, as well as in the written and verbal instructions given to the participants by the study personnel. Participants performed sensory evaluations as an one-on-one interview (4–7 year olds) or filled out test sheets themselves (8–17 year olds). The order of the sensory tests, as well as the order of presented samples within the tests, were randomised. Participants were not told which type of fruit juices they tasted. Samples, served in transparent plastic cups (2 cL) at room temperature (20 °C), were labelled with a 3-digit code. Inbetween tasting the different samples, participants rinsed their mouths with water.

The tasting session was designed to gather information on different aspects of liking without too much extra effort for the participants and included preference as well as acceptance methods (Fig. 1):

- two paired comparisons with forced choice to determine whether (a) Juice A is preferred pure or blended with bilberry juice, and (b) Juice G is preferred pure or blended with bilberry juice
- three paired comparisons to allow an indirect ranking of three blends of Juice GB with different amounts of Juice A
- age-adapted hedonic scales to measure the acceptance of two juices (AB and GB), and two respective smoothies on the group and on the individual level.

We have chosen three paired comparisons instead of a rank order test to keep the testing procedure consistent throughout all age groups, because a pretest showed that the younger children were not able to build a preference order out of three products (data not shown). The age-adapted hedonic scales we chose for

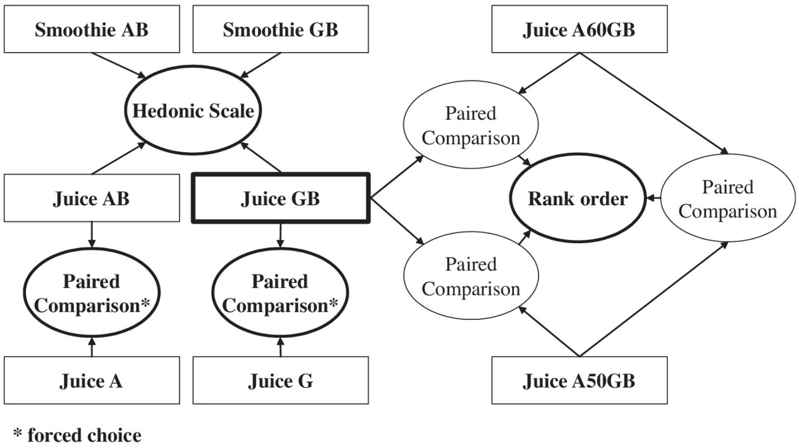

Fig. 1. Design of hedonic tests conducted with eight juice and smoothie samples. Juice and smoothie samples are depicted in rectangles (for compositions of samples see Table 2), sensory tests are figured as oval frames. Arrows display which kind of sensory tests were used to evaluate the different samples. Grape–bilberry juice (Juice GB) and apple–bilberry juice (Juice AB) as well as the respective smoothies grape–bilberry smoothie (Smoothie GB) and apple–bilberry smoothie (Smoothie AB) were evaluated on a hedonic scale. The liking of Juice GB and pure grape juice (Juice G) was compared in a paired comparison with forced choice as was in parallel, the liking of Juice AB and pure apple juice (Juice A). Juice GB and two mixtures of this juice with 50% apple juice (Juice A50GB) and 60% apple juice (Juice A60GB), respectively were tested in three paired comparisons to derive a rank order.

the hedonic ratings were a 5-point hedonic scale for 4–7 year olds (1 = "very bad", 3 = "indifferent", 5 = "very good"), and a 9-point hedonic scale (1 = "totally bad", 5 = "indifferent", 9 = "totally good") for the 8–17 year olds.

Statistical analysis

All calculations and statistical analyses were carried out using SAS (versions 9.1.3 and 9.2; Statistical Analysis System, Cary, NC, USA). Preferences in the two paired comparisons were tested using the binomial test. To test for differences in preferences by sex and age, the Chi-square-Test was performed. Preferences of the three blends of Juice GB with Juice A were tested using the Friedman Test for paired samples after building a rank order out of the three paired comparisons (see Fig. 1). For example, rank 1 was assigned to a juice which was declared to taste better in both paired comparisons with the other two juices. Those two juices both got rank 2.5 in the case they were liked equally in the paired comparison or got rank 2 and 3, respectively in the case one was preferred. To test for significant differences in average ratings on the hedonic scale for the two juices and two smoothies, the Friedman Test for paired samples and post-hoc pairwise Sign Tests for smoothie vs. juice and GB vs. AB were performed. The significance level for statistical tests was defined as $\alpha = 0.05$ and Bonferroni correction was applied for multiple comparisons.

To reveal the main determinants of the children's preferences for the four products rated using the hedonic scale, Internal Preference Mapping was performed using PROC PRINQUAL. This mapping technique applies principal component analysis (PCA) on preference data, in this case using the fruit juice samples as the objects, and the judgements of the participants as the variables.

Results

Preferences of the study sample

Juice A was preferred by significantly more participants than Juice AB (71% vs. 29%), whereas Juices G and GB were preferred equally frequently (51% vs. 49%). These results were independent from sex and age (data not shown).

Both in the older and in the younger age group, in 28% of the participants the results of the three paired comparisons could not be transformed into a clear rank order of the three juices (for example when GB was favoured over A60GB, A60GB was favoured over A50GB and A50GB was favoured over GB). In the remaining 72% of the sample no difference in rank for the three juices, neither in the younger (4–7 years) nor in the older participants (8–17 years), was determined (Table 3). The 28% of the participants whose results could not be transformed into a clear rank order, were not different from the rest of the sample in the respective age group, in terms of sex, age, siblings in the tasting session, time needed for the sensory tests and hedonic rating of Juice GB (data not shown).

Juice GB, the product highest in anthocyanins and sugar, received on average the highest rating of the four products evaluated on the 5-point and on the 9-point hedonic scale, applied by the younger and older participants, respectively (Table 4a). Smoothie AB, the product lowest in sugar, received the lowest rating. Both age groups rated juice significantly higher than the respective smoothie, and GB products were in both viscosities rated significantly higher than the respective AB products by the older age group (Table 4b). Results for the younger age group showed a similar tendency, but did not reach significance for comparison of viscosity (juice vs. smoothie), but only for comparison of fruit composition (GB vs. AB). For each of the four products in both age groups most ratings were in the positive range of the scale.

Table 3
Mean rank of three juices with different proportions of apple juice, derived from three paired comparisons.

Juice	Age group [years] 4–7 ($n = 105$) Mean rank	8–17 ($n = 221$)
GB	2.09	1.98
A50GB	1.97	2.00
A60GB	1.94	2.01
p (Friedman Test)	0.52	0.97

Table 4a
Mean liking scores of tested juice and smoothie samples in two age groups.

Sample	Age group [years]	
	4–7[a]	8–17[b]
n	105	221
Juice AB	3.55 (4)	6.01 (6)
Smoothie AB	3.22 (3)	5.24 (5)
Juice GB	3.95 (4)	6.78 (7)
Smoothie GB	3.68 (4)	5.95 (6)

Values presented are arithmetic means (medians).
[a] Measured on a 5-point hedonic scale (1 = "very bad", 3 = "indifferent", 5 = "very good").
[b] Measured on a 9-point hedonic scale (1 = "totally bad", 5 = "indifferent", 9 = "totally good").

Table 4b
Differences in liking scores of tested juices and smoothies in two age groups.

	Age group [years]	
	4–7 (n = 105) P^a	8–17 (n = 221) P^a
Juice vs. Smoothie		
Grape–bilberry	0.1175	<0.0001 (J > Sm)
Apple–bilberry	0.0226	<0.0001 (J > Sm)
Grape–bilberry vs. Apple–bilberry		
Juice	0.0099 (GB > AB)	<0.0001 (GB > AB)
Smoothie	0.0081 (GB > AB)	<0.0001 (GB > AB)

Friedman Test global $p < 0.0001$ for each age group.
[a] Pairwise Sign Tests with Bonferroni correction for multiple comparisons ($\alpha = 0.05/4 = 0.0125$).
J > Sm: juice was rated significantly higher than the respective smoothie.GB > AB: mixture grape–bilberry was rated significantly higher than mixture apple–bilberry.

Table 4c
Distribution of ratings on the hedonic scale.

Sample	Age group [years]	
	4–7[a] (n = 105) Percentage ratings in the negative/neutral/positive range of the scale	8–17[b] (n = 221)
Juice AB	20/20/60	22/18/60
Smoothie AB	35/20/45	37/21/52
Juice GB	10/22/68	7/11/82
Smoothie GB	22/20/58	19/19/62

[a] Measured on a 5-point hedonic scale (1–2 = "negative ratings", 3 = "indifferent rating", 4–5 = "positive ratings").
[b] Measured on a 9-point hedonic scale (1–4 = "negative ratings", 5 = "indifferent rating", 6–9 = "positive ratings").

Even so, only in one instance did a product receive more than 80% positive ratings: Juice GB in the older age group (Table 4c).

Individual preferences for the four products

PCA conducted for Internal Preference Mapping revealed fruit composition (GB vs. AB) as the first principal component (Fig. 2), accounting for the biggest part of variance in the rating data of both age groups (56% in older age group, 44% in younger age group). The second principal component in both age groups, accounting for 34% of the variance in the preference data of the older and for 40% in the younger age group, can be interpreted as the "viscosity-factor" (juice vs. smoothie). Excluding those participants who did not have any variance in their ratings, 152 out of 220 (69%) participants' judgements (variables) in the older age group and 64 out of 92 (70%) participants' judgements (variables) in the younger age group load positively on the first principal component, representing preference for GB products. Thus 30% of the participants in both age groups rated AB products higher than GB products. 136 of 220 (62%) participants' judgements in the older age group and 52 of 92 (57%) participants' judgements in the younger age group loaded positively on the second principal component, indicating that a major part of the sample preferred juices to smoothies. Vice versa, 38% of the participants in the older, and 43% in the younger, age group rated the smoothie higher than juice.

Discussion

Our sensory evaluation study showed that in this sample of children and adolescents with a wide age range:

- acceptance was not influenced by blending anthocyanin-rich Juice GB with the popular Juice A
- anthocyanin-rich products and in particular Juice GB were well accepted by children and adolescents, juices more than smoothies and GB more than AB
- on the individual level noticeable differences in preferences among subjects were revealed.

Familiarity with foods is positively associated with liking (Dinnella, Recchia, Tuorila, & Monteleone, 2011; Sabbe, Verbeke, Deliza, Matta, & Van Damme, 2009). Therefore, Juice A may have simply been preferred to Juice AB because it is a common fruit juice in Germany (Association of the German Fruit Juice Industry, 2011), and hence is well known and frequently consumed by children and adolescents (Alexy, Sichert-Hellert, Kersting, & Manz, 2001; German Nutrition Society, 2008). 71% of the DONALD participants who took part in the sensory evaluation study named apple juice as one of their favourite juices (data not shown). Interestingly, it was shown for grape, apple and bilberry juice that they were less well-known than the participants' preference suggested (Dinnella et al., 2011), indicating that these kinds of juices do not need to be very familiar to be liked. Since on the hedonic scale Juice AB got a lower mean rating than Juice GB this rather indicates that the particular mixture AB was not tasty. This may partly be due to the lower sugar content in comparison to the other products.

Juices G and GB were liked equally frequently, despite the probably for German children unfamiliar berry taste. This again indicates that the higher percentage of children in our sample who preferred Juice A to Juice AB was not due to a specific bilberry dislike, but rather to a dislike of the particular mixture AB.

The rank order test constructed by three paired comparisons revealed, that there was no clear preference for a higher or lower content of Juice A in the anthocyanin-rich Juice GB in this sample. Firstly, in more than one out of four participants it was not possible to derive a rank order from the three paired comparisons. Secondly, no difference in rank was found in the group of those who built a clear rank order. Keeping in mind the production of a product rich in anthocyanins, Juice GB may be promising on the market, supported also by its high ranking in the hedonic scale test.

The significantly higher rating not only of Juice GB, but also of Smoothie GB compared to their respective AB mixtures may be due to the higher sugar content, and therefore the sweeter taste, of GB products in comparison to the more sour taste of the AB products. As fruit juices are thought to be associated with weight gain (Libuda et al., 2008; Malik et al., 2006), sugar content should be reduced in a health-promoting product intended for children and adolescents. Nevertheless, it should be noted that recent studies have not found any evidence for an association between the consumption of 100% fruit juice and weight gain in children and adolescents (Nicklas, O'Neil, & Kleinman, 2008; O'Neil and Nicklas, 2008; O'Neil, Nicklas, & Kleinman, 2010). Furthermore, the

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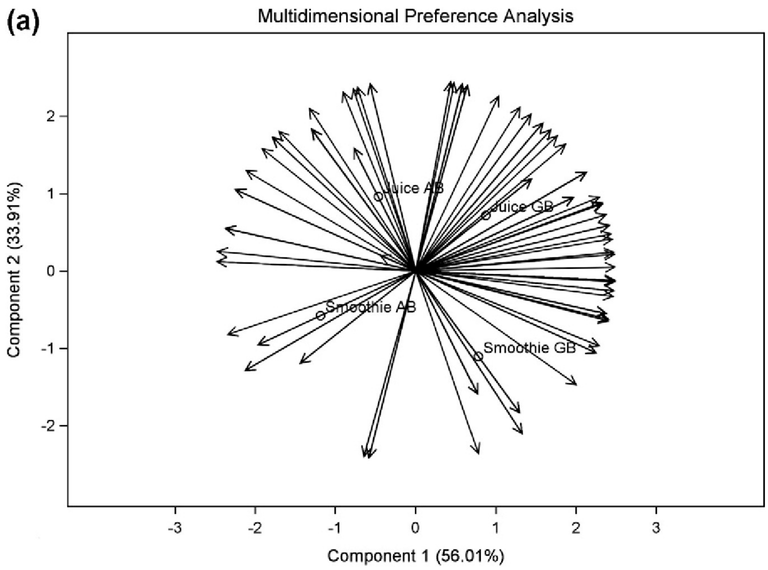

Fig. 2. Internal Preference Mapping of two juices and two smoothies evaluated by (a) 8–17 year old participants on a 9-point hedonic scale ($n = 221$) and (b) 4–7 year old participants on a 5-point hedonic scale ($n = 105$). The four fruit product points have coordinates that are the scores of the products on the first two principal components. Directions of arrows, each representing one participant- or more participants if the correlations are the same- are defined by the correlations of participants' judgements with the two principal components (i.e. factor loadings). Relative lengths of arrows represent the fit of the participants' judgement data to the two principal components (i.e. communalities).

consumption of 100% fruit juice was even positively associated with diet quality (Nicklas et al., 2008; O'Neil et al., 2010; O'Neil, Nicklas, Zanovec, & Fulgoni, 2011)

The participants' preference for juices rather than smoothies may be explained by the fact that the smoothies were not denoted as such, and participants may therefore have expected a juice. Children cannot respond as meaningfully as adults to a product removed from its real world context, like for example packaging and brand identity (Popper & Kroll, 2005). Therefore, another kind of presentation, for example in a little bottle typical for smoothies in the supermarkets, or even in a bowl with a spoon for high-viscosity smoothies, may increase acceptance and should be investigated. Nevertheless, smoothies also received high mean ratings, as well as positive ratings from 45% to 68% of the participants. Additionally, differences in viscosity (juice vs. smoothie) were not significant in younger children. It is not likely that this is due to the fewer discrimination possibilities on the 5-point scale, because "shrinking" the results of the 9-point scale to a 5-point scale leads to the same significant results in the older age group. The non-significance may therefore be rather due to a decreased ability to discriminate between such differences in the younger age group, or the fact that they truly liked both kinds of viscosity equally. Therefore, it may also be worthwhile to develop a smoothie, if one were aiming to make use of the advantages of smoothies in comparison to juices, such as the higher colonic availability of the polyphenols (Hagl et al., 2011).

Internal Preference Mapping suggested the presence of different consumer subgroups within our sample. On average, participants preferred Juice GB to the other three products rated, and this more complex analysis revealed in addition that on the individual level the majority of participants rated GB mixtures higher than AB mixtures, and juices higher than smoothies. As Juice GB was also the product that received the most positive ratings on the hedonic scale in both age groups, such a product may be the most promising for promotion to children and adolescents as consumers. However, Internal Preference Mapping also revealed that other groups of participants preferred AB to GB and/or smoothies to juices. This suggests that smoothies as well as products containing the liked and well known Juice A may also have a place on the market, for example for special target groups. Using Juice A may especially be an option in order to reduce the high sugar content of the anthocyanin-rich product, or to lower the price to get more people to buy the fruit juice (Andreyeva, Long, & Brownell, 2010). Moreover, the dark juices resulted in the children having purple coloured tongues and lips. This could be a possible argument for parents not to buy Juice GB.

Possible limitations of our study need to be named. Firstly, tasting sessions did not take place at the same time of day, nor at the same time of year for all subjects. However, no correlation was found between the time of day or the date and ratings on the hedonic scale (data not shown). Secondly, no sensory laboratory was available for the tasting sessions to ensure constant conditions. Nevertheless, all subjects were tested in the same room under the same conditions by the same personnel ensuring a standardised procedure. Thirdly, our DONALD participants are not a representative sample of German children and adolescents. However, products received similar ratings by a sample of school children in the sensory laboratory at Geisenheim Research Center (data not shown).

