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Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults

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Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults

Abstract

Background: Anthocyanins represent an important subgroup of non-nutritive components of food as evidence continues to build related to their beneficial bioactive effects. Using a recently developed Australian anthocyanin database, the present study aimed to estimate the intake of both total anthocyanins and their subclasses, identify food sources of anthocyanins, and determine associations between anthocyanin intake and measured blood pressure (BP).

Methods: The present study comprised was a secondary analysis of the 2011-12 National Nutrition and Physical Activity component of the Australian Health Survey. Anthocyanin intake was estimated using an Australian anthocyanin database. Usual anthocyanin intake, as estimated from 24-h diet recall data, was computed using multiple source methods, whereas food sources were determined by calculating contribution of food groups to total anthocyanin intake. Regression analysis, adjusted for covariates (age, gender, body mass index, high BP) diagnosis, smoking status and physical activity) assessed the relationship between anthocyanin intake and BP in adults aged ≥50 years.

Results: Mean anthocyanin intake was 24.17 ± 0.32 mg day −1 . Across age groups, berries were the top sources: blackberry (5-65%), cherry (2-24%), blueberry (2-13%) and raspberry (3-12%). There was a significant inverse association between anthocyanin intake and systolic BP (β = −0.04, F = 16.8, d.f. = 6, r^2 = 0.05, P < 0.01) and diastolic BP (β = 0.01, F = 5.35, d.f. = 6, R^2 = 0.013, P < 0.01), in models that adjusted for covariates.

Conclusions: In comparison with the world composite database, anthocyanin intake in the Australian population was above average [mean (SD): 24.17 (0.32) mg day^{-1} versus 18.05 (21.14) mg day^{-1}]. Berries comprised up the primary source of anthocyanins. Anthocyanin intake in older adults aged aged ≥50 years was inversely associated with BP.

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Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults

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Abstract

***Background**:* Anthocyanins represent an important sub-group of non-nutritive components of food as evidence continues to build related to their beneficial bioactive effects.

Objectives: Using a recently developed Australian anthocyanin database, this study aimed to estimate the intake of both total anthocyanins and their subclasses, identify food sources of anthocyanins, as well as determine associations between anthocyanin intake and measured Blood Pressure (BP).

Methods: This was a secondary analysis of the 2011-12 National Nutrition and Physical Activity component of the Australian Health Survey. Anthocyanin intake was estimated using an Australian anthocyanin database. Usual anthocyanin intake estimated from 24-h diet recall data was computed using Multiple Source Methods, while food sources were determined by calculating contribution of food groups to total anthocyanin intake. Regression analysis, adjusted for covariates (age, gender, body mass index, high blood pressure (BP) diagnosis, smoking status and physical activity) assessed the relationship between anthocyanin intake and BP in adults aged 50+y.

Results: Mean anthocyanins intake was 24.17±0.32mg/day. Across age-groups, berries were the top sources: blackberry(5-65%); cherry(2-24 %); blueberry(2-13%) and raspberry(3-12%). There was a significant inverse association between anthocyanin intake and systolic BP (β =-0.04, F(df)=16.8 (6), R-square=0.05, p<0.01) and diastolic BP β =0.01, F(df)=5.35 (6), R-square=0.013, p<0.01 (β= -0.01, p<0.01), in models that adjusted for covariates.

Conclusion: In comparison to the world composite database, anthocyanin intake in the Australian population was above average (24.17±0.32mg/day vs 18.05±21.14 mg/d). Berries made up the primary sources thereof. Anthocyanin intake in older adults aged 50+y was inversely associated with blood pressure.

Keywords: Anthocyanins, dietary intake, food sources, blood pressure, Australia Health Survey

Introduction

Incorporating fruits and vegetables in to the usual human diet has been shown to exert protective effects on health. These observed effects have been attributed to the presence of minerals, vitamins, phytochemicals and dietary fibre in these food groups [(1)]. Phytochemicals include phenols, terpenes, thiols, phytic acids, phytosterols and protease inhibitors. The phenols are the largest class of phytochemicals [(2)] and these bioactive compounds, independently and as a group in fruits and vegetables have been associated with reduced mortality [(3)] weight loss [(4)] cardiovascular diseases [(5)] and some cancers[(6)].

Anthocyanins are a subgroup of polyaromatic phenols. They are one of the major subgroups. Epidemiological evidence has shown that anthocyanins, aside from being responsible for the deep dark colours in fruits and vegetables, may also exert beneficial effects to health. To date, the most promising protective health effects of anthocyanins appear to be related to blood pressure regulation [(5, 7, 8)], vascular health [(9)], and cognitive function [(10, 11)]. Chronic inflammation as a result of raised blood pressure increases the risk of chronic diseases, including stroke, coronary heart disease, chronic kidney disease and heart failure [(12, 13)]. Anthocyanins as part of the overall diet plays an integral role in the regulation of chronic inflammation [(14)]. Fruits such as berries, with high anthocyanin contents, among others have been labelled as anti-inflammatory foods using the Dietary Inflammatory Index (DII) [(15)] which is a system to assess the quality of foods based on their inflammatory potential [(15)]

Despite emerging evidence regarding the beneficial health effects of anthocyanins, inter-individual variability in their metabolism, which in part may be related to differing microbiota profiles [(16, 17)], has hampered identification of optimal intakes, as well as elucidation of their role in cardio-metabolic health. The food matrix in fruits and vegetables may contribute to the wide inter-individual variability in the metabolism and subsequent effects of anthocyanins. Different cardiovascular and metabolic responses have been shown according to the food source of these compounds [(18, 19)]. The synergistic effect of other diverse compounds and nutrients present in the source foods has been explored by others [(20, 21)].

Accurate measurement of nutrient intakes, in this case anthocyanins, tailored specifically to individual countries and regions is an important consideration in both epidemiological and experimental studies [(22)]. Due to variations in climate, soil conditions and methods of plant harvesting, nutrient levels differ in foods produced across different regions [(23, 24)]. This variation in nutrient content has led to the development of country/region specific food composition databases. Currently the United States Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods and the European Phenol-Explorer food composition databases exist for the measurement of polyphenol (including anthocyanin) intakes [25, 26]. Comparing these two databases for the estimation of dietary polyphenol intake in Polish adults, Witkowska et al. (2015) [27] demonstrated significant discrepancies between the amount of flavonoid intake, estimated at 525 mg/d vs 403.5 mg/d, respectively ($p<0.001$). This discrepancy led to the first stage development of an Australian anthocyanin database to determine the amount of anthocyanins consumed by Australians [28] due to the difference in climate and agricultural practices in this region.

Following the development of an Australian anthocyanin database, the primary aim of this study was to use this newly developed anthocyanin database to estimate the intake of both total anthocyanins and their subclasses in a nationally representative sample of the Australian population and compare intakes to the world composite database and other population studies [29] as well as determine the top food contributors of anthocyanins in Australians.

Evidence from blood pressure intervention studies have shown that more significant effects are observed in older adults [7, 8] and elevated blood pressure as part of metabolic syndrome is common in people aged 50+years [30]. A secondary aim of this study was therefore to determine whether there was an association between anthocyanin intakes and blood pressure in Australians aged 50 years and older.

Materials and methods

This study was a secondary data analysis of the 2011–12 National Nutrition and Physical Activity Survey (NNPAS) data from the Australian Bureau of Statistics (ABS) using Basic Confidentialised Unit Record Files. The NNPAS 2011-12 is a component survey of the 2011-13 Australian Health Survey (AHS) which involved a total of 12,153 persons carried out between 29 May 2011 and 9 June 2012 in approximately 9,500 private dwellings selected throughout non-very remote areas of Australia.

This survey was conducted using a stratified multistage area sample of private dwellings. Survey aims were designed to provide detailed and broad level estimates for each state/territory and Australia, capital city/balance of state area, regions and sub-populations.

Within selected households, a random sub-sample of residents was selected as follows:

- one adult (aged 18 years and older), and (where applicable)
- one child aged 0-17 years (AHS)
- one child aged 2-17 years (NNPAS) [31].

Data collection for NNPAS involved face-to-face interview (and by telephone for the second NNPAS interview) to collect data on general demographic information (including age, sex, marital status and country of birth) for all individuals while detailed information was collected from one adult and one child aged 2-17 years. The survey sampling method covered about 97% of the people and households in Australia from which the study population was sampled.

A 24-hour dietary recall method was used to collect information on food, beverages and dietary supplements consumed, as well as some general information on dietary behaviours.

All participants (n=12,153) provided the first dietary recall through interviewer-administered format using the Automated Multiple-Pass Method[32] adapted to reflect the Australian food supply [33]. All respondents were invited to take part in a second 24hr dietary recall, conducted using computer assisted telephone interview. Data was collected from 63% of respondents (n=7735). To account for seasonal changes in health and nutritional intake, the NNPAS data collection was randomly spread over a 12-month period. Calculation of the person weights adjusted for this seasonal variation.

No ethics approval was required for this study because it was a secondary analysis. However, approval to carry out a secondary analysis with the NNPAS component of the AHS data was obtained by the researchers from the Australian Bureau of Statistics prior to conducting this study.

Estimation of anthocyanin intake

Estimation of anthocyanin intake occurred by applying a newly developed Australian anthocyanin database to the dietary records. The methods of the development of the database, including its strengths and weaknesses, are reported elsewhere [28]. Briefly, analytical values were systematically searched, and local researchers were contacted for unpublished data.

Following compilation of the data, values were borrowed from the USDA flavonoid database for selected foods as well as the European Phenol-Explorer database. Borrowed values were converted for Australian foods using a moisture conversion factor. There are a total of 5740 foods in the Australian Food and Nutrient Database (AUSNUT) 2011-13; of these, anthocyanin values were assigned to 318 individual foods.

The AUSNUT 2011–13 is a food composition database of food, dietary supplement and nutrient intake estimates that was compiled from participants' responses to the 2011–12 NNPAS. For the current analysis, the NNPAS data was expanded to include anthocyanin content of reported foods and total intakes were calculated based on the amount of food (g) consumed for each day of recall.

Using the two separate days of 24hr dietary recall data, usual anthocyanin intake was calculated using the multiple source method (MSM) [34, 35]. The MSM comprises three steps. First, for each respondent in the study sample, the probability of consumption of the response variable on a randomly selected day was calculated. Secondly, the usual amount of food group intake on reported consumption days was estimated, and finally, the usual overall intakes were calculated by multiplying probability of consumption of the response variable with usual amount of intake on consumption days. Intake values were calculated within the MSM model assuming all participants were habitual consumers, given anthocyanins are primarily found in fruit and vegetables, with age and gender included as covariates. Two variables were produced in the MSM output; usual daily intake of anthocyanins for all participants calculated by the MSM (e.g. measure of habitual intake) and the usual intake of anthocyanins in consumers from the 24-h dietary recall calculated by the MSM. Both of these variables were used in the statistical analysis (**Figure 1)**.