The strengths of our study include the wide age range of the participants covering preschool children to 17 year old adolescents and, at the same time, ensuring a constant testing environment throughout. The same products were used during the whole study period, and their sensory stability was ensured by repeated descriptive tests conducted by a panel at Geisenheim. By conducting individual tasting sessions distractions through peers were avoided, and every child and adolescent could focus on taste, and work at his own pace. Moreover, due to the constant presence of study personnel misunderstandings or missings in the test sheets were minimised.

Further research could take advantage of the DONALD design to study associations between usual or long-term food consumption habits and food preferences, using the results of this study. Furthermore, our results may be a starting point for a discussion on the nutritional aspects of product development, and the marketing/distribution of anthocyanin-rich fruit products, for example strategies using health claims (Sabbe et al., 2009) or school settings (Blanchette & Brug, 2005).

In conclusion, children and adolescents accept anthocyanin-rich fruit products, particularly grape–bilberry juice, very well, even in a population where anthocyanin-free apple and orange juices are the most popular juices. To deduce and to implement evidence-based recommendations for consumption, research on the efficacy of anthocyanins as well as studies on food development and marketing strategies to reach children and adolescents are fundamental.

References

Alexy, U., Sichert-Hellert, W., Kersting, M., & Manz, F. (2001). The foods most consumed by German children and adolescents: results of the DONALD Study. Dortmund Nutritional and Anthropometric Longitudinally Design. *Annals of Nutrition and Metabolism, 45*(3), 128–134.

Andreyeva, T., Long, M. W., & Brownell, K. D. (2010). The impact of food prices on consumption: a systematic review of research on the price elasticity of demand for food. *American Journal of Public Health, 100*(2), 216–222.

Association of the German Fruit Juice Industry. (2011). *The most popular varieties of fruit juice*. Available from http://www.fruchtsaft.net/en/index.php?menu_sel= 13&menu_sel2=3&menu_sel3=&menu_sel4=&msg=42 Retrieved June 2011.

Basu, A., Rhone, M., & Lyons, T. J. (2010). Berries: emerging impact on cardiovascular health. *Nutrition Reviews, 68*(3), 168–177.

Blanchette, L., & Brug, J. (2005). Determinants of fruit and vegetable consumption among 6–12-year-old children and effective interventions to increase consumption. *Journal of Human Nutrition and Dietetics, 18*(6), 431–443.

Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., et al. (2007). *Stellungnahme der Deutschen Gesellschaft für Ernährung e. V. Obst und Gemüse in der Prävention chronischer Krankheiten*. Available from http://www.dge.de/pdf/ws/Stellungnahme-OuG-Praevention-chronischer-Krankheiten-2007-09-29.pdf. Retrieved 08.06.2011.

Chun, O. K., Chung, S. J., Claycombe, K. J., & Song, W. O. (2008). Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. Adults. *Journal of Nutrition, 138*(4), 753–760.

Dinnella, C., Recchia, A., Tuorila, H., & Monteleone, E. (2011). Individual astringency responsiveness affects the acceptance of phenol-rich foods. *Appetite, 56*(3), 633–642.

German Nutrition Society. (2008). *Ernährungsbericht*. Bonn.

Hagl, S., Deusser, H., Soyalan, B., Janzowski, C., Will, F., Dietrich, H., et al. (2011). Colonic availability of polyphenols and D-(−)-quinic acid after apple smoothie consumption. *Molecular Nutrition & Food Research, 55*(3), 368–377.

Kohler, S., Kleiser, C., Richter, A., Stahl, A., Vohmann, C., Heseker, H., et al. (2007). Fluid intake of adolescents in Germany. Results collected in EsKiMo. *Ernährung-Wissenschaft und Praxis, 1*(10), 444–450.

Kroke, A., Manz, F., Kersting, M., Remer, T., Sichert-Hellert, W., Alexy, U., et al. (2004). The DONALD Study. History, current status and future perspectives. *European Journal of Nutrition, 43*(1), 45–54.

Libuda, L., Alexy, U., Sichert-Hellert, W., Stehle, P., Karaolis-Danckert, N., Buyken, A. E., et al. (2008). Pattern of beverage consumption and long-term association with body-weight status in German adolescents–results from the DONALD study. *British Journal of Nutrition, 99*(6), 1370–1379.

Lorson, B. A., Melgar-Quinonez, H. R., & Taylor, C. A. (2009). Correlates of fruit and vegetable intakes in US children. *Journal of the American Dietetic Association, 109*(3), 474–478.

Malik, V. S., Schulze, M. B., & Hu, F. B. (2006). Intake of sugar-sweetened beverages and weight gain: a systematic review. *American Journal of Clinical Nutrition, 84*(2), 274–288.

Max Rubner-Institut Federal Research Institute of Nutrition and Food. (2008). *Nationale Verzehrsstudie II Ergebnisbericht, Teil 2*. Available from http://www.was-esse-ich.de/uploads/media/NVSII_Abschlussbericht_Teil_2.pdf Retrieved 08.06.2011.

Mink, P. J., Scrafford, C. G., Barraj, L. M., Harnack, L., Hong, C. P., Nettleton, J. A., et al. (2007). Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *American Journal of Clinical Nutrition, 85*(3), 895–909.

Nicklas, T. A., O'Neil, C. E., & Kleinman, R. (2008). Association between 100% juice consumption and nutrient intake and weight of children aged 2 to 11 years. *Archives of Pediatrics & Adolescent Medicine, 162*(6), 557–565.

Nicklaus, S., Boggio, V., Chabanet, C., & Issanchou, S. (2004). A prospective study of food preferences in childhood. *Food Quality and Preference 15*(7–8), 805–818.

O'Neil, C. E., & Nicklas, T. A. (2008). A review of the relationship between 100% fruit juice consumption and weight in children and adolescents. *American Journal of Lifestyle Medicine, 2*(4), 315–354.

O'Neil, C. E., Nicklas, T. A., & Kleinman, R. (2010). Relationship between 100% juice consumption and nutrient intake and weight of adolescents. *American Journal of Health Promotion, 24*(4), 231–237.

O'Neil, C. E., Nicklas, T. A., Zanovec, M., & Fulgoni, V. L. 3rd., (2011). Diet quality is positively associated with 100% fruit juice consumption in children and adults in the United States: NHANES 2003–2006. *Nutrition Journal, 10*, 17.

Popper, R., & Kroll, J. J. (2005). Conducting sensory research with children. *Journal of Sensory Science, 20*(1), 75–87.

Sabbe, S., Verbeke, W., Deliza, R., Matta, V., & Van Damme, P. (2009). Effect of a health claim and personal characteristics on consumer acceptance of fruit juices with different concentrations of acai (Euterpe oleracea Mart.). *Appetite, 53*(1), 84–92.

Sichert-Hellert, W., & Kersting, M. (2004). Home-made carbonated water and the consumption of water and other beverages in children and adolescents: results of the DONALD study. *Acta Paediatrica, 93*(12), 1583–1587.

Stahl, A., Vohmann, C., Richter, A., Heseker, H., & Mensink, G. B. (2009). Changes in food and nutrient intake of 6- to 17-year-old Germans between the 1980s and 2006. *Public Health Nutrition, 12*(10), 1912–1923.

Wang, L. S., & Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters, 269*(2), 281–290.

Watzl, B. (2008). Anti-inflammatory effects of plant-based foods and of their constituents. *International Journal for Vitamin and Nutrition Research, 78*(6), 293–298.

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

Yngve, A., Wolf, A., Poortvliet, E., Elmadfa, I., Brug, J., Ehrenblad, B., et al. (2005). Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: the pro children cross-sectional survey. *Annals of Nutrition and Metabolism, 49*(4), 236–245.

3. GENERAL DISCUSSION

3.1 SYNOPSIS OF RESEARCH RESULTS

It was concluded after critical evaluation of the original literature [142] and anthocyanin analysis of bananas by the Geisenheim Research Center that the anthocyanidin content of bananas provided by the USDA [48] did not derive from anthocyanins, but rather from proanthocyanidins (Q1). This is also the case for the values for some nuts in the USDA DB, which are all provided by the same publication [142]. Therefore, in contrast to other estimations (e.g., in large US cohorts, EPIC subcohorts), in this thesis the values for bananas and nuts given by the USDA were excluded for anthocyanin intake estimations in children.

These estimations showed that anthocyanins were widely present in the diet of the young German sample from late infancy onwards (Q2, Q3). The median anthocyanidin intake estimated over the whole 20-year period from 1990 to 2009 was around 4 mg/day in older infants (from 9 months on) and around 6 mg/day in children and adolescents, with the mean intake being approximately twice as high (Q2, Q3). Pomaceous fruit (pears, apples) represented the main source of anthocyanins in infancy, whereas strawberries were the main source from the age of 18 months until the age of 18 years (Q2, Q3).

Median anthocyanidin density of the diet estimated over the whole 20-year period ranged from 0.6 mg/MJ in adolescent boys to 1.2 mg/MJ in 9-month-olds (Q2, Q3). Age and time trends were found to be present: density increased with age during infancy, followed by a slight decrease up to the age of 36 months (Q2). From early childhood into adolescence, density decreased further (Q3). Over the 20-year study period a decrease in anthocyanidin density in infants was observed, which may be attributable to a longer duration of full breastfeeding in recent times. In toddlers (18-36 months), a slight increase in anthocyanidin density of the diet was observed (Q2), whereas anthocyanidin density of children’s and adolescents’ (4-18 years) diets decreased slightly with time during the first half and increased slightly with time during the second half of the 20-year study period (Q3). Density was higher in girls than in boys during childhood and adolescence (Q3).

The sensory evaluation study in 4-17-year-olds showed that anthocyanin-rich products and in particular grape-bilberry juice were well accepted by children and adolescents, juices were liked better than smoothies and grape-bilberry liked better than apple-bilberry mixtures (Q4). Acceptance of the most popular grape-bilberry juice was not influenced by blending it with familiar apple juice. Internal Preference Mapping revealed that a group of participants rated smoothies higher than juices and/or apple-bilberry higher than grape-bilberry mixtures.

3.2 STRENGTHS AND WEAKNESSES OF THE DATA

Food consumption

An inherent weakness of intake estimations is the inaccuracy of food consumption data. The DONALD dietary records and the in-house food and nutrient database LEBTAB [139] were not designed with assessment of secondary plant metabolites as an objective. Therefore, specific information on the consumed food varieties regarding anthocyanin content, for example the colour of grapes, is not available. Further, it was not possible to quantify the amount of anthocyanins ingested in terms of food colourants, because this information has been entered into LEBTAB only since around 2005 and because of the "quantum satis" principle [50, 51].

Despite inherent difficulties, the dietary assessment method of the DONALD Study is a major strength. The detailed 3-day weighed dietary records [137] allow an exact assessment of amounts consumed and LEBTAB allows an exact breakdown of the recipes of foods [139], such that small amounts of anthocyanin-containing ingredients eaten can also be identified. In addition, the large number of subjects and records available and their distribution over the year should result in reliable mean intake values for different age groups, over the year.

Anthocyanin content of foods

Another inherent weakness of intake estimations is the technological [46], analytical [55] as well as biological [143] variability in food composition data, which is difficult to incorporate in databases. One may question if it is reasonable to apply a US database to foods consumed by a German population. However, values presented cover a wide range of varieties from different regions - in the first version of the database most values came from Europe and countries other than the US [56] - and foods are presently sold worldwide.

Strengths of the USDA DB include its wide application in previous intake estimations as well as in epidemiological studies, and the fact that it was the most comprehensive database available at the beginning of this work. Further, the exclusion of the content values for bananas and nuts from the present work should lead to more valid results, as they may have biased the results of former studies.

Sensory evaluation

A potential weakness is that tasting sessions did not take place at the same time of day, or at the same time of year for all subjects. However, no correlation was found between time of day or date and ratings on the hedonic scale. Further, no sensory laboratory was available for the tasting sessions to ensure constant conditions. Nevertheless, using the same room, conditions and personnel for all subjects ensured a standardised procedure.

Strengths of the sensory study include the wide age range of the participants, covering 4-17-year-olds and, at the same time, ensuring a constant testing environment throughout. The same products were used during the whole study period, and their sensory stability was ensured by repeated descriptive tests conducted by a sensory panel at the Geisenheim Research Center. By conducting individual tasting sessions, distractions due to the presence of peers were avoided, and every child and adolescent could focus on taste and work at his own pace. Moreover, due to the constant presence of study personnel, help was provided whenever needed and misunderstandings or missing values in the test sheets were minimised.

DONALD design

Limitations of the DONALD design are the convenient sampling scheme and the demanding study protocol, which lead to a non-representative sample with participants having a higher socioeconomic status as compared to the general German population [137, 144]. However, dietary recommendations derived from DONALD analyses are nearly the same as those derived from the nation-wide representative survey "EsKiMo" [107, 145], and the anthocyanin-rich products were rated similarly by a sample of school children (n = 25) in a sensory laboratory at the Geisenheim Research Center [146].

A strength of the DONALD study is its longitudinal structure, which provides both existing dietary data, collected over more than 25 years, and ongoing data assessment at the FKE. Firstly, in this way the very first anthocyanin intake estimations in Europe over a wide age range from infancy to adolescence were possible - these are age groups in which dietary patterns might be established. Secondly, due to the repeated measurements, age and time trends could be identified and analysed for the first time in individual intake data that have been collected and assessed using the same methods over the 20 years. Thirdly, children and adolescents of a wide age range were available for tasting sessions all at the same location. In summary, a major strength of this thesis was the use of these existing data and structures, thus using existing resources in an efficient manner. Additionally, further research could take advantage of the DONALD design to follow up trends in anthocyanin intake and to study associations between food consumption habits and food preferences, using the anthocyanin intake data and results of the sensory evaluation study.

3.3 HANDLING AND CONSEQUENCES OF INVALID FOOD COMPOSITION DATA

The USDA DB was the most comprehensive and most applied database on anthocyanin content of foods in recent years. Therefore, comparison with other intake estimations is facilitated at least on this point. However, as former intake estimations based on the USDA DB 2007 included the “false non-zero” values for bananas and nuts, falsely high intakes may have been estimated [147]. In the present intake estimation for German infants and toddlers, bananas would falsely be the predominant source of anthocyanins [105]. Likewise, bananas were presented to contribute 14-20% of anthocyanin intake across US cohorts [99, 147]. This indicates high banana consumption, and it is therefore particularly important to exclude the banana value for valid intake estimations.

Even more important is the exclusion of false values in epidemiological studies. If the inclusion of such values adds only random error, there will be a bias towards the null [147]. However, if for example banana consumption is associated with actual anthocyanin intake, this will lead to under- or overestimation of the effect and mistaken conclusions may be drawn.

In contrast to other pitfalls in the estimation of anthocyanin intake, which will hardly be avoidable, such as lack of information on consumed varieties or change of content during storage, identified systematical issues must be avoided. In this context it is regrettable that the updated version of the USDA DB, which was released in 2011, still contains anthocyanidin values for bananas and nuts [57]. Therefore, the responsibility of recognising and excluding invalid values unfortunately rests with the users of the database, which is problematic, since for example epidemiologists and nutritional scientists are unlikely to be experts in anthocyanin analyses.

Unfortunately, in recent studies using the USDA DB it is not stated whether the false values were excluded or not, despite the publication of the two letters indicating them [105, 106]. For example, in a study on diabetes [14] and in a study on stroke [103], reference is made to the flavonoid data used in the study on hypertension that gave occasion to the first letter [105], because bananas were found to be one of the main sources of anthocyanins [99]. However, in the two recent studies on diabetes and stroke respectively, bananas are not mentioned as a source of anthocyanins, but the exclusion of the value is not clearly stated either [14, 103]. Likewise, in a study on CVD mortality [104], it is not indicated whether the value for bananas was excluded or not.

In contrast to the USDA DB, the values for bananas and nuts were not included in Phenol-Explorer, as the analytical method was not seen as appropriate. In Phenol-Explorer, it is possible to request mean content values per category of analytical method, which may be useful in some cases.

Better monitoring of databases and more rapid realisation of updates are required, including an explanation for the changes made. Users of the database must be able to rely on the tabulated values. Additionally, a database should meet the following requirements, summarised in the term “ACQUIRE”: accessible, comprehensive, queryable, user-friendly, interactive, referenced and expandable [55].

3.4 ANTHOCYANIN INTAKE ESTIMATIONS

Anthocyanins were widely distributed in the diet of the study population, but intake distribution was found to be skewed to the right. This is characteristic for most dietary components [148] and in particular for components that are not uniformly distributed in foods, but rather are present in high amounts in a limited number of foods, which is true of anthocyanins [88]. Several other anthocyanin intake estimations present mean values much higher than median values and 10-36% “non-consumers” [88]. These “non-consumers” are, however, replaced by “low-consumers” in the DONALD sample, because the detailed dietary assessment made it possible to include anthocyanins on the ingredient level.

Comparison between different published intake estimations is difficult for several reasons. Firstly, different dietary assessment methods are used. Food consumption per capita described on the basis of food balance sheets is likely to be overestimated as losses due to waste and processing are not considered [53]. For example, in the UK, a mean anthocyanidin intake of 69 mg/day was estimated using FAO Food Balance sheets [95], which is much higher than the mean intake estimated using 24HR in the British EPIC cohort (22-31 mg/day) [49]. In Finland too, an estimation based on the average berry consumption was higher (82 mg/day) [64] than estimations using a 48HR (47 mg/day) [85] or a 4-day food record (4DFR) (6 mg/day) [86].

On the individual dietary assessment level, food that is only eaten occasionally, such as blueberries, may be better represented by an FFQ than by methods covering a smaller time frame [88]. However, FFQ is a primarily qualitative measure of which foods are consumed and the accuracy of FFQ is lower than that of other methods [54]. In addition, many FFQ used in epidemiological studies are not validated or at least not validated for the measurement of anthocyanin intake [149]. In most cases, anthocyanin intake is estimated retrospectively in studies which have already been conducted or are still running (Table 1).

Mullie et al. (2008) compared an FFQ designed to assess flavonoid intake (conducted twice) with a 4DFR [88]. While total flavonoid intake, measured with the two methods, did not differ, a statistically significant difference in anthocyanin intake between FFQ and 4DFR was found. The two methods misclassified 29% of the 45 participants in a non-adjacent quartile. A weak correlation between the two FFQ and the 4DFR was found (Spearman's rank correlation coefficients r = 0.33 and r = 0.34 respectively, and p = 0.02 for both) [88].

Nevertheless, a vague classification of our results should be made. The very low estimated mean intake of 0.0-0.7 mg/day in 2-18-year-old Australians [75] might be due to the use of a 24HR on a relatively small number of subjects (n = 3007, 5 age groups, [150]). However, intake may also truly be low due to less anthocyanin-rich foods available, as is the case in Fiji [76]. This estimation would not be plausible for a young German population consuming a mixed diet. In DONALD, mean as well as median intake was higher than 3 mg/day even as early as age 9 months [96, 101].

Compared to estimations in German adults, it can be stated that the estimation in the DONALD sample lies between an earlier estimation in a subcohort of the German National Nutrition Survey in the 1990s (mean: 7 mg/day) [63] and the recent estimation conducted in the EPIC study (mean: 30-41 mg/day) [49]. The earlier estimation had less anthocyanin data (for example, no data for strawberries), which might explain the lower value. The recent estimation might differ from the young DONALD sample on the grounds of wine consumption.

Overall, most estimations in European adults are higher than in the USA, Oceania and Japan, which are in the range of or below estimations in DONALD (Table 1). This might indicate that the differences are due to the differences in food patterns across continents rather than to age.

Density was estimated to be 7 mg/MJ in a Finnish study [85], 1.5 mg/1,000 kcal (= 0.36 mg/MJ) in a US [84] and 0.3 mg/MJ [63] in the formerly mentioned German study. DONALD values lie below the Finnish, but above the US and German values. This seems plausible considering the high berry consumption and high absolute anthocyanin intake in Finland on the one hand and the lower estimated mean intake in the USA as well as in the early German adult population on the other hand. Nevertheless, the question remains, whether the absolute anthocyanin intake in the young DONALD sample will remain stable into adulthood and will, along with increasing energy intake, lead to a lower density in adulthood. The decreasing trend in anthocyanidin density found in adolescence [101] seems to be indicative of this. In the Australian cross-sectional study, absolute anthocyanin intake was higher in adults than in children and adolescents due to wine consumption [75]. However, density values were not provided in this study. The decreasing trend over time in infancy in

DONALD is attributable to a longer duration of full breastfeeding in recent times. The increase in anthocyanidin density in children's diets since 2001 may be due to the increase in fruit and vegetable consumption [110], perhaps attributable to the "5-a-day campaign" [151].

Main sources of anthocyanins in infants and toddlers were pomaceous fruit, but from the age of 18 months onwards, strawberries became the major source in all age groups. This may be due to the change from infant food, which is mainly based on apples and pears in baby jars and home-made porridges in Germany, to foods in the adult diet, such as jam and fruit yoghurts, which are rarely given to infants and, in Germany, often contain strawberries. However, anthocyanin sources on the level of foods could be further clarified in additional analyses. The identified main sources also indicate that frequency of consumption seems to be more relevant than level of anthocyanin content at the group level.

To make intake estimations and epidemiological studies more valid and more comparable, the different existing databases providing anthocyanin content data for foods [57, 58, 60] should be compared and their particular strengths and weaknesses must be studied.

A better approach to estimating anthocyanin intake at the group level might be to use the original analytical data instead of the tabulated mean values for anthocyanin content in foods and thereby account for biological variances. These data on anthocyanin distribution in foods could be combined with the distribution of consumed amounts of foods by use of parametric or non-parametric simulation to estimate an anthocyanin intake distribution. Biomarkers, for example anthocyanins or metabolites in urine [152], might facilitate and improve intake estimations at the individual level. However, assessment of biomarkers reflecting short-term intake (e.g., 24h-urine collection) may not be easier or less expensive than dietary assessment in epidemiological studies. Further, biomarkers reflecting long-term intake are lacking [153].

3.5 ANTHOCYANIN-RICH FOOD PRODUCTS FOR CHILDREN

Grape-bilberry juice received the highest mean rating score on the hedonic scale, and blendings of this juice with apple juice were equally preferred by the participants. Together with the high anthocyanin content, this makes grape-bilberry juice a promising product to promote for children. That grape-bilberry received higher ratings than apple-bilberry mixtures may be due to the higher sugar content, and the resulting sweeter taste of the former, in comparison to the more sour taste of the apple-bilberry products. At the Geisenheim Research Center, more than half of a sample of children and adolescents rated the apple-bilberry mixtures as "not sweet enough" [146]. Preference of juices over smoothies may be attributable to the unfamiliarity of smoothies (about two thirds of the participants indicated that they do not know what smoothies are; unpublished data). Another explanation may be that smoothies were not denoted as such and the unexpected texture may have decreased liking.

In 30% of the sample, preferences were reversed, with apple-bilberry being liked better than grape-bilberry and/or smoothies liked better than juices. This is in line with previous research revealing that a proportion of children prefer sour taste, which was related to the willingness to try unknown food [154] and to higher fruit consumption in boys [155].

Consumer tests involving children are routinely conducted in food product development, but hardly any results are published, because they are usually owned by the companies [131]. Therefore, comparison with other studies is limited here. In a sensory evaluation study with 123 adults, juices with different amount of açaí (anthocyanin-rich berries grown in Brazil) were scored on a 9-point hedonic scale [156]. Two commercial juices received the highest mean rating scores, which were in the range of the mean rating score for grape-bilberry juice in the DONALD sample (≈7). This indicates good preselection of fruit juice samples for this thesis and shows the potential for grape-bilberry juice to become a marketable product. In a consumer study with 153 children aged between 6 and 13, it was investigated whether liking of black currant juice is influenced by different packaging materials and storage temperatures [157]. Juices were liked equally from glass and plastic bottles, but products that were stored in the fridge (4°C) were preferred compared to those stored at 21°C due to better flavour, taste and aftertaste. These results suggest that storage in cooling shelves should be considered in food development and the marketing process of anthocyanin-rich juices.

In developing an anthocyanin-rich product suitable for children, in the interest of public health, the following factors need to be considered: 1) from a nutritional point of view, the composition and amount of anthocyanin-rich products that can be recommended for children and adolescents (behavioural prevention) and 2) from an economic, social and political point of view, possible strategies to make a large part of the population benefit from such health-promoting products (environmental prevention).

3.6 PREVENTION

Behavioural prevention

Behavioural prevention aims to avoid behaviour (including dietary habits) that is hazardous to health [158]. To develop positive behaviour, knowledge is required and this has to be provided by science in terms of dietary recommendations. It is difficult to translate nutrient-based targets to the population, which is why food-based rather than nutrient-based dietary guidelines should be developed [159]. Within the scope of “5-a-day” and the additional existing recommendation to eat colourful [160], combined with the possibility of replacing one portion of fruit or vegetable by a juice [117], it may already be reasonable to recommend one purple fruit juice a day. This seems promising for the purpose of increasing fruit and vegetable as well as anthocyanin intake, because juice is popular among German children [114, 115] and they currently consume fruits that are mostly low in anthocyanins [107, 120].

Especially when giving food-based dietary recommendations and when a juice or smoothie should replace one portion of fresh fruit and the positive health effects expected from this, its composition needs to be considered. Regarding anthocyanin content, grape-bilberry juice provides about 80 mg anthocyanins/100 mL [161], which is more than tenfold the estimated median daily intake in the DONALD sample [96, 101] and therefore has the potential to significantly increase intake. By contrast, commercial grape juices contain only about 2 mg anthocyanins/100 mL [146]. However, the efficacy of anthocyanins and questions concerning loss of anthocyanins during storage [46] as well as bioavailability influenced by food matrix [38] and individual gut microbiota composition [162] are yet to be clarified.

Regarding sugar content, it is still a point of discussion whether consumption of sugar sweetened beverages and 100% fruit juices is associated with being overweight, as it was at least in subgroups in some studies [163, 164]. However, a review including 21 studies did not find evidence for such an association in children and adolescents [165]. By contrast, 100% fruit juice consumption was positively associated with diet quality in children and adolescents in the NHANES [166-169]. However, as fruit juice consumption may only be a marker for a healthy lifestyle, and as no causal inference is possible on the basis of a cross-sectional study, this association should be interpreted cautiously. Nevertheless, it can be stated that fruit juices, in moderate amounts, can be part of a healthy diet.

The sugar content of 100 g of fresh fruit is similar to the sugar content of 100 mL of juice from the same fruit [170]. Given that the amount of juice or smoothie recommended per day should not provide more sugar than a portion of fresh fruit, a portion of juice could range from 50 mL for one-year-olds (= one fifth of the recommended amount for fruit and vegetables [171]) up to 140 mL for 17-year-old boys. In the consensus statement of the American Heart Association, the amounts of sweet beverages allowed for children and adolescents are more liberal than this calculation (max. 6 oz/day (equivalent to 177 mL/day) for children aged 1-6 years and max. 12 oz (355 mL) for children aged 7-18 years) [172].

As apple products may reach special target groups and smoothies contain more fibre than juices [173], and a larger amount of polyphenols reaches the colon after smoothie than after juice consumption [174], apple-bilberry mixtures and smoothies should also be further studied as potential health-promoting products. Smoothies might be better liked in the future due to increasing popularity or given a different kind of presentation, such as in bottles typical of smoothies.

Environmental prevention

Environmental prevention deals with technical, organisational and social characteristics of the environment and the role they play in development of diseases [158]. Determinants of food intake are motivation, ability and opportunity, the latter being determined by the social as well as the physical environment [175]. One part of the environment is provided by the parents [175]. Parent-reported accessibility of fruits, vegetables and 100% fruit juices was related to the child's consumption of these foods [176] and familiarity is known to increase liking of new foods [156]. Therefore, availability and accessibility of anthocyanin-rich fruits at home may be one way to increase children's liking and intake. In cooperation with companies, using their experiences in marketing, it would be possible to promote a new health-promoting product and to influence consumer decision-making in a positive way. But such a strategy may only reach families of a higher social class, since, in general, socio-economic status of a family is positively associated with fruit and vegetable intake [177] and public health relevance would be small. Another part of the environment is determined by school, which provides accessibility and availability of foods [175]. In a similar way to projects providing milk or the school fruit scheme [178], schools may be an optimal setting for providing health-promoting food products. This could be especially effective in combination with behavioural prevention [179], such as lessons on health-promoting diets or secondary plant metabolites, as it was in a study on the promotion of drinking water [180].

3.7 CONCLUSION

In this thesis, detailed food consumption data from a German open cohort study were used to estimate, for the very first time in Europe, anthocyanin intake of 0-18-year-olds. Further, the longitudinal data were used to identify changes of intake with age and time. To improve accuracy of the estimations, the false non-zero values for bananas and nuts provided by the applied USDA Database for the Flavonoid Content of Selected Foods have been excluded. However, inherent difficulties in estimating intake of secondary plant metabolites always need to be considered.

The results of this thesis indicate that anthocyanins are widely present in the diet of German children, and that their density in the diet decreases with age and is lower in the diets of boys as compared to girls. This reveals scope for increase in anthocyanins in the diets of young age groups. In the sensory evaluation study, anthocyanin-rich grape-bilberry juice was found to be well-liked by children and adolescents of a wide age range. This suggests that it may be a suitable product for increasing young age groups' fruit intake in general and anthocyanin intake in particular by means of both behavioural and environmental prevention. However, to deduce and implement evidence-based dietary recommendations in the future, research on the efficacy of anthocyanins is fundamental.

3.8 FUTURE PROSPECTS

Additional research on anthocyanin intake of young age groups in other countries would be interesting in the context of health-promotion. Intake estimations could be further improved by dietary assessment instruments that are validated for anthocyanin intake on the one hand and the expansion of food composition databases and their steady evaluation by users on the other hand. Development of yield and retention factors is in progress [47] and may improve accuracy of composition data, even though change of polyphenol content during storage and food production is supposed to often be negligible compared to biological differences between plant varieties [46]. As an alternative to intake estimations based on food consumption and food composition data, the development of biomarkers [152, 153, 181] may be a further step in increasing the accuracy of secondary plant metabolite intake assessments.

If health-promoting effects of anthocyanins are confirmed, anthocyanin-rich products will be developed to include more anthocyanins in the average diet. In addition to anthocyanin content, stability, bioavailability, and sugar content also need to be considered. The preferences of children and adolescents should be taken into account as they are an important age group in the context of health-promotion.

4. REFERENCES

1. Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 2008;54(4):733-49.
2. Watzl B, Rechkemmer G. Flavonoide. Ernährungsumschau. 2001;48(12):498-502.
3. He J, Giusti MM. Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol. 2010;1:163-87.
4. Marquart LC. Die Farben der Blüthen. Habicht; 1835.
5. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, et al. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr. 2007;85(3):895-909.
6. Chun OK, Chung SJ, Claycombe KJ, Song WO. Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. J Nutr. 2008;138(4):753-60.
7. Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. Cancer Lett. 2008;269(2):281-90.
8. Frankenfeld CL, Cerhan JR, Cozen W, Davis S, Schenk M, Morton LM, et al. Dietary flavonoid intake and non-Hodgkin lymphoma risk. Am J Clin Nutr. 2008;87(5):1439-45.
9. Rossi M, Bosetti C, Negri E, Lagiou P, La Vecchia C. Flavonoids, proanthocyanidins, and cancer risk: a network of case-control studies from Italy. Nutr Cancer. 2010;62(7):871-7.
10. Prior RL, Wu X. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. Free Radic Res. 2006;40(10):1014-28.
11. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, et al. Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr. Epub 2012 Jun 9.
12. Tavani A, Spertini L, Bosetti C, Parpinel M, Gnagnarella P, Bravi F, et al. Intake of specific flavonoids and risk of acute myocardial infarction in Italy. Public Health Nutr. 2006;9(3):369-74.
13. Welch A, MacGregor A, Jennings A, Fairweather-Tait S, Spector T, Cassidy A. Habitual flavonoid intakes are positively associated with bone mineral density in women. J Bone Miner Res. Epub 2012 May 1.
14. Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, et al. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. Am J Clin Nutr. 2012;95(4):925-33.
15. Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A. Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology. 2012;78(15):1138-45.
16. Wang H, Cao G, Prior RL. Oxygen Radical Absorbing Capacity of Anthocyanins. J Agric Food Chem. 1997;45(2):304-9.
17. Tsuda T. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. Mol Nutr Food Res. 2011;56(1):159-70.
18. Phenol-Explorer: Database on polyphenol content in foods. [cited 2012 May]; Available from: http://www.phenol-explorer.eu/.

19. Andersen ØM, Jordheim M. The anthocyanins. In: Andersen ØM, Markham KR, editors. Flavonoids Chemistry, Biochemistry and Applications. Boca Raton: Taylor & Francis Group; 2006. p. 471-551.
20. Clifford MN. Anthocyanins – nature, occurrence and dietary burden. J Sci Food Agric. 2000;80(7):1063-72.
21. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep. 2009;26(8):1001-43.
22. McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. Mol Nutr Food Res. 2007;51(6):702-13.
23. Del Rio D, Borges G, Crozier A. Berry flavonoids and phenolics: bioavailability and evidence of protective effects. Br J Nutr. 2010;104 Suppl 3:S67-90.
24. Davies KM. Modifying Anthocyanin Production in Flowers. In: Gould K, Davies K, Winefield C, editors. Anthocyanins Biosynthesis, Functions, and Applications. New York: Springer; 2009. p. 49-83.
25. Steyn WJ. Prevalence and Functions of Anthocyanins in Fruits. In: Gould K, Davies K, Winefield C, editors. Anthocyanins Biosynthesis, Functions, and Applications. New York: Springer; 2009. p. 85-105.
26. Gleason FK, Chollet R. Plant Biochemistry. 1. ed. Sudbury, MA: Jones & Bartlett Learning; 2012.
27. Petersen M, Hans J, Matern U. Biosynthesis of phenylpropanoids and related compounds. In: Wink M, editor. Biochemistry of plant secondary metabolism. 2. ed. Chichester: Wiley-Blackwell; 2010. p. 182-257.
28. Schopfer P, Brennicke A. Pflanzenphysiologie. 7. ed. Heidelberg: Spektrum; 2010.
29. Aaby K, Mazur S, Nes A, Skrede G. Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. Food Chem. 2012;132(1):86-97.
30. Kalt W. Effects of Production and Processing Factors on Major Fruit and Vegetable Antioxidants. J Food Sci. 2005;70(1):R11-R9.
31. Mallery SR, Budendorf DE, Larsen MP, Pei P, Tong M, Holpuch AS, et al. Effects of human oral mucosal tissue, saliva, and oral microflora on intraoral metabolism and bioactivation of black raspberry anthocyanins. Cancer Prev Res (Phila). 2011;4(8):1209-21.
32. Passamonti S, Vrhovsek U, Vanzo A, Mattivi F. The stomach as a site for anthocyanins absorption from food. FEBS Lett. 2003;544(1-3):210-3.
33. Talavera S, Felgines C, Texier O, Besson C, Lamaison JL, Remesy C. Anthocyanins are efficiently absorbed from the stomach in anesthetized rats. J Nutr. 2003;133(12):4178-82.
34. Cai H, Thomasset SC, P-Berry D, Garcea G, Brown K, Steward WP, et al. Determination of anthocyanins in the urine of patients with colorectal liver metastases after administration of bilberry extract. Biomed Chromatogr. 2011;25(6):660-3.
35. Passamonti S, Vrhovsek U, Mattivi F. The interaction of anthocyanins with bilitranslocase. Biochem Biophys Res Commun. 2002;296(3):631-6.
36. He J, Wallace TC, Keatley KE, Failla ML, Giusti MM. Stability of black raspberry anthocyanins in the digestive tract lumen and transport efficiency into gastric and small intestinal tissues in the rat. J Agric Food Chem. 2009;57(8):3141-8.
37. Matuschek MC, Hendriks WH, McGhie TK, Reynolds GW. The jejunum is the main site of absorption for anthocyanins in mice. J Nutr Biochem. 2006;17(1):31-6.