Statistical Analysis

Statistical analysis was carried out using SAS (release 9.4, 2012; SAS Institute). Daily anthocyanin intake was calculated and expressed as mean and standard error (SE). The total population analysis was based on usual daily anthocyanin intake for all participants and the consumer population analysis was based on the usual intake of anthocyanins in consumers only. Intake of the major sub-classes of anthocyanins (cyanidins, delphinidins, malvidins, pelargonidin, peonidins and petunidins) were also calculated. Weighting factors (person weights and replicate weights produced by ABS [36]) were applied to the data in order to generalise results to the total Australian population at the time the survey and to account for sampling discrepancies.

Mean anthocyanin intake for the total population and across sub-groups (age-groups, sex, Body Mass Index (BMI,)) level of education, smoking status and level of physical activity) were calculated. Age-groups were categorised according to those used in the National Health and Medical Research Council Nutrient Reference Values (NRVs) for Australia and New Zealand [37]. For the purpose of the analysis, categories of BMI, education, smoking and physical activity were grouped according to the ABS classifications with similar classes grouped together (e.g. underweight Class 3, underweight Class 2 and underweight Class 1 grouped as ‘underweight’) and individuals aged less than 15y were classified as ‘not applicable’ to maintain the integrity of the weighting factors applied [31].

Linear regression analysis was used to determine the relationship between anthocyanin intake and blood pressure in adults aged 50+ y (Figure 1), while adjusting for age and gender (model 1) and adjusting for age, gender, BMI, physical activity, smoking status and whether diagnosed with high blood pressure (by a health professional and/or measured BP >=140/90 mmHg) (model 2). Inclusion criteria for this analysis were: i) adults aged 50 years and above and ii) who had a valid blood pressure measurement

Level of significance was set at 0.05 and calculated from t-test for pair-wise comparisons or ANOVA to determine if there are differences in any of the subgroups where appropriate.

Results

Dietary anthocyanin intake from the NNPAS was estimated at 24.17 ± 0.32 mg/d for the total population (n = 12,153) and 37.68mg/d for the consumer population (n = 8,958). Mean intakes for total and subclasses of anthocyanins according to sociodemographic (gender, age-group, level of education) and lifestyle (BMI, smoking status and physical activity) are reported in **Table 1**. There were more adults than children (n = 9,341 vs 2,812) with no statistically significant difference in anthocyanin intake. Respondents who had high physical activity levels consumed more anthocyanins compared to those who had a sedentary lifestyle (30.04mg/d vs 20.42mg/d, <0.001). A similar trend was also observed for respondents with a Bachelor’s degree compared to those with a diploma or lower (29mg/d vs 24mg/d, t-test p-value <0.001).

The top ten food sources stratified by age are shown in **Table 2**. Berries are highly concentrated sources of anthocyanins [28, 38] and were the top contributors to total anthocyanin intake across all age-groups. The top ten food sources were reported as these made up more than 50% of the total anthocyanin intake across all age-groups.

There was a significant inverse association between measured systolic and diastolic blood pressure and anthocyanin intake (**Table 3)**. This was evident for both systolic ($\beta = -0.04$, F (df) = 7.77 (2), R square = 0.01, $p=0.001$) and diastolic ($\beta = -0.01$, F (df) = 5.72 (2), R square = 0.01, $p=0.005$) blood pressure (Model 1: adjusted for age and gender). After controlling for further confounders (Model 2: age, gender, BMI, physical activity, high BP diagnosis, smoking status and physical activity), the association remained significant ($\beta = -0.04$, F (df) = 16.8 (6), R square = 0.05, $p<0.01$ for systolic BP; and $\beta = 0.01$, F (df) = 5.35 (6), R square = 0.013, $p<0.01$ for diastolic BP).

Discussion

Using nationally representative dietary survey data, this study reports an observed wide distribution of anthocyanin intake in the Australian population. The estimated mean intake was 24.17 ± 0.32mg/d which is midway between estimates reported for other populations that range between 2.9 and 42.79mg/d (**Table 4),** and was above average in comparison to the world composite database [29]. There were variations in anthocyanin intake across subgroups analysed, for example a higher anthocyanin intake in the 51-70y age-group (27.75 ± 0.65 mg/d) compared to the lowest consumption (18.56 ± 0.87 mg/d) in the 14-18y age-group ($p<0.001$). Adults with a post-graduate qualification had higher anthocyanin intakes (30.09 ± 1.47 mg/d) compared to those without school qualifications (20.57 ± 0.43mg/d) ($p<0.001$) while highly active adults (30.04 ± 1.11mg/d) had higher intakes compared to sedentary respondents (20.42 ± 0.73mg/d) ($p<0.001$). These results are consistent with previous reports on the significant association between socio-demographic and lifestyle factors and fruit and vegetable consumption [39-42]. Surprisingly, ex-smokers reportedly consumed significantly higher daily anthocyanins (27.68 ± 0.88 mg/d), more than both smokers (18.21 ± 0.68 mg/d) and non-smokers (24.33 ± 0.45 mg/d). Zamora-Ros et al.(2010) [43] similarly reported that ex-smokers consumed more anthocyanins (11.16mg/d) than non-smokers (10.62mg/d).

Estimation of anthocyanin intake in the Australian population is an important preliminary step in understanding anthocyanin-health relationships. Despite increased research interest on the observed health benefits of anthocyanins provided by food and beverages, the accompanying increased prevalence of consumption of processed foods translates to a reduced consumption of dietary anthocyanins [44]. In the 1970s, average daily dietary anthocyanin intake in the USA. was estimated at 215 mg/d in the summer and 180 mg/d during winter [45]. Current estimates show that dietary anthocyanin intake ranges between 3-43mg/day across countries and tends to be higher in Southern compared to Northern European countries (**Table 4).**

Consumption of anthocyanin subclasses were also reported for the total and consumer populations. Cyanidins and malvidins were the most prevalent anthocyanins in both the general and consumer populations and across subgroups. In the 51-70y age-group, malvidins were the most prevalent anthocyanins. This could be explained by the difference in consumption pattern of the major food contributors of anthocyanins in this age-group being red wine, which has a high content of malvidins. A similar trend was also observed in other Australian population studies [46, 47] with red wine as a major contributor of anthocyanins in older participants.