38. Yang M, Koo SI, Song WO, Chun OK. Food matrix affecting anthocyanin bioavailability: review. Curr Med Chem. 2011;18(2):291-300.
39. Vitaglione P, Donnarumma G, Napolitano A, Galvano F, Gallo A, Scalfi L, et al. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. J Nutr. 2007;137(9):2043-8.
40. Kahle K, Kraus M, Scheppach W, Ackermann M, Ridder F, Richling E. Studies on apple and blueberry fruit constituents: do the polyphenols reach the colon after ingestion? Mol Nutr Food Res. 2006;50(4-5):418-23.
41. González-Barrio R, Borges G, Mullen W, Crozier A. Bioavailability of anthocyanins and ellagitannins following consumption of raspberries by healthy humans and subjects with an ileostomy. J Agric Food Chem. 2010;58(7):3933-9.
42. Wu X, Pittman HE, 3rd, Prior RL. Fate of anthocyanins and antioxidant capacity in contents of the gastrointestinal tract of weanling pigs following black raspberry consumption. J Agric Food Chem. 2006;54(2):583-9.
43. Keppler K, Humpf HU. Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. Bioorg Med Chem. 2005;13(17):5195-205.
44. Kalt W, Blumberg JB, McDonald JE, Vinqvist-Tymchuk MR, Fillmore SA, Graf BA, et al. Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. J Agric Food Chem. 2008;56(3):705-12.
45. Janle EM, Lila MA, Grannan M, Wood L, Higgins A, Yousef GG, et al. Pharmacokinetics and tissue distribution of 14C-labeled grape polyphenols in the periphery and the central nervous system following oral administration. J Med Food. 2010;13(4):926-33.
46. Amarowicz R, Carle R, Dongowski G, Durazzo A, Galensa R, Kammerer D, et al. Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. Mol Nutr Food Res. 2009;53 Suppl 2:S151-83.
47. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A. Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: an application of the phenol-explorer database. J Agric Food Chem. 2010;58(8):4959-69.
48. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Flavonoid Content of Selected Foods, Release 2.1. 2007 [updated 2007; cited 2009 Aug]; Available from: http://www.ars.usda.gov/Services/docs.htm?docid=6231.
49. Zamora-Ros R, Knaze V, Luján-Barroso L, Slimani N, Romieu I, Touillaud M, et al. Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr. 2011;106(7):1090-9.
50. European Parliament, Council of the European Union. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Official Journal of the European Union. 2008(L 354):16-33.
51. European Commission. Commission regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Official Journal of the European Union. 2011;54(L 295):1-177.
52. Commission of the European Communities. Report from the commission on dietary food additive intake in the European Union. 2001 [updated 2001; cited 2012 May]; Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2001:0542:FIN:EN:PDF.

53. Agudo A. Measuring intake of fruit and vegetables. World Health Organization; 2005 [updated 2005; cited 2012 Apr]; Available from: http://www.who.int/dietphysicalactivity/publications/f&v_intake_measurement.pdf.
54. Gibson RS. Principles of nutritional assessment. New York: Oxford University Press; 1990.
55. Scalbert A, Andres-Lacueva C, Arita M, Kroon P, Manach C, Urpi-Sarda M, et al. Databases on food phytochemicals and their health-promoting effects. J Agric Food Chem. 2011;59(9):4331-48.
56. Holden JM, Bhagwat SA, Haytowitz DB, Gebhardt SE, Dwyer JT, Peterson J, et al. Development of a database of critically evaluated flavonoids data: application of USDA's data quality evaluation system. J Food Compost Anal. 2005;18:829-44.
57. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Flavonoid Content of Selected Foods, Release 3.0. 2011 [updated 2011; cited 2011 Nov]; Available from: http://www.ars.usda.gov/Services/docs.htm?docid=6231.
58. Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford). 2010:bap024.
59. Kiely M, Black LJ, Plumb J, Kroon PA, Hollman PC, Larsen JC, et al. EuroFIR eBASIS: application for health claims submissions and evaluations. Eur J Clin Nutr. 2010;64 Suppl 3:S101-7.
60. EuroFIR. eBasis. BioActive Substances in Food Information System. [cited 2012 May]; Available from: http://ebasis.eurofir.org/.
61. Gry J, Black L, Eriksen FD, Pilegaard K, Plumb J, Rhodes M, et al. EuroFIR-BASIS - a combined composition and biological activity databse for bioactive compounds in plant-based foods. Trends Food Sci Technol. 2007;18(8):434-44.
62. Kühnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev Nutr Diet. 1976;24:117-91.
63. Linseisen J, Radtke J, Wolfram G. Flavonoidzufuhr Erwachsener in einem bayrischen Teilkollektiv der Nationalen Verzehrsstudie. Zeitschrift für Ernährungswissenschaft. 1997;36(4):403-12.
64. Heinonen M. Antioxidant activity and antimicrobial effect of berry phenolics--a Finnish perspective. Mol Nutr Food Res. 2007;51(6):684-91.
65. Peterson J, Lagiou P, Samoli E, Lagiou A, Katsouyanni K, La Vecchia C, et al. Flavonoid intake and breast cancer risk: a case--control study in Greece. Br J Cancer. 2003;89(7):1255-9.
66. Lagiou P, Samoli E, Lagiou A, Peterson J, Tzonou A, Dwyer J, et al. Flavonoids, vitamin C and adenocarcinoma of the stomach. Cancer Causes Control. 2004;15(1):67-72.
67. Lagiou P, Samoli E, Lagiou A, Tzonou A, Kalandidi A, Peterson J, et al. Intake of specific flavonoid classes and coronary heart disease--a case-control study in Greece. Eur J Clin Nutr. 2004;58(12):1643-8.
68. Melby MK, Murashima M, Watanabe S. Phytochemical intake and relationship to past health history in Japanese women. Biofactors. 2004;22(1-4):265-9.
69. Kita J, Tada J, Ito M, Shirakawa M, Murashima M, Zhuo XG, et al. Intake of phytochemicals among Japanese, calculated by the new FFF database. Biofactors. 2004;22(1-4):259-63.

70. Bosetti C, Spertini L, Parpinel M, Gnagnarella P, Lagiou P, Negri E, et al. Flavonoids and breast cancer risk in Italy. Cancer Epidemiol Biomarkers Prev. 2005;14(4):805-8.
71. Rossi M, Negri E, Talamini R, Bosetti C, Parpinel M, Gnagnarella P, et al. Flavonoids and colorectal cancer in Italy. Cancer Epidemiol Biomarkers Prev. 2006;15(8):1555-8.
72. Lagiou P, Samoli E, Lagiou A, Skalkidis Y, Katsouyanni K, Petridou E, et al. Flavonoid classes and risk of peripheral arterial occlusive disease: a case-control study in Greece. Eur J Clin Nutr. 2006;60(2):214-9.
73. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 2006;54(11):4069-75.
74. Centers for Disease Control and Prevention/National Center for Health Statistics. National Health and Nutrition Examination Survey 1999–2012 Survey Content Brochure. [cited 2012 Jun]; Available from: http://www.cdc.gov/nchs/data/nhanes/survey_content_99_12.pdf.
75. Johannot L, Somerset SM. Age-related variations in flavonoid intake and sources in the Australian population. Public Health Nutr. 2006;9(8):1045-54.
76. Lako J, Wattanapenpaiboon N, Wahlqvist M, Trenerry C. Phytochemical intakes of the Fijian population. Asia Pac J Clin Nutr. 2006;15(2):275-85.
77. Rossi M, Garavello W, Talamini R, La Vecchia C, Franceschi S, Lagiou P, et al. Flavonoids and risk of squamous cell esophageal cancer. Int J Cancer. 2007;120(7):1560-4.
78. Rossi M, Garavello W, Talamini R, Negri E, Bosetti C, Dal Maso L, et al. Flavonoids and the risk of oral and pharyngeal cancer: a case-control study from Italy. Cancer Epidemiol Biomarkers Prev. 2007;16(8):1621-5.
79. Garavello W, Rossi M, McLaughlin JK, Bosetti C, Negri E, Lagiou P, et al. Flavonoids and laryngeal cancer risk in Italy. Ann Oncol. 2007;18(6):1104-9.
80. Bosetti C, Rossi M, McLaughlin JK, Negri E, Talamini R, Lagiou P, et al. Flavonoids and the risk of renal cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2007;16(1):98-101.
81. Fink BN, Steck SE, Wolff MS, Britton JA, Kabat GC, Schroeder JC, et al. Dietary flavonoid intake and breast cancer risk among women on Long Island. Am J Epidemiol. 2007;165(5):514-23.
82. Gammon MD, Neugut AI, Santella RM, Teitelbaum SL, Britton JA, Terry MB, et al. The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. Breast Cancer Res Treat. 2002;74(3):235-54.
83. Fink BN, Steck SE, Wolff MS, Britton JA, Kabat GC, Gaudet MM, et al. Dietary flavonoid intake and breast cancer survival among women on Long Island. Cancer Epidemiol Biomarkers Prev. 2007;16(11):2285-92.
84. Chun OK, Chung SJ, Song WO. Estimated dietary flavonoid intake and major food sources of U.S. adults. J Nutr. 2007;137(5):1244-52.
85. Ovaskainen ML, Törrönen R, Koponen JM, Sinkko H, Hellström J, Reinivuo H, et al. Dietary intake and major food sources of polyphenols in Finnish adults. J Nutr. 2008;138(3):562-6.
86. Mursu J, Nurmi T, Tuomainen TP, Salonen JT, Pukkala E, Voutilainen S. Intake of flavonoids and risk of cancer in Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. Int J Cancer. 2008;123(3):660-3.

87. Mursu J, Voutilainen S, Nurmi T, Tuomainen TP, Kurl S, Salonen JT. Flavonoid intake and the risk of ischaemic stroke and CVD mortality in middle-aged Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Br J Nutr. 2008;100(4):890-5.

88. Mullie P, Clarys P, Deriemaeker P, Hebbelinck M. Estimation of daily human intake of food flavonoids. Int J Food Sci Nutr. 2008;59(4):291-8.

89. Cutler GJ, Nettleton JA, Ross JA, Harnack LJ, Jacobs DR, Jr., Scrafford CG, et al. Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. Int J Cancer. 2008;123(3):664-71.

90. Bobe G, Sansbury LB, Albert PS, Cross AJ, Kahle L, Ashby J, et al. Dietary flavonoids and colorectal adenoma recurrence in the Polyp Prevention Trial. Cancer Epidemiol Biomarkers Prev. 2008;17(6):1344-53.

91. Somerset SM, Johannot L. Dietary flavonoid sources in Australian adults. Nutr Cancer. 2008;60(4):442-9.

92. Bobe G, Peterson JJ, Gridley G, Hyer M, Dwyer JT, Brown LM. Flavonoid consumption and esophageal cancer among black and white men in the United States. Int J Cancer. 2009;125(5):1147-54.

93. Dilis V, Trichopoulou A. Antioxidant intakes and food sources in Greek adults. J Nutr. 2010;140(7):1274-9.

94. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventós RM, Berenguer T, Jakszyn P, Barricarte A, et al. Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). J Am Diet Assoc. 2010;110(3):390-8.

95. Beking K, Vieira A. An assessment of dietary flavonoid intake in the UK and Ireland. Int J Food Sci Nutr. 2010;62(1):17-9.

96. Drossard C, Alexy U, Bolzenius K, Kunz C, Kersting M. Anthocyanins in the diet of infants and toddlers: intake, sources and trends. Eur J Nutr. 2011;50(8):705-11.

97. Lesser S, Bai JC, Heseker H, Niggemeier C, Overzier S, Paker-Eichelkrauth S, et al. Dietary flavonoid intake in elderly living in German nursing homes - the cross-sectional multicentre ERNSTES Study. 5th International Conference on Polyphenols and Health, Conference Proceedings; 2011 17-20 October; Sitges, Barcelona, Spain. 2011. p. 93.

98. Pérez-Jiménez J, Fezeu L, Touvier M, Arnault N, Manach C, Hercberg S, et al. Dietary intake of 337 polyphenols in French adults. Am J Clin Nutr. 2011;93(6):1220-8.

99. Cassidy A, O'Reilly EJ, Kay C, Sampson L, Franz M, Forman J, et al. Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nutr. 2011;93:338-47.

100. Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, et al. Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. women. J Nutr. 2011;141(4):618-25.

101. Drossard C, Bolzenius K, Kunz C, Kersting M. Anthocyanins in the diet of children and adolescents: intake, sources and trends. Eur J Nutr. Epub 2012 May 15.

102. Kesse-Guyot E, Fezeu L, Andreeva VA, Touvier M, Scalbert A, Hercberg S, et al. Total and specific polyphenol intakes in midlife are associated with cognitive function measured 13 years later. J Nutr. 2012;142(1):76-83.

103. Cassidy A, Rimm EB, O'Reilly EJ, Logroscino G, Kay C, Chiuve SE, et al. Dietary flavonoids and risk of stroke in women. Stroke. 2012;43(4):946-51.

104. McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, Dwyer JT. Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. Am J Clin Nutr. 2012;95(2):454-64.

105. Drossard C, Fröhling B, Dietrich H, Kersting M. Anthocyanin analysis in banana fruit--a mistake. Am J Clin Nutr. 2011;93(4):865-6; author reply 6-7.

106. Drossard C, Kersting M. Banana as a relevant source of anthocyani(di)ns in European populations? Br J Nutr. 2012;107:765-6; author reply 7.

107. Mensink GBM, Heseker H, Richter A, Stahl A, Vohmann C. Forschungsbericht Ernährungsstudie als KiGGS-Modul (EsKiMo). 2007 [updated 2007; cited 2012 Mar]; Available from: http://www.bmelv.de/cae/servlet/contentblob/378624/publicationFile/25912/EsKiMoStudie.pdf.

108. Association of the German Fruit Juice Industry. The most popular varieties of fruit juice. 2011 [updated 2011; cited 2011 Jun]; Available from: http://www.fruchtsaft.net/en/index.php?menu_sel=13&menu_sel2=3&menu_sel3=&menu_sel4=&msg=42.

109. German Nutrition Society. Ernährungsbericht 2008. Bonn; 2008

110. Stahl A, Vohmann C, Richter A, Heseker H, Mensink GB. Changes in food and nutrient intake of 6- to 17-year-old Germans between the 1980s and 2006. Public Health Nutrition. 2009;12(10):1912-23.

111. Yngve A, Wolf A, Poortvliet E, Elmadfa I, Brug J, Ehrenblad B, et al. Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: The Pro Children Cross-sectional Survey. Ann Nutr Metab. 2005;49(4):236-45.

112. Lorson BA, Melgar-Quinonez HR, Taylor CA. Correlates of fruit and vegetable intakes in US children. J Am Diet Assoc. 2009;109(3):474-8.

113. Diethelm K, Jankovic N, Moreno LA, Huybrechts I, De Henauw S, De Vriendt T, et al. Food intake of European adolescents in the light of different food-based dietary guidelines: results of the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr. 2012;15(3):386-98.

114. Sichert-Hellert W, Kersting M. Home-made carbonated water and the consumption of water and other beverages in children and adolescents: results of the DONALD study. Acta Paediatrica. 2004;93(12):1583-7.

115. Kohler S, Kleiser C, Richter A, Stahl A, Vohmann C, Heseker H, et al. Fluid intake of adolescents in Germany. Results collected in EsKiMo. Ernährung-Wissenschaft und Praxis. 2007;1(10):444-50.

116. Watzl B. Smoothies - Wellness aus der Flasche? Ernährungsumschau. 2008;55(6):352-3.

117. German Nutrition Society. Smoothies - Obst aus der Flasche. 2007 [updated 2007; cited 2011 Jul]; Available from: http://www.dge.de/modules.php?name=News&file=article&sid=764.