The highest contributing foods (top 10) of dietary anthocyanin in the Australian population were berries, principally blueberries, blackberries, raspberries, and cherries. Berries are known to contain a very high concentration of anthocyanins [48]. Given this, anthocyanin research has generally focused on berries at the expense of other high anthocyanin foods, such as plums and eggplants which also made up some of the top 10 contributors of anthocyanins in the Australian population. Accordingly, it might be worth further exploration to gather epidemiological evidence of the health-related benefits related to these foods.

Results from this study showed a significant association between anthocyanin intake in older adults and lower blood pressure. This result is in agreement with current evidence from clinical and epidemiological studies on the effect of dietary anthocyanins on blood pressure in acute settings and over the longer term [7, 8, 49] . Anthocyanins have also been classified as nutraceuticals in their ability, as part of a food component, to provide health and medical benefits [50]. However, it is unlikely that these health benefits are the independent effects of anthocyanins instead a synergistic effect with other polyphenols [51]. This emphasises the importance of studying anthocyanin food sources, consistent with this study, rather than isolated anthocyanins. A review by Pascual-Teresa et al. (2010) [52] observed that evidence related to the protective effects of anthocyanins, as part of the diet, against cardiovascular disease risks has been consistent over the years. Dietary patterns high in fruits and vegetables such as blueberries, apples, and leafy greens that are high in natural antioxidants and polyphenols (anthocyanins) have been shown to reduce the risk of high blood pressure [53] and other chronic diseases evident in the Dietary Inflammatory Index [14]. This evidence has been consistent with similar dietary patterns. Using the Dietary Inflammatory Index, Steck et. al (2014) [54] found that the Mediterranean diet showed anti-inflammatory potential based on the resulting DII scores. In addition, the Nordic diet significantly reduced 24-h ambulatory diastolic blood pressure (DBP) and mean arterial pressure in comparison to a control diet based on mean nutrient intakes in Nordic countries [55]. Other dietary patterns that emphasise high fruit and vegetable intake (the Dietary Approach to Stop Hypertension), low-carbohydrate, Palaeolithic, high-protein, low-glycaemic index, low-sodium, and low-fat diets) were also found to significantly reduce blood pressure (systolic blood pressure (−8.73 to −2.32 mmHg) and DBP (−4.85 to −1.27 mmHg)) in comparison to control/usual diets [56]. Attributable risk related to the polyphenols present in these diets (i.e. anthocyanins) cannot be disentangled from other health-promoting components of key foods included in these blood pressure lowering dietary patterns.

Previous studies have estimated the dietary flavonoid intake in selected Australian populations using weighed food records [47] and from National Nutrition Survey (1995) data using a single 24-h dietary recall questionnaire [46]. Our results using the latest nationally representative survey data differ significantly from earlier studies with higher values reported in the current study. This is possibly due to differences in the method of dietary assessments as well as the use of the MSM to calculate usual daily intake from two repeated 24-h dietary recalls, it could also be as a result of difference/change in population characteristics. Our analysis indicates that the Australian population consume intermediate quantities of anthocyanins compared to other populations (Table 4), but that are similar to estimates from some European countries including Belgium, Norway, Sweden and Denmark [57, 58]. A need for tailored databases for nutritional epidemiological studies cannot be overemphasised. Following the development of the first Australian food composition database in the mid-1980s, comparative analysis showed that using the UK and US databases overestimated specific nutrient contents by up to 60% [59]. As a result, it is imperative that databases be tailored to the specific food supply of the population under study. However, development of nutrient databases is fraught with incomplete coverage of all nutrients hence, borrowing and calculating nutrient values is a known validated method in the absence of analytical values [(60)].

To our knowledge, this is the first time that dietary anthocyanin intake in the Australian population has been estimated using an Australian-specific anthocyanin database, this being a major methodological strength. Another notable strength is the use of a representative sample of the Australian population, as well as the validated method (MSM) of calculating usual nutrient intake from repeated 24h dietary recalls [(34)]. Some of the limitations of this study include the use of 24-h diet recall questionnaires in the NNPAS (1-2). The single 24-h diet recall has been deemed insufficient because of the retrospective method of dietary assessment and an inability to describe the typical diet from a single day's intake. In addition, the recall is dependent on the memory and cooperation of the participant [(61)]. Four repeated 24-h diet recalls have been recommended as the most appropriate method for large surveys,[(62)] while two were applied in the NNPAS. This study applied a regression model to these data to better represent usual intake although no adjustments were made to potential misreporting of the data. A further limitation relates to the effects of processing and storage [(63)] which was not taken into consideration in the borrowed data used from USDA and Phenol-Explorer food composition databases. Finally, the omission of anthocyanin intake from dietary supplements is a notable limitation of this study. It is however unlikely that such supplements are widely used in Australia, and composition of supplements and extracts in the AUSNUT food composition database did not include polyphenols (anthocyanins). Each of these elements could have led to both over- and underestimation of anthocyanin intake.