118. Lesschaeve I, Noble AC. Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. Am J Clin Nutr. 2005;81 Suppl 1:330S-5S.

119. Vidal S, Francis L, Noble A, Kwiatkowski M, Cheynier V, Waters E. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Analytica Chimica Acta. 2004;513:57-65.

120. Alexy U, Sichert-Hellert W, Kersting M, Manz F. The foods most consumed by German children and adolescents: results of the DONALD Study. Dortmund

Nutritional and Anthropometric Longitudinally Design. Ann Nutr Metab. 2001;45(3):128-34.

121. Dietrich H. Herstellung von roten Traubensäften und Anthocyanextrakten aus dem Trester anthocanreicher Traubensorten. Deutsche Lebensmittel-Rundschau. 2009;105:695-702.
122. Birch LL. Development of food preferences. Annu Rev Nutr. 1999;19:41-62.
123. Beauchamp GK, Mennella JA. Early flavor learning and its impact on later feeding behavior. J Pediatr Gastroenterol Nutr. 2009;48 Suppl 1:S25-30.
124. Westenhöfer J. Establishing good dietary habits. Public Health Nutr. 2001;4(1A):125-9.
125. Birch LL. Development of food acceptance patterns in the first years of life. Proc Nutr Soc. 1998;57(4):617-24.
126. Nicklaus S, Boggio V, Chabanet C, Issanchou S. A prospective study of food preferences in childhood. Food Qual Prefer. 2004;15(7-8):805-18.
127. Mikkilä V, Räsänen L, Raitakari OT, Pietinen P, Viikari J. Consistent dietary patterns identified from childhood to adulthood: the cardiovascular risk in Young Finns Study. Br J Nutr. 2005;93(6):923-31.
128. Mela DJ. Why do we like what we like? J Sci Food Agric. 2001;81(1):10-6.
129. Léon F, Couronne T, Marcuz MC, Köster EP. Measuring food liking in children: a comparison of non verbal methods. Food Qual Prefer. 1999;10(2):93-100.
130. Liem DG, de Graaf C. Sweet and sour preferences in young children and adults: role of repeated exposure. Physiol Behav. 2004;83(3):421-9.
131. Guinard J-X. Sensory and consumer testing with children. Trends Food Sci Technol. 2001;11(8):273-83.
132. Popper R, Kroll JJ. Conducting sensory research with children. J Sens Stud. 2005;20(1):75-87.
133. Popper R, Kroll JJ. Consumer testing of food products using children. In: Kilcast D, Angus F, editors. Developing children's food products. Cambridge: Woodhead Publishing; 2011. p. 163-87.
134. Lawless HT, Heymann H. Sensory Evaluation of Food. Heldman DR, editor. New York: Chapman & Hall; 1998.
135. University of Giessen. BMBF Verbundprojekt Anthocyane in Fruchtsäften aus Beerenobst. 2011 [updated 2011; cited 2012 Feb]; Available from: http://www.uni-giessen.de/cms/fbz/afb.
136. University of Giessen. Organigramm. 2011 [updated 2011; cited 2012 Feb]; Available from: http://www.uni-giessen.de/cms/fbz/afb/organigramm.
137. Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U, et al. The DONALD Study. History, current status and future perspectives. Eur J Nutr. 2004;43(1):45-54.
138. Kersting M, Sichert-Hellert W, Lausen B, Alexy U, Manz F, Schöch G. Energy intake of 1 to 18 year old German children and adolescents. Z Ernährungswiss. 1998;37(1):47-55.
139. Sichert-Hellert W, Kersting M, Chahda C, Schäfer R, Kroke A. German food composition database for dietary evaluations in children and adolescents. J Food Compost Anal. 2007;20:63-70.
140. Singer JD. Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. J Educ Behav Stat. 1998;24(4):323-55.

141. SAS/STAT(R) 9.2 User's Guide. Second Edition. Examples: PRINQUAL Procedure. [cited 2011 Apr]; Available from: http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_prinqual_sect028.htm.

142. Harnly JM, Doherty RF, Beecher GR, Holden JM, Haytowitz DB, Bhagwat S, et al. Flavonoid content of U.S. fruits, vegetables, and nuts. J Agric Food Chem. 2006;54(26):9966-77.

143. Lätti AK, Jaakola L, Riihinen KR, Kainulainen PS. Anthocyanin and flavonol variation in bog bilberries (Vaccinium uliginosum L.) in Finland. J Agric Food Chem. 2010;58(1):427-33.

144. Buyken AE, Alexy U, Kersting M, Remer T. Die DONALD Kohorte. Ein aktueller Überblick zu 25 Jahren Forschung im Rahmen der Dortmund Nutritional and Anthropometric Longitudinally Designed Study. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz. 2012;55(6/7):875-84.

145. Kersting M, Alexy U. Die DONALD Studie. Ernährungsumschau. 2008;55(1):16-9.

146. Fröhling B. Getränkeforschung: Functional Food Fruchtsaft - Entwicklung von Produkten mit Zusatznutzen im Rahmen der ANTHONIA-Studie. Wissenschaftsmagazin der Forschungsanstalt Geisenheim. 2011;2(1):16-9.

147. Cassidy A, Kay C, Rimm EB. Reply to C Drossard et al. Am J Clin Nutr. 2011;93(4):866-7.

148. Tooze JA, Kipnis V, Buckman DW, Carroll RJ, Freedman LS, Guenther PM, et al. A mixed-effects model approach for estimating the distribution of usual intake of nutrients: the NCI method. Stat Med. 2010;29(27):2857-68.

149. Somerset S. Weaknesses in using quasi-quantitative food-frequency questionnaire data to link habitual intake of flavonoids to hypertension incidence. Am J Clin Nutr. 2011;93(5):1153-4; author reply 4.

150. Magarey A, Daniels LA, Smith A. Fruit and vegetable intakes of Australians aged 2-18 years: an evaluation of the 1995 National Nutrition Survey data. Aust N Z J Public Health. 2001;25(2):155-61.

151. 5 am Tag e.V. 5 am Tag – Obst und Gemüse. 2011 [updated 2011; cited 2012 Jan]; Available from: http://www.5amtag.org/fileadmin/user_upload/Vereinsseite/Mitgliedschaft/1_Kompaktinfos.pdf.

152. Pérez-Jiménez J, Hubert J, Hooper L, Cassidy A, Manach C, Williamson G, et al. Urinary metabolites as biomarkers of polyphenol intake in humans: a systematic review. Am J Clin Nutr. 2010;92(4):801-9.

153. Zamora-Ros R, Rabassa M, Llorach R, González CA, Andres-Lacueva C. Application of Dietary Phenolic Biomarkers in Epidemiology: Past, Present, and Future. J Agric Food Chem. Epub 2012 Feb 22.

154. Liem DG, Westerbeek A, Wolterink S, Kok FJ, de Graaf C. Sour taste preferences of children relate to preference for novel and intense stimuli. Chem Senses. 2004;29(8):713-20.

155. Liem DG, Bogers RP, Dagnelie PC, de Graaf C. Fruit consumption of boys (8--11 years) is related to preferences for sour taste. Appetite. 2006;46(1):93-6.

156. Sabbe S, Verbeke W, Deliza R, Matta VM, Van Damme P. Consumer liking of fruit juices with different acai (Euterpe oleracea Mart.) concentrations. J Food Sci. 2009;74(5):S171-6.

157. Patterson NJ, Beeren CJM. Case studies of consumer testing of food products using children. In: Kilcast D, Angus F, editors. Developing children's food products. Cambridge: Woodhead Publishing; 2011. p. 188-203.

158. Oberender PO, Hebborn A, Zerth J. Wachstumsmarkt Gesundheit. 2. ed. Stuttgart: Lucius & Lucius; 2006.

159. Mozaffarian D, Ludwig DS. Dietary guidelines in the 21st century--a time for food. JAMA. 2010;304(6):681-2.

160. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC; 2007

161. Drossard C, Fröhling B, Bolzenius K, Dietrich H, Kunz C, Kersting M. Liking of anthocyanin-rich juices by children and adolescents. Appetite. 2012;58(2):623-8.

162. Gill CI, McDougall GJ, Glidewell S, Stewart D, Shen Q, Tuohy K, et al. Profiling of phenols in human fecal water after raspberry supplementation. J Agric Food Chem. 2010;58(19):10389-95.

163. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr. 2006;84(2):274-88.

164. Libuda L, Alexy U, Sichert-Hellert W, Stehle P, Karaolis-Danckert N, Buyken AE, et al. Pattern of beverage consumption and long-term association with body-weight status in German adolescents--results from the DONALD study. Br J Nutr. 2008;99(6):1370-9.

165. O'Neil CE, Nicklas TA. A Review of the Relationship Between 100% Fruit Juice Consumption and Weight in Children and Adolescents. Am J Lifestyle Med. 2008;2(4):315-54.

166. Nicklas TA, O'Neil CE, Kleinman R. Association between 100% juice consumption and nutrient intake and weight of children aged 2 to 11 years. Arch Pediatr Adolesc Med. 2008;162(6):557-65.

167. O'Neil CE, Nicklas TA, Kleinman R. Relationship between 100% juice consumption and nutrient intake and weight of adolescents. Am J Health Promot. 2010;24(4):231-7.

168. O'Neil CE, Nicklas TA, Zanovec M, Fulgoni VL, 3rd. Diet quality is positively associated with 100% fruit juice consumption in children and adults in the United States: NHANES 2003-2006. Nutr J. 2011;10:17.

169. O'Neil CE, Nicklas TA, Zanovec M, Kleinman RE, Fulgoni VL. Fruit juice consumption is associated with improved nutrient adequacy in children and adolescents: the National Health and Nutrition Examination Survey (NHANES) 2003-2006. Public Health Nutr. 2012:1-8.

170. Souci SW, Fachmann W, Kraut H. Food Composition and Nutrition Tables. 7. revised and completed ed. Stuttgart; Germany: Medpharm Scientific Publishers; 2008.

171. Research Institute of Child Nutrition. Empfehlungen für die Ernährung von Kindern und Jugendlichen Die Optimierte Mischkost optimiX. 2010.

172. Gidding SS, Dennison BA, Birch LL, Daniels SR, Gillman MW, Lichtenstein AH, et al. Dietary recommendations for children and adolescents: a guide for practitioners: consensus statement from the American Heart Association. Circulation. 2005;112(13):2061-75.

173. Ruxton CHS. Smoothies: one portion or two? British Nutrition Foundation Nutrition Bulletin. 2008;33:129-32.

174. Hagl S, Deusser H, Soyalan B, Janzowski C, Will F, Dietrich H, et al. Colonic availability of polyphenols and D-(-)-quinic acid after apple smoothie consumption. Mol Nutr Food Res. 2011;55(3):368-77.

175. Brug J, Tak NI, te Velde SJ, Bere E, de Bourdeaudhuij I. Taste preferences, liking and other factors related to fruit and vegetable intakes among schoolchildren: results from observational studies. Br J Nutr. 2008;99 Suppl 1:S7-S14.

176. Cullen KW, Baranowski T, Owens E, Marsh T, Rittenberry L, de Moor C. Availability, accessibility, and preferences for fruit, 100% fruit juice, and vegetables influence children's dietary behavior. Health Educ Behav. 2003;30(5):615-26.

177. Rasmussen M, Krølner R, Klepp KI, Lytle L, Brug J, Bere E, et al. Determinants of fruit and vegetable consumption among children and adolescents: a review of the literature. Part I: Quantitative studies. Int J Behav Nutr Phys Act. 2006;3:22.

178. de Sa J, Lock K. Will European agricultural policy for school fruit and vegetables improve public health? A review of school fruit and vegetable programmes. Eur J Public Health. 2008;18(6):558-68.

179. Pudel V. Verhältnisprävention muss Verhaltensprävention ergänzen. Ernährungsumschau. 2003;53(3):95-8.

180. Muckelbauer R, Libuda L, Clausen K, Reinehr T, Kersting M. A simple dietary intervention in the school setting decreased incidence of overweight in children. Obes Facts. 2009;2(5):282-5.

181. Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. Br J Nutr. 2008;99(1):12-22.

5. APPENDIX

5.1 PUBLICATIONS RELATED TO DROSSARD ET AL., AJCN 2011

Cassidy A, O'Reilly ÉJ, Kay C, Sampson L, Franz M, Forman JP, Curhan G, Rimm EB.

Habitual intake of flavonoid subclasses and incident hypertension in adults.

American Journal of Clinical Nutrition 2011 93(2):338-47.

First published online 2010 Nov 24.

DOI: 10.3945/ajcn.110.006783

Freely accessible at
http://www.ajcn.org/content/93/2/338.long

Cassidy A, Kay C, Rimm EB.

Reply to C Drossard et al.

American Journal of Clinical Nutrition 2011 93(4):866.

First published online 2011 Feb 2.

DOI: 10.3945/ajcn.110.010744

Freely accessible at
http://www.ajcn.org/content/93/4/866.full.pdf+html?sid=3943385f-396b-4dde-b9fa-68635046ace1

5.2 PUBLICATIONS RELATED TO DROSSARD ET AL., BJN 2012

Zamora-Ros R, Knaze V, Luján-Barroso L, Slimani N, Romieu I, Touillaud M, Kaaks R, Teucher B, Mattiello A, Grioni S, Crowe F, Boeing H, Förster J, Quirós JR, Molina E, Huerta JM, Engeset D, Skeie G, Trichopoulou A, Dilis V, Tsiotas K, Peeters PH, Khaw KT, Wareham N, Bueno-de-Mesquita B, Ocké MC, Olsen A, Tjønneland A, Tumino R, Johansson G, Johansson I, Ardanaz E, Sacerdote C, Sonestedt E, Ericson U, Clavel-Chapelon F, Boutron-Ruault MC, Fagherazzi G, Salvini S, Amiano P, Riboli E, González CA.
Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study.
British Journal of Nutrition (2011) 106(7):1090–1099.
First published online 2011 Apr 12.
DOI: 10.1017/S0007114511001437

Zamora-Ros R, Knaze V, González CA.
Response: Banana is not a food source of delphini(di)ns in the EPIC study.
British Journal of Nutrition (2012) 107(5):767.
First published online 2011 Nov 7.
DOI: 10.1017/S0007114511004442

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British Journal of Nutrition (2011), **106**, 1090–1099
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British Journal of Nutrition

Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study

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Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; FCDB, food composition database; 24-HDR, 24 h dietary recall.

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Abstract

Anthocyanidins are bioactive flavonoids with potential health-promoting effects. These may vary among single anthocyanidins considering differences in their bioavailability and some of the mechanisms involved. The aim of the present study was to estimate the dietary intake of anthocyanidins, their food sources and the lifestyle factors (sex, age, BMI, smoking status, educational level and physisical activity) involved among twenty-seven centres in ten European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Anthocyanidin intake and their food sources for 36 037 subjects, aged between 35 and 74 years, in twenty-seven redefined centres were obtained using standardised 24 h dietary recall software (EPIC-SOFT). An *ad hoc* food composition database on anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin) was compiled using data from the US Department of Agriculture and Phenol-Explorer databases and was expanded by adding recipes, estimated values and cooking factors. For men, the total anthocyanidin mean intake ranged from 19·83 (SE 1·53) mg/d (Bilthoven, The Netherlands) to 64·88 (SE 1·86) mg/d (Turin, Italy), whereas for women the range was 18·73 (SE 2·80) mg/d (Granada, Spain) to 44·08 (SE 2·45) mg/d (Turin, Italy). A clear south to north gradient intake was observed. Cyanidins and malvidins were the main anthocynidin contributors depending on the region and sex. Anthocyanidin intake was higher in non-obese older females, non-smokers, and increased with educational level and physical activity. The major food sources were fruits, wine, non-alcoholic beverages and some vegetables. The present study shows differences in both total and individual anthocyanidin intakes and various lifestyle factors throughout Europe, with some geographical variability in their food sources.

Key words: Anthocyanidins: Intake: Food sources: EPIC-Europe

Anthocyanidins are water-soluble plant pigments that form one subgroup of flavonoids. They mainly provide the red, blue and purple colours to fruits, vegetables and flowers. Chemically, they are derivative salts of the flavilium cation. Anthocyanins are glycosides of anthocyanidins, and their sugar moiety (glucose, galactose, rhamnose, xylose and fructose) is mostly bounded to the C3 position of the C-ring[1]. Diglycosides have also been reported, but in smaller amounts[1].

In nature, more than 500 anthocyanins derived from thirty-one anthocyanidins have been identified[2]. However, only six anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin) occur ubiquitously and have dietary importance. They are found in fruits, such as berries, red grapes, cherries, and plums; in vegetables, such as red cabbage, red onions, radish and aubergines; and also in fruit and vegetable products, such as juices and wines[3,4]. The anthocyanidin content is enhanced during the ripening process. Moreover, these flavonoids are found mainly in the skin of fruit, except in berries where they are in the skin and flesh[5].

Some epidemiological studies suggest that the consumption of anthocyanidins decreases the risk of total mortality[6] and CVD[7,8] due, in part, to their antioxidant and anti-inflammatory activities[9]. There is also much *in vitro* and *in vivo* evidence in animal models about their anti-carcinogenic properties[9,10], but findings in human subjects are still controversial. Anthocyanidin intake has been associated with a decreased risk of some cancers, especially digestive system cancers[11–15], but, in other epidemiological studies, these significant associations were not observed[6,16–25].

All anthocyanidins are poorly absorbed (usually less than 0·1 %, but up to 5 % has been reported), highly metabolised (more than 65 % is detected in glucuronidated and methylated forms in serum) and rapidly excreted in urine (about 4 h elimination half-life)[9]. Differences in the chemical structure of some anthocyanidins also determine their bioavailability; for example, pelargonidin-3-glucoside has an 8-fold higher apparent absorption rate than cyanidin-3-glucoside[26]. In the same way, several activities of anthocyanidins depend on their chemical structure[9]. For example, delphynidins and cyanidins are able to inhibit lipopolysaccharide-induced cyclo-oxygenase-2 expression, but pelargonidins, peonidins and malvidins are not[27]. For these reasons, further studies are needed, comparing individual anthocyanidin bioavailability and metabolic actions.

To date, there are few population-based descriptive studies of anthocyanidin intake[28,29], especially in European countries[30,31]. The previous studies mainly reported associations between anthocyanidins and markers of disease risk. In general, these studies evaluated anthocyanidins as a group rather than exploring individual anthocyanidins; furthermore, main food sources were not reported. The aims of the present study were to estimate the consumption of the six most important anthocyanidins and their main food sources across the ten European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study and across population subgroups.

Materials and methods

Study population

EPIC is an ongoing prospective cohort study designed to investigate the associations between diet, lifestyle and cancer throughout ten western European countries: Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, The Netherlands and the UK[32,33]. The cohort includes approximately 366 000 women and 153 000 men, most aged 35–70 years, who were enrolled between 1992 and 2000. Participants were mostly recruited from the general population residing within defined geographical areas, with some exception: women members of a health insurance scheme for state school employees (France); women attending breast cancer screening (Utrecht in The Netherlands and Florence in Italy); mainly blood donors (centres in Italy and Spain); and a cohort consisting predominantly of vegetarians (the 'health-conscious' cohort in Oxford, UK)[33]. The initial twenty-three EPIC administrative centres were redefined into twenty-seven geographical regions relevant to the analysis of dietary consumption patterns[34]. Of the twenty-seven EPIC centres redefined for dietary analysis, nineteen had both male and female participants, and eight recruited only women (France, Norway, Utrecht in The Netherlands and Naples in Italy).

For calibration purposes, a standardised 24 h dietary recall (24-HDR) interview was administered to a stratified random sample (36 994) by age, sex and centre, and weighted for expected cancer cases in each stratum. A total of 36 037 subjects with 24-HDR data were included in this analysis, after exclusion of 941 subjects aged less than 35 years of age or over 74 years because of low participation in these age categories, and sixteen subjects were excluded due to missing FFQ data. Approval for the EPIC study was obtained from all ethical review boards of participating institutions. All participants provided written informed consent.

Dietary and lifestyle information

The 24-HDR was administered in a face-to-face interview, except in Norway, where it was obtained by telephone interview[35]. A computerised interview program (EPIC-SOFT) was developed specifically for the calibration study[36,37]. A complete description of the rationale, methodology and population characteristics of the 24-HDR calibration study has been described elsewhere[34]. The original diet and health survey from which information used in the present study was obtained had ethical approval from all ethical review boards of participating institutions.

Data on other lifestyle factors, including educational level, anthropometry, physical activity and smoking history, were collected at baseline through standardised questionnaires and clinical examinations, and have been described elsewhere[33,34,38]. Data on age, as well as on body weight and height, were self-reported by the participants during the 24-HDR interview. The mean time interval between completion of the baseline questionnaire measures and the 24-HDR interview varied by country, and ranged from 1 d to 3 years later[34].

Food composition database

In order to estimate the anthocyanidin (cyanidin, delphynidin, malvidin, pelargonidin, peonidin and petunidin) intake from the 24-HDR, a food composition database (FCDB) was developed, which contained 1877 food items (annex table 1; see supplementary material available online at http://www.journals.cambridge.org/bjn). Anthocyanidins are expressed as anthocyanidin aglycones per 100 mg fresh weight and are calculated as the sum of the available forms (glycosides and aglycones) in the literature.

Our database is based on the US Department of Agriculture (USDA) database[3] and expanded with values from Phenol-Explorer[39]. Approximately, 5 and 1 % of our database come from USDA and Phenol-Explorer databases, respectively. To date, these two databases are the most complete and updated databases on flavonoids and polyphenols and they evaluate and compile the most worldwide food composition data published. There are no large differences on the anthocyanidin data between the two databases.

One cannot assume that foods that are not in either of the databases do not contain anthocyanidins. Therefore, for our FCDB we calculated estimated values (89 %) including logical zeros (26 %), estimations based on similar food items (15 %), application of retention factors (29 %) and recipes (19 %). First, logical zeros were applied when no anthocyanidins are expected in a food (for example, animal foods or plant foods without colour, because anthocyanidins are plant pigments). Second, estimations based on similar food items were applied when it was possible to extrapolate the composition from one food to another similar one (for example, different varieties of blueberries). Third, when there was no analytical data for cooked food, retention factors were applied. These were 70, 35 and 25 % after frying, cooking in a microwave oven, and boiling, respectively[40]. Crozier *et al.*[40] calculated these retention factors for flavonols, but these are quite similar to the average of anthocyanidin retention factors available in the literature by each cooking method[41–45], although further investigation is needed in this regard. Recipes were applied when it was feasible to deconstruct the food item into a list of available ingredients in our FCDB. Finally, only 4 % of our FCDB had missing values, which are calculated as a zero by default.

Statistical analyses

Dietary intake data are presented as means (least square means) and standard errors stratified by sex and study centre and ordered according to a geographical south to north gradient. The mean intake data were adjusted for age. The contribution of each food group to the total intake of anthocyanidins was calculated as a percentage. Differences in anthocyanidin intake stratified by sex were also compared according to the categories of age, educational level, smoking status, level of physical activity, BMI and European region (south: all centres in Greece, Spain, Italy and the south of France centre; central: all of France other than the south centre, all centres in Germany, The Netherlands and the UK; north: all centres in Denmark, Sweden and Norway). These models were adjusted for age, region, BMI and energy intake. All models were weighted by season and day of the week of the 24-HDR using generalised linear models to

Table 1. Adjusted* daily intakes (mg/d) of total and single anthocyanidins by sex and centre ordered from south to north
(Mean values with their standard errors)

Men

Country and centre	n	Anthocyanidins (mg/d) Mean	SE	Cyanidin (mg/d) Mean	SE	Delphinidin (mg/d) Mean	SE	Malvidin (mg/d) Mean	SE	Pelargonidin (mg/d) Mean	SE	Peonidin (mg/d) Mean	SE	Petunidin (mg/d) Mean	SE
Greece	1314	37·87	1·34	14·54	0·79	2·21	0·32	17·30	0·63	0·59	0·31	2·06	0·13	1·15	0·08
Spain															
Granada	214	38·50	3·31	13·92	1·95	2·38	0·79	17·21	1·55	1·08	0·76	2·20	0·31	1·71	0·20
Murcia	243	36·46	3·11	16·73	1·83	2·09	0·74	13·60	1·46	0·91	0·71	1·64	0·29	1·48	0·18
Navarra	444	39·45	2·30	10·39	13·52	2·94	0·55	20·20	1·08	1·00	0·53	2·50	0·21	2·42	0·14
San Sebastian	490	47·49	2·19	16·00	1·29	3·25	0·52	20·93	1·03	2·26	0·50	2·47	0·20	2·58	0·13
Asturias	386	39·55	2·47	14·76	1·45	2·27	0·59	15·90	1·16	2·59	0·56	2·08	0·23	1·96	0·15
Italy															
Ragusa	168	44·39	3·74	19·53	2·20	3·05	0·89	16·85	1·75	0·97	0·85	2·38	0·35	1·60	0·22
Naples															
Florence	271	44·46	2·94	19·54	1·73	2·71	0·70	16·05	1·38	2·40	0·67	2·04	0·27	1·72	0·17
Turin	676	64·88	1·86	25·44	1·10	4·05	0·44	26·42	0·87	2·70	0·43	3·61	0·17	2·67	0·11
Varese	327	55·48	2·68	23·12	1·58	4·16	0·64	21·91	1·26	1·07	0·61	2·79	0·25	2·43	0·16
France															
South coast															
South															
North-east															
North-west															
Germany															
Heidelberg	1034	29·79	1·51	12·31	0·89	2·31	0·36	9·78	0·71	2·82	0·34	1·33	0·14	1·24	0·09
Potsdam	1233	33·74	1·38	16·86	0·81	2·54	0·33	7·38	0·65	4·85	0·32	1·25	0·13	0·87	0·08
The Netherlands															
Bilthoven	1024	19·83	1·53	9·24	0·90	2·53	0·36	4·37	0·72	2·25	0·35	0·74	0·14	0·69	0·09
Utrecht															
UK															
General population	403	21·79	2·42	7·83	1·42	1·81	0·58	6·33	1·13	2·15	0·55	2·83	0·22	0·85	0·14
Health-conscious	113	27·82	4·56	14·60	2·68	2·41	1·09	5·01	2·14	4·20	1·04	0·83	0·42	0·76	0·27
Denmark															
Copenhagen	1356	31·60	1·32	10·16	0·77	3·35	0·31	13·28	0·62	1·29	0·30	1·68	0·12	1·82	0·08
Aarhus	567	28·02	2·04	11·67	1·20	2·18	0·48	9·83	0·95	1·62	0·46	1·33	0·19	1·40	0·12
Sweden															
Malmö	1421	20·22	1·32	6·39	0·77	4·00	0·31	5·92	0·62	1·60	0·30	0·94	0·12	1·37	0·08
Umeå	1344	21·24	1·32	7·27	0·78	5·41	0·32	4·72	0·62	1·34	0·30	0·92	0·12	1·58	0·08
Norway															
South and east															
North and west															

Women

Country and centre	n	Anthocyanidins (mg/d) Mean	SE	Cyanidin (mg/d) Mean	SE	Delphinidin (mg/d) Mean	SE	Malvidin (mg/d) Mean	SE	Pelargonidin (mg/d) Mean	SE	Peonidin (mg/d) Mean	SE	Petunidin (mg/d) Mean	SE
Greece	1373	25·77	1·31	12·42	0·77	1·28	0·31	9·53	0·61	0·65	0·30	1·31	0·12	0·57	0·08
Spain															
Granada	300	18·73	2·80	11·66	1·65	0·85	0·67	3·87	1·31	1·28	0·64	0·76	0·26	0·31	0·17
Murcia	304	21·40	2·79	11·17	1·64	1·20	0·66	5·59	1·30	2·33	0·64	0·69	0·26	0·43	0·17
Navarra	271	22·76	2·94	9·73	1·73	0·83	0·70	8·78	1·38	1·75	0·67	1·06	0·27	0·60	0·17
San Sebastian	244	26·29	3·11	13·20	1·83	0·93	0·74	7·18	1·46	3·54	0·70	0·80	0·29	0·65	0·18
Asturias	324	25·16	2·69	15·41	1·58	0·77	0·64	5·03	1·26	2·52	0·62	0·94	0·25	0·49	0·16
Italy															
Ragusa	138	33·93	4·13	21·68	2·43	1·98	0·98	7·56	1·94	0·84	0·94	1·27	0·38	0·59	0·25
Naples	403	27·55	2·41	15·62	1·42	1·55	0·58	6·04	1·13	2·38	0·55	1·40	0·22	0·57	0·14
Florence	784	30·29	1·73	14·03	1·02	1·47	0·41	9·56	0·81	3·03	0·40	1·41	0·16	0·80	0·10
Turin	392	44·08	2·45	23·06	1·44	2·29	0·58	11·44	1·15	4·08	0·56	2·11	0·23	1·11	0·15
Varese	794	40·04	1·72	21·16	1·01	2·09	0·41	12·33	0·81	1·97	0·39	1·60	0·16	0·89	0·10
France															
South coast	620	38·42	1·95	15·86	1·15	2·29	0·46	12·55	0·91	4·59	0·45	1·98	0·18	1·15	0·12
South	1425	40·78	1·28	18·56	0·76	1·99	0·31	11·40	0·60	5·80	0·29	1·98	0·12	1·04	0·08
North-east	2059	38·11	1·07	17·60	0·63	2·36	0·26	9·77	0·50	5·58	0·24	1·80	0·10	1·01	0·06
North-west	631	32·37	1·93	15·39	1·14	1·57	0·46	7·28	0·90	5·91	0·44	1·39	0·18	0·82	0·11
Germany															
Heidelberg	1087	36·02	1·48	16·29	0·87	3·31	0·35	8·66	0·70	5·33	0·34	1·29	0·14	1·14	0·09
Potsdam	1061	40·80	1·49	20·61	0·88	3·41	0·36	9·02	0·70	5·06	0·34	1·61	0·14	1·08	0·09
The Netherlands															
Bilthoven	1086	23·27	1·49	12·37	0·88	3·32	0·36	3·86	0·70	2·35	0·34	0·69	0·14	0·68	0·09
Utrecht	1870	24·59	1·13	12·03	0·66	2·63	0·27	4·70	0·53	3·54	0·26	0·94	0·10	0·74	0·07
UK															
General population	571	24·07	2·03	8·31	1·19	1·39	0·48	7·67	0·95	2·76	0·46	3·18	0·19	0·75	0·12
Health-conscious	196	30·78	3·46	13·68	2·04	2·26	0·82	8·42	1·62	2·70	0·79	2·82	0·32	0·89	0·21
Denmark															
Copenhagen	1484	26·97	1·26	10·20	0·74	2·56	0·30	10·00	0·59	1·58	0·29	1·30	0·12	1·33	0·08
Aarhus	510	26·23	2·15	12·38	1·26	1·80	0·51	8·52	1·01	1·30	0·49	1·10	0·20	1·12	0·13
Sweden															
Malmö	1711	20·13	1·19	6·51	0·70	3·52	0·28	6·23	0·56	1·73	0·27	0·93	0·11	1·22	0·07
Umeå	1574	22·26	1·22	8·55	0·72	5·77	0·29	4·40	0·57	1·37	0·28	0·82	0·11	1·36	0·07
Norway															
South and east	1004	27·81	1·55	9·46	0·91	4·16	0·37	9·20	0·72	2·22	0·35	1·25	0·14	1·53	0·09
North and west	793	25·31	1·74	9·32	1·02	3·98	0·41	7·78	0·81	2·00	0·40	0·98	0·16	1·25	0·10

* Adjusted for age and weighted by season and day of recall.

control for different distributions of 24-HDR interviews across seasons and days of the week. All analyses were conducted using SPSS Statistics software (version 17.0; SPSS Inc., Chicago, IL, USA).

Results

The mean intakes and for single and total anthocyanidins stratified by centre and sex, adjusted for age, and weighted by season and day of the week are shown in Table 1. For men, the total anthocyanidin intake ranged from 19·83 mg/d (Bilthoven, The Netherlands) to 64·88 mg/d (Turin, Italy), whereas for women the range was from 18·73 mg/d (Granada, Spain) to 44·08 mg/d (Turin, Italy). The main anthocyanidin contributors (Table 2) were malvidin (42·7 % in men and 29·4 % in women) and cyanidin (38·0 % in men and 49·9 % in women) in the southern region, cyanidin (45·6 % in men and 46·8 % in women) in the central region, and cyanidin (34·0 % in men and 36·8 % in women) and malvidin (33·0 % in men and 30·5 % in women) in the northern European region.

Table 3 shows the assessment of the effect of certain lifestyle factors on anthocyanidin intake adjusted for sex, age, BMI and energy intake (where appropriate) and weighted by season and day of the week. In south European countries, men consumed more anthocyanidins than women of these countries, whereas in north European countries, they consumed similar amounts, and in central European countries women ingested greater quantities than men. The difference in intake between the sexes in south European countries was due to malvidin intake which in men was two-fold that of women. A geographical gradient of increasing total anthocyanidin, cyanidin, malvidin and peonidin intakes from north to south Europe was observed. However, there was an inverse regional gradient for delphynidin intake. Older individuals consumed more anthocyanidins, with a maximum intake in those aged 55–64 years. There were positive trends when assessing total anthocyanidin intakes and educational level, smoking status (comparing current *v.* never or former smokers), BMI (obese *v.* normal or overweight) and physical activity.

Table 2. Percentage contribution* of intakes of individual anthocyanidins in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort by European region and sex

		Region		
Anthocyanidin	Sex	South	Central	North
Cyanidin	Men	38·0	45·6	34·0
	Women	49·9	46·8	36·8
Delphinidin	Men	6·4	8·7	16·2
	Women	4·9	8·2	15·9
Malvidin	Men	42·7	25·6	33·0
	Women	29·4	23·8	30·5
Pelargonidin	Men	3·3	12·0	5·7
	Women	8·9	13·7	7·0
Peonidin	Men	5·4	4·7	4·8
	Women	4·6	4·7	4·3
Petunidin	Men	4·2	3·3	6·3
	Women	2·3	2·8	5·4

* Adjusted for age and weighted by season and day of recall.