The cross-sectional nature of this study limits interpretation of the inverse association found between anthocyanin intake and blood pressure. However, evidence is beginning to emerge from experimental studies that support a protective role of anthocyanins on CVD risk in both young and older adults [(64)]. For two foods, plum and red cabbage, the Australian analytical data only reported total anthocyanins but not anthocyanin subclasses. Many fruits and vegetables that are high in anthocyanins are seasonal, available only in summer and autumn in Australia, therefore contribution to total intake will depend on the time of year that surveys are conducted. For example, red cabbage is a rich source of anthocyanins but accounted for less than 1% (0.2%) of total anthocyanin intake in the Australian population. This could also be as a result of not many people consuming red cabbage.

Although different methods have been described for estimating usual intake, the MSM method involves similar steps to the National Cancer Institute (NCI) method but uses different modelling procedures and handling of non-consumers [65]. Comparison of the different methods, including those from Iowa State University (ISU), NCI, MSM and Statistical Program to Assess Dietary Exposure (SPADE), showed that with small sample sizes (n = 150), the ISU, MSM and SPADE methods were more reliable and showed more precise estimates than the NCI method, mainly for the 10th and 90th percentiles. The observed differences between methods became less significant with larger sample sizes (n = 300 and n = 500) [66].

Conclusion

In conclusion, this study estimates for the first time, mean daily intake of anthocyanin in the Australian population, according to sociodemographic characteristics, using an Australian-specific anthocyanin food composition database. Given the rapidly emerging evidence base related to the beneficial effects of anthocyanins on cardiovascular risk factors, it is timeous to assess population-level intake of this flavonoid subgroup. Anthocyanin intake was similar to that reported in southern European countries, and higher than in Northern Europe and USA. Identification of major dietary sources of anthocyanins (blackberry, raspberry, blueberry, cherry, red cabbage, eggplant and red wine) allows for focused dietary messaging.

Transparency Declaration

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is compliant with STROBE- nut guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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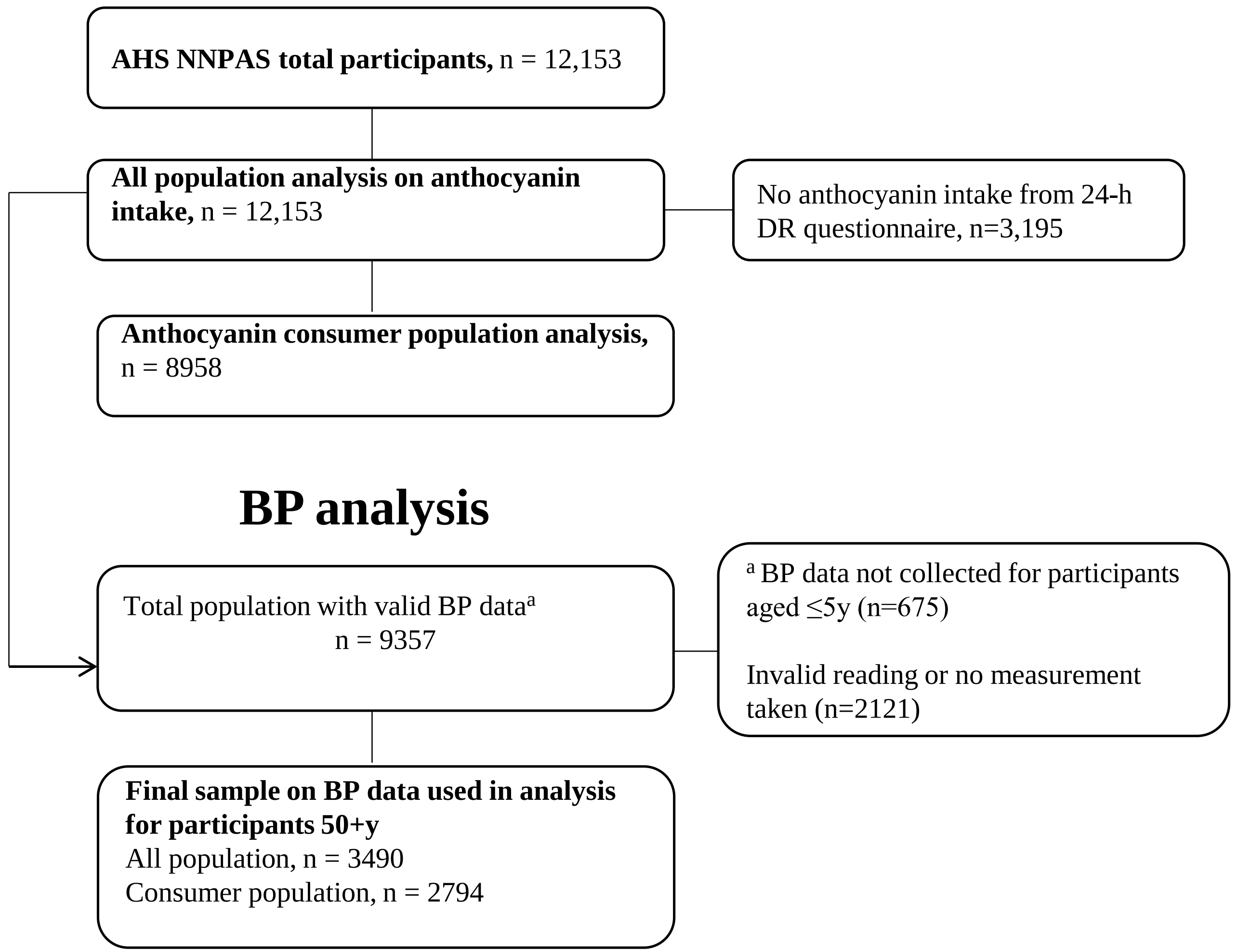

Figure 1: Participant flowchart for secondary analysis of the Australian Health Survey, NNPAS component