The main food sources of anthocyanidin intake by European region were also studied (Table 4). The group of fruits, nuts and seeds (mainly non-citrus fruit such as grapes, apples and pears) contributed most of the total anthocyanidin intake. In south, central and north European countries this food group contributed 61·2, 52·9 and 38·1 %, respectively. Other major food sources were wine (contributions ranged from 14·4 to 24·5 %), followed by non-alcoholic beverages, such as carbonated, soft and isotonic drinks in northern European countries (15·8 %) and fruit and vegetable juices in central European countries (13·4 %), and some types of vegetables (ranging from 4·8 to 9·7 %). The major food sources of cyanidins were fruits and non-alcoholic beverages derived from either fruits and vegetables or carbonated, soft and isotonic drinks. For delphynidins, the main contributors in southern countries were wine, bananas, grapes and fruiting vegetables, mainly aubergine. However, in central and northern countries the richest sources were banana, non-alcoholic beverages, berries and wine. Malvidins were almost exclusively derived from grape and wine products. The main contributors to pelargonidins were berries, followed by root vegetables and dairy products with berries as ingredients. We identified fruits, wine and non-alcoholic beverages (only in the north and central European countries) as the most abundant sources of peonidins and petunidins.

Discussion

To our knowledge, this is the first study to estimate the intake of anthocyanidins and their main food sources in a large adult European cohort, evaluating differences across ten European countries and the most important determinant factors. The use of a unique FCDB on anthocyanidins and the same methodology in the dietary assessment for the whole cohort provided more comparable results across the countries. The FCDB was compiled at the end of 2009 using the most updated and available worldwide databases[3,39] on flavonoids and polyphenols. Furthermore, our database was expanded with recipes, estimations by food or food group and the application of cooking factors[40]. However, the use of different FCDB and different food surveys limits the comparisons between studies.

In men, there were great differences in anthocyanidin intakes across EPIC centres, ranging from 19·83 mg/d in Bilthoven to 64·88 mg/d in Turin. Indeed, the south European region had the highest consumption of total anthocyanidins, and the two main individual anthocyanidins (cyanidins and malvidins). Moreover, regional trends of increasing anthocyanidin, cyanidin, malvidin and peonidin intakes from northern to southern countries were also observed. Meanwhile, women from central and southern regions were the highest anthocyanidin consumers. Individuals aged 55–64 years, who had a university degree, non-smokers (former or never smokers), those doing moderate or active physical activity and those that were overweight (BMI 25 to $< 30\,kg/m^2$) had the highest anthocyanidin consumption. Part of these differences was due to the differences in the consumption pattern of the major food sources in the European countries. For example, in

Table 3. Adjusted* daily intakes (mg/d) of total and single anthocyanidins by sex and selected characteristics
(Mean values with their standard errors)

		Anthocyanidins (mg/d)			Cyanidin (mg/d)			Delphinidin (mg/d)			Malvidin (mg/d)			Pelargonidin (mg/d)			Peonidin (mg/d)			Petunidin (mg/d)		
Stratification variable	*n*	Mean	SE	*P*	Mean	SE	*P*	Mean	SE	*P*	Mean	SE	*P*	Mean	SE	*P*	Mean	SE	*P*	Mean	SE	*P*
Sex				< 0·001			< 0·001			0·004			0·274			< 0·001			0·017			0·016
Male	13028	29·44	0·53		12·01	0·31		2·26	0·13		10·27	0·25		2·19	0·12		1·49	0·05		1·23	0·03	
Female	23009	33·52	0·39		15·09	0·23		2·71	0·09		9·94	0·18		3·02	0·09		1·64	0·04		1·13	0·02	
European region				< 0·001			< 0·001			< 0·001			< 0·001			< 0·001			< 0·001			< 0·001
South	11285	37·42	0·46		16·35	0·27		2·11	0·11		13·57	0·22		2·27	0·11		1·87	0·04		1·25	0·03	
Central	12988	29·79	0·44		13·64	0·26		2·48	0·11		7·52	0·21		3·81	0·10		1·41	0·04		0·93	0·03	
North	11764	23·45	0·45		8·21	0·27		3·83	0·11		7·55	0·21		1·40	0·10		1·05	0·04		1·40	0·03	
Age (non-adjusted for age) (years)				< 0·001			< 0·001			< 0·001			< 0·001			< 0·001			0·049			< 0·001
35–44	3335	26·43	0·89		11·75	0·53		1·99	0·21		8·23	0·42		2·08	0·20		1·40	0·08		1·00	0·05	
45–54	12595	29·67	0·48		12·86	0·28		2·14	0·12		9·71	0·22		2·32	0·11		1·52	0·04		1·13	0·03	
55–64	14940	33·44	0·45		14·20	0·27		2·76	0·11		10·74	0·21		2·88	0·11		1·62	0·04		1·33	0·03	
54–74	5167	33·34	0·77		14·41	0·46		2·85	0·18		10·34	0·36		2·85	0·18		1·63	0·07		1·27	0·05	
BMI (kg/m^2)				< 0·001			0·030			0·261			0·002			0·039			0·004			0·009
< 25	16854	31·74	0·46		13·80	0·27		2·55	0·11		9·95	0·22		2·69	0·11		1·53	0·04		1·21	0·03	
25 to < 30	13766	32·04	0·46		13·57	0·27		2·49	0·11		10·52	0·22		2·63	0·11		1·65	0·04		1·18	0·03	
≥ 30	5417	28·82	0·70		12·57	0·41		2·24	0·17		9·29	0·33		2·24	0·16		1·42	0·07		1·07	0·04	
Level of schooling				< 0·001			< 0·001			0·010			< 0·001			0·027			< 0·001			< 0·001
None	1709	23·91	1·37		10·00	0·81		2·09	0·33		6·96	0·65		2·67	0·32		1·15	0·13		1·04	0·08	
Primary completed	10469	27·83	0·54		12·63	0·32		2·21	0·13		8·22	0·26		2·44	0·13		1·33	0·05		1·00	0·03	
Technical/professional	8038	32·24	0·63		13·94	0·37		2·45	0·15		10·54	0·30		2·45	0·15		1·66	0·06		1·20	0·04	
Secondary school	7152	33·99	0·63		14·30	0·38		2·70	0·15		11·42	0·30		2·58	0·15		1·72	0·06		1·26	0·04	
University degree	8155	36·10	0·60		14·89	0·35		2·82	0·14		12·20	0·28		2·95	0·14		1·85	0·06		1·39	0·04	
Smoking status				0·020			< 0·001			0·336			< 0·001			0·181			0·369			< 0·001
Never smoker	17483	31·37	0·44		14·05	0·26		2·39	0·11		9·57	0·21		2·73	0·10		1·53	0·04		1·10	0·03	
Former smoker	10288	32·44	0·52		13·62	0·31		2·61	0·13		10·81	0·24		2·52	0·12		1·60	0·05		1·28	0·03	
Current smoker	7726	30·42	0·59		12·47	0·35		2·50	0·14		10·17	0·28		2·48	0·14		1·60	0·06		1·19	0·04	
Physical activity				< 0·001			0·002			0·450			< 0·001			0·041			0·066			0·039
Inactive	7463	29·88	0·61		13·33	0·37		2·28	0·15		9·05	0·29		2·58	0·14		1·54	0·06		1·09	0·04	
Moderately inactive	11969	32·05	0·50		13·78	0·30		2·37	0·12		10·37	0·23		2·81	0·11		1·57	0·05		1·15	0·03	
Moderately active	8400	33·39	0·59		14·05	0·35		2·33	0·14		11·24	0·27		2·84	0·13		1·72	0·06		1·22	0·03	
Active	6380	33·15	0·67		15·28	0·40		2·59	0·16		10·11	0·32		2·39	0·16		1·60	0·06		1·18	0·04	

* Adjusted for sex, age, region, energy intake, and BMI (where appropriate) and weighted by season and day of recall.

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Table 4. Percentage contribution of food groups and some main foods to the intake of total and single anthocyanidins by European region*

	Anthocyanidins (%)			Cyanidins (%)			Delphinidins (%)			Malvidins (%)			Pelargonidins (%)			Peonidins (%)			Petunidins (%)		
Food groups and foods†	South	Central	North	South	Central	North	South	Central	North	South	Central	North	South	Central	North	South	Central	North	South	Central	North
Potatoes and other tubers	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Vegetables	9·7	8·8	4·8	15·2	11·4	5·5	24·5	8·6	1·0	0·0	0·0	0·0	16·7	19·9	39·4	0·0	0·0	0·1	0·0	0·0	0·0
Leafy vegetables	7·1	3·8	0·4	14·3	7·8	1·0	7·0	2·5	0·1	0·0	0·0	0·0	0·0	0·0	0·1	0·0	0·0	0·0	0·0	0·0	0·0
Fruiting vegetables	0·9	0·4	0·1	0·0	0·0	0·0	16·7	4·9	0·5	0·0	0·0	0·0	0·1	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Root vegetables	1·2	2·8	2·7	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	16·5	19·8	39·2	0·0	0·0	0·0	0·0	0·0	0·0
Cabbages	0·4	1·6	1·5	0·8	3·5	4·2	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Other and mixed vegetables	0·1	0·1	0·1	0·1	0·1	0·2	0·7	1·2	0·4	0·0	0·0	0·0	0·1	0·0	0·1	0·0	0·0	0·1	0·0	0·0	0·0
Legumes	0·1	0·1	0·1	0·1	0·0	0·1	0·1	0·0	0·0	0·0	0·0	0·0	0·8	0·3	0·6	0·0	0·0	0·0	0·2	0·1	0·0
Fruits, nuts and seeds	61·2	52·9	38·1	76·6	56·8	55·5	25·9	33·8	20·2	46·5	47·6	32·5	73·9	66·3	38·4	54·9	44·6	27·7	25·8	33·1	17·5
Citrus fruits	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Apples and pears	14·2	10·0	12·7	30·1	21·3	34·9	0·3	0·1	0·0	0·0	0·0	0·0	0·2	0·1	0·1	0·1	0·3	0·4	0·0	0·0	0·0
Grapes	18·9	13·0	10·6	0·9	0·7	0·7	20·8	9·4	3·7	46·3	44·5	28·0	0·1	0·0	0·1	30·3	21·7	18·5	25·1	18·5	7·7
Stone fruits	14·8	10·0	2·8	29·6	20·0	7·6	0·0	0·0	0·0	0·0	0·0	0·0	1·5	0·6	0·1	16·3	14·1	0·8	0·0	0·0	0·0
Berries	6·3	16·5	10·1	2·6	9·6	8·3	2·2	22·5	15·8	0·1	2·9	4·2	68·1	61·5	34·5	0·4	7·1	6·1	0·6	14·4	9·7
Other and mixed fruits	2·5	2·3	1·1	3·5	3·2	1·4	2·6	1·7	0·5	0·0	0·0	0·0	4·0	4·1	3·7	7·8	1·5	1·8	0·1	0·2	0·1
Olives	4·5	0·8	0·9	9·6	1·7	2·5	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Nuts and seeds	0·2	0·1	0·1	0·3	0·3	0·2	0·0	0·1	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Dairy products	0·5	1·5	0·8	0·1	0·2	0·2	0·1	0·3	0·0	0·0	0·0	0·0	6·5	9·3	10·8	0·0	0·0	0·0	0·0	0·1	0·0
Cereal, cakes and confectionery	1·0	6·5	4·5	1·4	7·4	6·4	1·3	19·7	3·8	0·2	2·4	2·0	1·9	2·8	3·4	1·3	5·1	7·7	0·9	9·0	6·4
Meat, fish and eggs	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Non-alcoholic beverages	1·7	13·6	19·7	3·1	21·7	26·5	0·1	16·7	49·0	0·7	3·3	0·3	0·0	0·8	5·5	0·9	21·9	7·4	0·4	7·9	19·7
Fruit and vegetable juices	1·7	13·4	3·9	3·0	21·5	8·6	0·1	16·4	3·3	0·7	3·2	0·2	0·0	0·8	0·8	0·9	21·7	2·0	0·4	7·7	1·4
Carbonated, soft, and isotonic drinks	0·0	0·2	15·8	0·1	0·2	17·9	0·0	0·3	45·8	0·0	0·1	0·1	0·0	0·0	4·7	0·0	0·2	5·4	0·0	0·2	18·3
Coffee, tea and herbal teas	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Alcoholic beverages	25·5	15·9	25·4	3·4	2·3	4·0	47·6	19·5	15·3	52·2	45·2	55·6	0·0	0·1	0·0	42·6	27·3	46·6	72·1	47·2	39·2
Wine	24·5	14·4	24·5	2·0	1·2	2·6	45·9	17·9	15·0	51·8	42·3	55·7	0·0	0·0	0·0	42·0	25·5	45·7	70·5	44·1	38·5
Beer, cider	0·6	0·3	0·4	1·2	0·7	1·2	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·1	0·0	0·0	0·0	0·0
Liqueurs and spirits	0·4	1·2	0·5	0·2	0·4	0·2	1·7	1·6	0·3	0·5	2·9	1·0	0·0	0·1	0·0	0·6	1·8	0·8	1·6	3·1	0·7
Soups, bouillons	0·0	0·3	5·9	0·0	0·1	1·7	0·0	0·9	10·3	0·0	0·4	7·3	0·0	0·0	0·0	0·0	0·4	9·5	0·0	1·3	16·4
Miscellaneous	0·3	0·5	0·7	0·2	0·1	0·2	0·4	0·5	0·4	0·4	1·0	1·2	0·2	0·6	1·8	0·3	0·6	1·1	0·5	1·1	0·8

*Values are percentages derived from models adjusted for age and sex and weighted by season and day of recall. There were differences between European regions for all food sources ($P < 0·001$), except for food sources where anthocyanidin contributions are less than 0·2 % for all regions (NS differences).

† Leafy vegetables include red leaf lettuce, red chicory, radicchio and trevise (red Treviso lettuce); fruiting vegetables include aubergines; root vegetables include beetroot, red radish and black radish; cabbages include red cabbage and Chinese cabbage; stone fruits include plums, peaches, nectarines, apricots, mangoes and paraguayos; other and mixed fruits include cherries, red fruit not specified, sour cherries, persimmon, sharon fruit and pomegranate; cereal, cakes and confectionery include fruit cakes, biscuits with jam and plum cake; fruit and vegetable juices include blackcurrant juice, cranberry juice, redcurrant juice, cherry juice, peach juice, apricot juice, plum juice and beetroot juice; carbonated, soft and isotonic drinks include blackcurrant syrups, syrups of fruits and berries, cherry coke, pommac and jaffa; soups and bouillons include bilberry soup, berry soup and elderberry soup.

southern countries, a high intake of wine[46], non-citrus fruits (especially grapes, stone fruits, apples and pears, and olives) and leafy vegetables[47] was observed. However, in central and northern countries the main contributors were non-citrus fruits (mainly berries, apples and pears, and grapes), wine and, finally, non-alcoholic beverages (juices and soft drinks of anthocyanidin-rich fruits). The large differences in anthocyanidin intakes between men (45·47 mg/d) and women (31·73 mg/d) in the southern region (Italy, Spain, Greece) were due to the high consumption of red wine, which is very rich in malvidins, as observed in a previous Spanish cohort[30]. The present results are comparable with previously published data of intakes in the southern European region; median intakes of 9·3 to 28·0 mg/d have been reported[7,12–15,17,18,21,24,25,48–50] although a Greek cohort was found to consume 52·6 mg/d[22]. Two previous studies in northern countries (Finland) also reported great differences in mean intakes; 5·9 mg/d in the Kuopio Ischaemic Heart Disease Risk Factor Study[23] and 47 mg/d in the FINDIET 2002 Study[31]. In non-European countries, lower intakes have been observed than in European countries. For example, in the USA mean intakes were found to range from less than 1 to 10·1 mg/d[6,11,16,19,20,29], while in Australia 2·9 mg/d[28], and in Japan 11·3 mg/d[51] were reported.

Cyanidins were the most prevalent anthocyanidins (34–50 %) except in men from the southern European region. Cyanidin intake ranged from 8·2 to 16·4 mg/d; these values are slightly higher than our previous results in Spain (6·2 mg/d)[30] and in Greece (4 mg/d)[50], lower than Finland (25 mg/d)[31] and much higher than in Australia (0·42 mg/d)[28]. In Finland the main contributors were berries and their derived products (88 %)[31], whereas in the present study berries and berry products (juices, soft drinks and soups) represented approximately 6, 31 and 37 % in southern, central and northern countries, respectively. In the present study, leafy vegetables, apples and pears, and stone fruits were also major food sources of cyanidins. Malvidin was the main anthocyanidin in men from the southern European region, which is in line with findings from our previous study in Spain[30] and in Australia[28]. In the entire cohort and in the literature the main contributors were red wine and red grapes. Delphynidin was usually the third most abundant anthocyanidin (5·6 and 16·0 % in southern and northern countries, respectively). Moreover, a geographical trend was observed, with increasing intakes from south (0·8 mg/d women in Navarra, Spain) to north (5·8 mg/d women in Umeå, Sweden), as has previously been observed in Spain (2·5 mg/d)[30] and Finland (14 mg/d)[31]. Pelargonidins (3·3–13·7 %), peonidins (4·3–5·4 %) and petunidins (2·3–6·3 %) were the least abundant anthocyanidins, similar to findings reported in previous papers[28,30,31].