Table 1: Anthocyanin intakes by demographic and lifestyle factors for the Australian population (and consumer population) in 2011–12 NNPAS

Stratification variable	N	Anthocyanins(mg/d)			Cyanidins (mg/d)			Delphinidin (mg/d)			Malvidin (mg/d)			Pelargonidin (mg/d)			Peonidin (mg/d)			Petunidin (mg/d)		
		Mean	SE	P	Mean	SE	P	Mean	SE	P	Mean	SE	P	Mean	SE	P	Mean	SE	P	Mean	SE	P
Total NNPAS population **(consumer population)**	12153 **(8958)**	24.17 **(37.68)**	0.32 **(0.41)**		9.33 **(15.22)**	0.13 **(0.23)**		3.03 **(5.07)**	0.07 **(0.12)**		7.61 **(14.14)**	0.20 **(0.42)**		0.12 **(0.21)**	0.01 **(0.02)**		1.12 **(1.99)**	0.02 **(0.05)**		1.05 **(1.98)**	0.04 **(0.08)**	
Gender				<0.001 **(<0.001)**			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001
Male	5702 **(4030)**	23.62 **(38.27)**	0.41 **(0.63)**		9.41 **(16.04)**	0.17 **(0.33)**		2.83 **(4.89)**	0.06 **(0.11)**		7.46 **(14.46)**	0.29 **(0.67)**		0.11 **(0.21)**	0.01 **(0.03)**		1.08 **(1.98)**	0.03 **(0.07)**		1.00 **(1.97)**	0.04 **(0.09)**	
Female	6451 **(4928)**	24.72 **(37.14)**	0.47 **(0.67)**		9.27 **(14.49)**	0.18 **(0.29)**		3.22 **(5.24)**	0.13 **(0.22)**		7.76 **(13.85)**	0.23 **(0.48)**		0.13 **(0.21)**	0.01 **(0.02)**		1.16 **(2.00)**	0.04 **(0.08)**		1.10 **(2.00)**	0.06 **(0.13)**	
Age group (yrs.)				<0.001 **(<0.001)**			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001
Children (≤18)	2812 **(2071)**	22.46 35.25	0.58 0.90		10.97 **(17.96)**	0.28 **(0.45)**		2.51 **(4.04)**	0.11 **(0.18)**		4.86 **(9.33)**	0.33 **(0.72)**		0.11 **(0.20)**	0.01 **(0.02)**		0.89 **(1.58)**	0.05 **(0.09)**		0.57 **(1.08)**	0.07 **(0.14)**	
2-3	464 **(378)**	21.51 **(31.11)**	1.40 **(1.86)**		7.54 **(11.06)**	0.43 **(0.83)**		3.45 **(5.59)**	0.28 **(0.44)**		6.34 **(11.18)**	0.89 **(1.53)**		0.14 **(0.27)**	0.02 **(0.04)**		0.99 **(1.67)**	0.13 **(0.21)**		0.83 **(1.49)**	0.16 **(0.27)**	
4-8	789 **(633)**	26.06 **(37.04)**	1.30 **(1.78)**		12.31 **(18.60)**	0.75 **(1.17)**		3.12 **(4.59)**	0.34 **(0.52)**		6.51 **(11.55)**	0.67 **(1.25)**		0.16 **(0.26)**	0.02 **(0.03)**		1.51 **(1.87)**	0.11 **(0.19)**		0.83 **(1.40)**	0.19 **(0.31)**	
9-13	787 **(576)**	22.92 **(36.29)**	0.91 **(1.40)**		12.02 **(20.01)**	0.53 **(0.86)**		2.11 **(3.42)**	0.12 **(0.21)**		4.38 **(8.54)**	0.49 **(1.14)**		0.09 **(0.16)**	0.01 **(0.02)**		0.83 **(1.50)**	0.06 **(0.11)**		0.43 **(0.87)**	0.06 **(0.15)**	
14-18	772 **(484)**	18.56 **(33.69)**	0.87 **(1.74)**		9.80 **(18.13)**	0.51 **(1.09)**		1.94 **(3.29)**	0.12 **(0.22)**		3.06 **(6.41)**	0.44 **(1.22)**		0.07 **(0.14)**	0.01 **(0.02)**		0.64 **(1.25)**	0.06 **(0.15)**		0.36 **(0.72)**	0.07 **(0.18)**	
Adults (≥19)	9341 **(6887)**	24.66 38.38	0.35 0.44		8.87 **(14.43)**	0.13 **(0.23)**		3.17 **(5.37)**	0.07 **(0.13)**		8.39 **(15.52)**	0.23 **(0.47)**		0.12 **(0.21)**	0.01 **(0.02)**		1.18 **(2.11)**	0.03 **(0.06)**		1.19 **(2.24)**	0.04 **(0.08)**	
19-30	1592 **(1017)**	19.24 **(34.24)**	0.68 **(1.07)**		8.48 **(15.73)**	0.29 **(0.60)**		2.42 **(4.60)**	0.13 **(0.25)**		4.62 **(10.08)**	0.45 **(1.43)**		0.16 **(0.33)**	0.04 **(0.09)**		0.78 **(1.61)**	0.05 **(0.15)**		0.57 **(1.29)**	0.06 **(0.16)**	
31-50	3565 **(2543)**	25.66 **(40.55)**	0.66 **(0.91)**		9.58 **(15.79)**	0.27 **(0.45)**		3.22 **(5.48)**	0.14 **(0.25)**		8.40 **(15.84)**	0.41 **(0.85)**		0.11 **(0.17)**	0.01 **(0.01)**		1.19 **(2.13)**	0.05 **(0.09)**		1.24 **(2.35)**	0.07 **(0.15)**	
51-70	2907 **(2300)**	27.75 **(40.20)**	0.65 **(0.97)**		8.88 **(13.39)**	0.24 **(0.42)**		3.55 **(5.71)**	0.13 **(0.27)**		10.97 **(18.96)**	0.49 **(0.98)**		0.11 **(0.18)**	0.01 **(0.02)**		1.46 **(2.46)**	0.06 **(0.11)**		1.54 **(2.75)**	0.08 **(0.16)**	