Anthocyanidins have been shown to have protective effects in clinical and epidemiological studies, especially against some chronic diseases. In a US breast cancer case–control study, a reduction of all mortality at 6 years of follow-up after a high intake of anthocyanidins and other flavonoids[6] was reported. Concerning CVD, an Italian case–control study observed a significant inverse trend between acute myocardial infarction and anthocyanidin intake, and an OR of 0·45 (95 % CI 0·26, 0·78) when comparing extreme quintiles[7]. However, in two Greek case–control studies no associations were found between anthocyanidin consumption and peripheral arterial occlusive disease[48] or CHD[49]. Indeed, in a recent meta-analysis, Hooper *et al.* concluded that there were insufficient data from clinical trials to confirm the beneficial effects on CVD[52]. Several epidemiological studies have suggested contradicting results regarding cancer. However, these differences can be explained, in part, by low anthocyanidin bioavailability (less than 5 %)[9] and the wide range of anthocyanidin intakes among studies. Overall, all cancers studied not related to the digestive system (breast, ovarian, prostate, lung, pancreatic, liver, renal cancers, and diffuse and follicular β-cell lymphomas) have not been significantly associated with anthocyanidin intake[6,17–20,22–25,53]. Concerning cancers of the digestive system, when the mean consumption of anthocyanidins is low (< 20 mg/d), non-significant associations have been reported for upper aero-digestive and colorectal cancer, colorectal and oesophageal squamous cell cancer in the Iowa Women's Health Study[19], the Kuopio Ischaemic Heart Disease Risk Factor Study[23] and a US case–control study[11], respectively. However, when their mean intake is high (southern European countries), a protective effect against colorectal, oral cavity, pharyngeal and laryngeal oesophageal cancers comparing extreme quintiles has been observed, although the trend analysis has usually not been significant[12–15]. Gastric cancer has only been studied in a Greek case–control study, in which no statistical association with anthocyanidin intake was shown, even though the mean intake was slightly high (20·4 mg/d)[21]. More recently, anthocyanidins have been shown to reach some brain regions after consumption of blueberries in rats[54]; therefore they are able to cross the haemato–encephalic barrier. This finding suggests the potential role of anthocyanidins as anti-inflammatory and antioxidant agents against the deleterious effects of ageing and its related neurodegenerative diseases[55] and in improving memory function in older adults[56]. Further basic and epidemiological investigation is needed to confirm these potential effects against cancer and cardiovascular and neurodegenerative diseases, but taking into account possible differences among individual anthocyanidins.

To our knowledge, this is a unique study and the largest to date describing anthocyanidin intake across several European countries. However, as not all the EPIC cohorts are representative of the population, the observed level of intake cannot be extrapolated to the general population of each region. Another limitation of the present study is an underestimation of the real anthocyanidin intake, because there are some food items with missing composition data. However, our database was compiled from the most updated flavonoid databases, with only 10 % of missing values. Indeed, the major strength of the present study is the use of a unique and specifically developed FCDB, for that allowed results to be compared across countries. Further underestimation may be due to the omission of dietetic supplements in this analysis. However, there are few consumers of herb or plant supplements in the present study (up to 5 % in Denmark, the highest consumer country)[57].

The present study generated data for total and individual anthocyanidin intakes among twenty-seven centres in ten European countries, according to sex, age and some lifestyle factors. Main food sources and differences among European regions were also identified. These descriptive data will be valuable for future aetiological research focused on the relationships between anthocyanidins and chronic diseases.

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R. Z.-R. and C. A. G. designed the research; R. Z.-R. and V. K. conducted the research; R. Z.-R. and L. L.-B. performed the statistical analysis; R. Z.-R. wrote the manuscript; all authors critically reviewed and approved the final manuscript.

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References

1. Clifford MN (2000) Anthocyanins – nature, occurrence and dietary burden. *J Sci Food Agric* **80**, 1126–1137.
2. Anderson OM & Jordheim M (2006) The anthocyanins. In *Flavonoids: Chemistry, Biochemistry and Applications*, pp. 472–551 [OM Anderson and KR Markham, editors]. Boca Raton, FL: CRC Press/Taylor & Francis Group.
3. US Departament of Agriculture (2007) *USDA Database for the Flavonoid Content of Selected Foods*, Release 2.1 ed. Beltsville, MD: USDA.
4. Perez-Jimenez J, Neveu V, Vos F, *et al.* (2010) Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: an application of the Phenol-Explorer database. *J Agric Food Chem* **58**, 4959–4969.
5. Manach C, Scalbert A, Morand C, *et al.* (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727–747.
6. Fink BN, Steck SE, Wolff MS, *et al.* (2007) Dietary flavonoid intake and breast cancer survival among women on Long Island. *Cancer Epidemiol Biomarkers Prev* **16**, 2285–2292.
7. Tavani A, Spertini L, Bosetti C, *et al.* (2006) Intake of specific flavonoids and risk of acute myocardial infarction in Italy. *Public Health Nutr* **9**, 369–374.
8. Mink PJ, Scrafford CG, Barraj LM, *et al.* (2007) Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am J Clin Nutr* **85**, 895–909.
9. Prior RL & Wu X (2006) Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radic Res* **40**, 1014–1028.
10. Wang LS & Stoner GD (2008) Anthocyanins and their role in cancer prevention. *Cancer Lett* **269**, 281–290.
11. Bobe G, Peterson JJ, Gridley G, *et al.* (2009) Flavonoid consumption and esophageal cancer among black and white men in the United States. *Int J Cancer* **125**, 1147–1154.
12. Rossi M, Garavello W, Talamini R, *et al.* (2007) Flavonoids and the risk of oral and pharyngeal cancer: a case–control study from Italy. *Cancer Epidemiol Biomarkers Prev* **16**, 1621–1625.
13. Garavello W, Rossi M, McLaughlin JK, *et al.* (2007) Flavonoids and laryngeal cancer risk in Italy. *Ann Oncol* **18**, 1104–1109.
14. Rossi M, Garavello W, Talamini R, *et al.* (2007) Flavonoids and risk of squamous cell esophageal cancer. *Int J Cancer* **120**, 1560–1564.
15. Rossi M, Negri E, Talamini R, *et al.* (2006) Flavonoids and colorectal cancer in Italy. *Cancer Epidemiol Biomarkers Prev* **15**, 1555–1558.
16. Bobe G, Sansbury LB, Albert PS, *et al.* (2008) Dietary flavonoids and colorectal adenoma recurrence in the Polyp Prevention Trial. *Cancer Epidemiol Biomarkers Prev* **17**, 1344–1353.
17. Bosetti C, Spertini L, Parpinel M, *et al.* (2005) Flavonoids and breast cancer risk in Italy. *Cancer Epidemiol Biomarkers Prev* **14**, 805–808.
18. Bosetti C, Rossi M, McLaughlin JK, *et al.* (2007) Flavonoids and the risk of renal cell carcinoma. *Cancer Epidemiol Biomarkers Prev* **16**, 98–101.
19. Cutler GJ, Nettleton JA, Ross JA, *et al.* (2008) Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. *Int J Cancer* **123**, 664–671.
20. Fink BN, Steck SE, Wolff MS, *et al.* (2007) Dietary flavonoid intake and breast cancer risk among women on Long Island. *Am J Epidemiol* **165**, 514–523.
21. Lagiou P, Samoli E, Lagiou A, *et al.* (2004) Flavonoids, vitamin C and adenocarcinoma of the stomach. *Cancer Causes Control* **15**, 67–72.
22. Lagiou P, Rossi M, Lagiou A, *et al.* (2008) Flavonoid intake and liver cancer: a case–control study in Greece. *Cancer Causes Control* **19**, 813–818.
23. Mursu J, Nurmi T, Tuomainen TP, *et al.* (2008) Intake of flavonoids and risk of cancer in Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Int J Cancer* **123**, 660–663.

24. Peterson J, Lagiou P, Samoli E, *et al*. (2003) Flavonoid intake and breast cancer risk: a case–control study in Greece. *Br J Cancer* **89**, 1255–1259.
25. Rossi M, Negri E, Lagiou P, *et al.* (2008) Flavonoids and ovarian cancer risk: a case–control study in Italy. *Int J Cancer* **123**, 895–898.
26. Felgines C, Talavera S, Gonthier MP, *et al.* (2003) Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. *J Nutr* **133**, 1296–1301.
27. Hou DX, Yanagita T, Uto T, *et al.* (2005) Anthocyanidins inhibit cyclooxygenase-2 expression in LPS-evoked macrophages: structure–activity relationship and molecular mechanisms involved. *Biochem Pharmacol* **70**, 417–425.
28. Johannot L & Somerset SM (2006) Age-related variations in flavonoid intake and sources in the Australian population. *Public Health Nutr* **9**, 1045–1054.
29. Chun OK, Chung SJ & Song WO (2007) Estimated dietary flavonoid intake and major food sources of US adults. *J Nutr* **137**, 1244–1252.
30. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventos RM, *et al.* (2010) Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). *J Am Diet Assoc* **110**, 390–398.
31. Ovaskainen ML, Torronen R, Koponen JM, *et al.* (2008) Dietary intake and major food sources of polyphenols in Finnish adults. *J Nutr* **138**, 562–566.
32. Riboli E & Kaaks R (1997) The EPIC project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* **26**, Suppl. 1, S6–S14.
33. Riboli E, Hunt KJ, Slimani N, *et al.* (2002) European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* **5**, 1113–1124.
34. Slimani N, Kaaks R, Ferrari P, *et al.* (2002) European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutr* **5**, 1125–1145.
35. Brustad M, Skeie G, Braaten T, *et al.* (2003) Comparison of telephone vs face-to-face interviews in the assessment of dietary intake by the 24 h recall EPIC SOFT program – the Norwegian calibration study. *Eur J Clin Nutr* **57**, 107–113.
36. Slimani N, Ferrari P, Ocke M, *et al.* (2000) Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr* **54**, 900–917.
37. Slimani N, Deharveng G, Unwin I, *et al.* (2007) The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr* **61**, 1037–1056.
38. Haftenberger M, Schuit AJ, Tormo MJ, *et al.* (2002) Physical activity of subjects aged 50–64 years involved in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* **5**, 1163–1176.
39. Neveu V, Perez-Jimenez J, Vos F, *et al.* (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)* **2010**, bap024.
40. Crozier A, Lean MEJ, McDonald MS, *et al.* (1997) Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuces, and celery. *J Agric Food Chem* **45**, 590–595.
41. Hiemori M, Koh E & Mitchell AE (2009) Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *japonica* var. SBR). *J Agric Food Chem* **57**, 1908–1914.
42. Mulinacci N, Ieri F, Giaccherini C, *et al.* (2008) Effect of cooking on the anthocyanins, phenolic acids, glycoalkaloids, and resistant starch content in two pigmented cultivars of *Solanum tuberosum* L. *J Agric Food Chem* **56**, 11830–11837.
43. Steed LE, Truong VD, Simunovic J, *et al.* (2008) Continuous flow microwave-assisted processing and aseptic packaging of purple-fleshed sweetpotato purees. *J Food Sci* **73**, 455–462.
44. Xu B & Chang SK (2008) Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *J Agric Food Chem* **56**, 7165–7175.
45. Oliveira C, Amaro LF, Pinho O, *et al.* (2010) Cooked blueberries: anthocyanin and anthocyanidin degradation and their radical-scavenging activity. *J Agric Food Chem* **58**, 9006–9012.
46. Sieri S, Krogh V, Saieva C, *et al.* (2009) Alcohol consumption patterns, diet and body weight in 10 European countries. *Eur J Clin Nutr* **63**, Suppl. 4, S81–S100.
47. Agudo A, Slimani N, Ocke MC, *et al.* (2002) Vegetable and fruit consumption in the EPIC cohorts from 10 European countries. *IARC Sci Publ* **156**, 99–103.
48. Lagiou P, Samoli E, Lagiou A, *et al.* (2006) Flavonoid classes and risk of peripheral arterial occlusive disease: a case–control study in Greece. *Eur J Clin Nutr* **60**, 214–219.
49. Lagiou P, Samoli E, Lagiou A, *et al.* (2004) Intake of specific flavonoid classes and coronary heart disease – a case–control study in Greece. *Eur J Clin Nutr* **58**, 1643–1648.
50. Dilis V & Trichopoulou A (2010) Antioxidant intakes and food sources in Greek adults. *J Nutr* **140**, 1274–1279.
51. Melby MK, Murashima M & Watanabe S (2008) Phytochemical intake and relationship to past health history in Japanese women. *Biofactors* **22**, 265–269.
52. Hooper L, Kroon PA, Rimm EB, *et al.* (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **88**, 38–50.
53. Frankenfeld CL, Cerhan JR, Cozen W, *et al.* (2008) Dietary flavonoid intake and non-Hodgkin lymphoma risk. *Am J Clin Nutr* **87**, 1439–1445.
54. Andres-Lacueva C, Shukitt-Hale B, Galli RL, *et al.* (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutr Neurosci* **8**, 111–120.
55. Shukitt-Hale B, Lau FC & Joseph JA (2008) Berry fruit supplementation and the aging brain. *J Agric Food Chem* **56**, 636–641.
56. Krikorian R, Shidler MD, Nash TA, *et al.* (2010) Blueberry supplementation improves memory in older adults. *J Agric Food Chem* **58**, 3996–4000.
57. Skeie G, Braaten T, Hjartaker A, *et al.* (2009) Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study. *Eur J Clin Nutr* **63**, Suppl. 4, S226–S238.

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Letter to the editor – Reply

Response: Banana is not a food source of delphini(di)ns in the EPIC study

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We thank Dr Drossard for her interest and comments concerning our recent paper[1]. We fully agree with Drossard *et al.*[2] regarding the incorrect delphinidin value of bananas (7·39 mg/100 g) in the USDA database[3]. Phenol-Explorer[4] does not use this value (which comes from Harnly *et al.*[5]) due to its poor quality and provides no further delphinidin data for bananas. Kitdamrongsont *et al.*[6] analysed the anthocyanidin content in some varieties of wild bananas in Thailand and clearly showed that red, pink, blue and purple bananas contain delphinidins, but not the yellow or green-yellow varieties. Indeed, anthocyanidins are common plant pigments (red, blue, violet), and so logically foods without these colours may not contain anthocyanidins. Therefore, the delphinidin content in yellow and green-yellow bananas should be zero. In our study, we did not use the USDA value, but rather we used the content of delphinidins in banana as zero based on the above reasons[6]. Therefore, banana does not appear in the food composition table on anthocyanidins of the most abundant raw food sources (annex Table 1). In the table of food sources (Table 4), banana is included in the group of 'Other and mixed fruits' because it was not shown to be a relevant contributor of anthocyanidins. However, in our text, there is a mistake when describing the food sources of delphinidins by region where banana is listed as one of the main contributors by error. Therefore, for the southern countries the richest food sources of delphinidins should be wine, grapes and fruiting vegetables, mainly aubergine, whereas for the central and northern countries the main contributors should be non-alcoholic beverages, berries and wine. In conclusion, banana is not a food source of delphinidins in the European Prospective Investigation into Cancer and Nutrition (EPIC) study because Europeans mainly consume yellow bananas, which have not yet been shown to contain delphinidins.

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References

1. Drossard C & Kersting M (2012) Banana as a relevant source of anthocyani(di)ns in European populations? *Br J Nutr* **107**, 765–766.
2. Drossard C, Frohling B, Dietrich H, *et al.* (2011) Anthocyanin analysis in banana fruit – a mistake. *Am J Clin Nutr* **93**, 865–866.
3. US Departament of Agriculture (2007) *USDA Database for the Flavonoid Content of Selected Foods*. Release 2.1 ed. Beltsville, MD: USDA.
4. Neveu V, Perez-Jimenez J, Vos F, *et al.* (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)* **2010**, bap024.
5. Harnly JM, Doherty RF, Beecher GR, *et al.* (2006) Flavonoid content of US fruits, vegetables, and nuts. *J Agric Food Chem* **54**, 9966–9977.
6. Kitdamrongsont K, Pothavorn P, Swangpol S, *et al.* (2008) Anthocyanin composition of wild bananas in Thailand. *J Agric Food Chem* **56**, 10853–10857.

5.3 LIST OF PRESENTATIONS

Drossard C, Kersting M.
Age and time trends in anthocyanidin density of the diet of children and adolescents.
49 th Scientific Congress of the German Nutrition Society (DGE), Freising-Weihenstephan.
Proc Germ Nutr Soc (2012) 17: 10. (oral presentation)

Drossard C, Kersting M.
Liking of anthocyanin-rich fruit juices and smoothies in children and adolescents: Results of sensory evaluations in the DONALD Study.
5th International Conference on Polyphenols and Health (2011) Sitges, Spain.
Proceedings of Conference (2011): 162. (poster)

Drossard C, Kronwald J, Kersting M.
Beliebtheit anthocyanreicher Fruchtsäfte bei Kindern und Jugendlichen: Ergebnisse sensorischer Tests in der DONALD Studie.
48 th Scientific Congress of the German Nutrition Society (DGE), Potsdam.
Proc Germ Nutr Soc (2011) 15: 22. (oral presentation)

Drossard C, Alexy U, Kersting M.
Anthocyanidinaufnahme bei Säuglingen und Kleinkindern.
47 th Scientific Congress of the German Nutrition Society (DGE), Jena.
Proc Germ Nutr Soc. (2010) 14: 37. (oral presentation)

Drossard C, Alexy U, Kersting M.
Age and time trends in anthocyanidin intake in infants and toddlers in Germany.
4th International Conference on Polyphenols and Health (2009), Harrogate, UK.
Proceedings of Conference (2009): 260. (poster)