Stratification variable	N	Anthocyanins(mg/d)			Cyanidins (mg/d)			Delphinidin (mg/d)			Malvidin (mg/d)			Pelargonidin (mg/d)			Peonidin (mg/d)			Petunidin (mg/d)		
		Mean	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**
71+	1277 **(1027)**	24.61 **(33.64)**	1.15 **(1.37)**		7.29 **(10.80)**	0.32 **(0.48)**		3.64 **(5.37)**	0.22 **(0.33)**		9.60 **(14.16)**	0.61 **(0.99)**		0.14 **(0.23)**	0.05 **(0.09)**		1.27 **(1.91)**	0.09 **(0.16)**		1.39 **(2.14)**	0.15 **(0.24)**	
BMI (kg/m^2)				<0.001 **(<0.001)**			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001
< 25	4876 **(3668)**	24.38 **(37.76)**	0.59 **(0.83)**		9.91 **(16.04)**	0.21 **(0.39)**		3.02 **(4.99)**	0.14 **(0.22)**		7.29 **(13.73)**	0.30 **(0.64)**		0.14 **(0.24)**	0.02 **(0.03)**		1.12 **(1.99)**	0.04 **(0.08)**		1.03 **(1.96)**	0.07 **(0.13)**	
25 to < 30	3044 **(2274)**	25.51 **(39.10)**	0.58 **(0.87)**		9.21 **(14.92)**	0.25 **(0.41)**		3.10 **(5.24)**	0.09 **(0.19)**		8.96 **(16.70)**	0.45 **(0.96)**		0.12 **(0.21)**	0.02 **(0.04)**		1.25 **(2.24)**	0.05 **(0.10)**		1.24 **(2.36)**	0.06 **(0.14)**	
≥ 30	2258 **(1607)**	23.23 **(37.02)**	0.76 **(1.06)**		8.53 **(14.09)**	0.30 **(0.53)**		3.08 **(5.34)**	0.15 **(0.28)**		7.36 **(13.36)**	0.46 **(0.99)**		0.09 **(0.17)**	0.02 **(0.04)**		1.06 **(1.91)**	0.06 **(0.12)**		1.00 **(1.90)**	0.07 **(0.18)**	
Measurement not taken	1975 **(1409)**	22.46 **(35.68)**	0.69 **(1.09)**		8.96 **(14.74)**	0.31 **(0.56)**		2.86 **(4.71)**	0.13 **(0.27)**		6.51 **(11.67)**	0.43 **(0.92)**		0.09 **(0.16)**	0.01 **(0.01)**		0.97 **(1.62)**	0.05 **(0.10)**		0.82 **(1.47)**	0.06 **(0.13)**	
Level of education				<0.001 **(<0.001)**			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001
Not applicable	2180 **(1676)**	23.64 **(35.87)**	0.70 **(1.08)**		11.36 **(18.06)**	0.35 **(0.61)**		2.65 **(4.21)**	0.14 **(0.22)**		5.39 **(10.10)**	0.38 **(0.81)**		0.12 **(0.22)**	0.01 **(0.02)**		0.96 **(1.68)**	0.06 **(0.11)**		0.62 **(1.16)**	0.09 **(0.17)**	
Post-Grad	770 **(629)**	30.09 **(44.42)**	1.47 **(1.92)**		10.08 **(15.66)**	0.57 **(0.81)**		3.70 **(5.88)**	0.22 **(0.40)**		11.46 **(20.61)**	0.86 **(1.68)**		0.12 **(0.20)**	0.02 **(0.02)**		1.57 **(2.69)**	0.10 **(0.20)**		1.71 **(3.09)**	0.14 **(0.28)**	
Bachelors	1615 **(1304)**	29.90 **(44.07)**	1.22 **(1.63)**		10.84 **(16.84)**	0.35 **(0.54)**		3.75 **(6.13)**	0.27 **(0.46)**		10.07 **(18.23)**	0.65 **(1.27)**		0.17 **(0.32)**	0.04 **(0.08)**		1.46 **(2.52)**	0.09 **(0.17)**		1.54 **(2.86)**	0.13 **(0.26)**	
Diploma/TAFE Courses	3252 **(2341)**	24.61 **(39.10)**	0.56 **(0.77)**		8.74 **(14.39)**	0.21 **(0.38)**		3.07 **(5.23)**	0.12 **(0.19)**		8.71 **(16.56)**	0.35 **(0.74)**		0.12 **(0.19)**	0.02 **(0.03)**		1.22 **(2.24)**	0.04 **(0.10)**		1.21 **(2.35)**	0.06 **(0.14)**	
No Non-School Qualification	4190 **(2893)**	20.57 **(32.99)**	0.43 **(0.71)**		8.08 **(13.54)**	0.22 **(0.44)**		2.72 **(4.68)**	0.07 **(0.17)**		6.07 **(11.02)**	0.25 **(0.59)**		0.10 **(0.18)**	0.02 **(0.04)**		0.89 **(1.55)**	0.03 **(0.06)**		0.80 **(1.48)**	0.04 **(0.08)**	
Level not determined	146 **(115)**	29.06 **(41.95)**	2.40 **(3.71)**		9.32 **(14.21)**	1.04 **(1.69)**		4.43 **(7.32)**	0.67 **(0.35)**		10.25 **(17.42)**	1.60 **(3.00)**		0.09 **(0.15)**	0.02 **(0.04)**		1.35 **(2.15)**	0.17 **(0.31)**		1.53 **(2.70)**	0.27 **(0.53)**	
Smoking status				<0.001 **(<0.001)**			<0.001			<0.001			<0.001			<0.01			<0.001			<0.001
Not applicable	2180 **(1676)**	23.64 **(35.87)**	0.70 **(1.08)**		11.36 **(18.06)**	0.35 **(0.61)**		2.65 **(4.21)**	0.14 **(0.22)**		5.39 **(10.10)**	0.38 **(0.81)**		0.12 **(0.22)**	0.01 **(0.02)**		0.96 **(1.68)**	0.06 **(0.11)**		0.62 **(1.16)**	0.09 **(0.17)**	
Smoker	1813	18.21	0.68		7.28	0.34		2.20	0.11		5.27	0.33		0.15	0.05		0.78	0.04		0.73	0.05	

Stratification variable	N	Anthocyanins(mg/d)			Cyanidins (mg/d)			Delphinidin (mg/d)			Malvidin (mg/d)			Pelargonidin (mg/d)			Peonidin (mg/d)			Petunidin (mg/d)		
		Mean	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**	**Mean**	**SE**	**P**
	(1073)	**(32.61)**	**(1.10)**		**(13.38)**	**(0.68)**		**(4.14)**	**(0.25)**		**(10.52)**	**(0.87)**		**(0.26)**	**(0.11)**		**(1.43)**	**(0.10)**		**(1.47)**	**(0.13)**	
Ex-smoker	3096	27.68	0.88		9.40	0.28		3.38	0.12		10.44	0.56		0.11	0.01		1.42	0.07		1.52	0.09	
	(2365)	**(41.65)**	**(1.34)**		**(14.81)**	**(0.45)**		**(5.42)**	**(0.21)**		**(18.77)**	**(1.10)**		**(0.17)**	**(0.01)**		**(2.48)**	**(0.13)**		**(2.82)**	**(0.18)**	
Never smoked	5064	24.33	0.45		9.18	0.20		3.24	0.11		7.65	0.27		0.12	0.01		1.12	0.03		1.06	0.05	
	(3844)	**(37.41)**	**(0.70)**		**(14.79)**	**(0.33)**		**(5.46)**	**(0.20)**		**(14.01)**	**(0.58)**		**(0.22)**	**(0.03)**		**(1.97)**	**(0.07**		**(1.96)**	**(0.10)**	
Physical activity				<0.001			<0.001			<0.001			<0.001			ns			<0.001			<0.001
				(<0.001)																		
Not applicable	2718	22.64	0.60		10.99	0.28		2.54	0.11		4.98	0.35		0.11	0.01		0.90	0.05		0.57	0.08	
	(2010)	**(35.28)**	**(0.93)**		**(17.91)**	**(0.48)**		**(4.09)**	**(0.19)**		**(9.53)**	**(0.75)**		**(0.20)**	**(0.02)**		**(1.60)**	**(0.10)**		**(1.09)**	**(0.15)**	
High	1328	30.04	1.11		11.34	0.48		3.92	0.35		9.58	0.72		0.11	0.01		1.45	0.09		1.47	0.14	
	(1066)	**(43.83)**	**(1.24)**		**(17.63)**	**(0.67)**		**(6.33)**	**(0.53)**		**(17.28)**	**(1.26)**		**0.20)**	**(0.02)**		**(2.49)**	**(0.17)**		**(2.73)**	**(0.24)**	
Moderate	2574	25.99	0.69		9.17	0.28		3.32	0.10		9.33	0.40		0.13	0.02		1.31	0.05		1.39	0.07	
	(1950)	**(39.50)**	**(0.96)**		**(14.55)**	**(0.43)**		**(5.53)**	**(0.20)**		**(17.07)**	**(0.83)**		**(0.21)**	**(0.04)**		**(2.30)**	**(0.10)**		**(2.62)**	**(0.15)**	
Low	3351	23.57	0.57		8.44	0.20		2.99	0.10		8.12	0.38		0.10	0.01		1.13	0.05		1.08	0.06	
	(2461)	**(37.13)**	**(0.83)**		**(13.92)**	**(0.37)**		**(5.06)**	**(0.19)**		**(15.05)**	**(0.78)**		**(0.19)**	**(0.03)**		**(2.01)**	**(0.09)**		**(2.01)**	**(0.12)**	
Sedentary	2075	20.42	0.73		7.40	0.27		2.68	0.15		6.44	0.45		0.15	0.04		0.90	0.05		0.86	0.07	
	(1398)	**(34.05)**	**(1.27)**		**(12.56)**	**(0.49)**		**(4.74)**	**(0.31)**		**(12.04)**	**(1.04)**		**0.25)**	**(0.08)**		**(1.63)**	**(0.13)**		**(1.62)**	**(0.17)**	
Not stated	107	26.59	4.93		12.55	3.28		3.28	0.67		7.15	1.54		0.21	0.12		0.99	0.17		1.00	0.22	
	(73)	**(39.30)**	**(6.77)**		**(19.03)**	**(5.12)**		**(4.86)**	**(1.15)**		**(11.82)**	**(3.13)**		**(0.37)**	**(0.23)**		**(1.57)**	**(0.32)**		**(1.78)**	**(0.45)**	

p values significant at <0.05 using ANOVA

Table 2: Top ten food sources of anthocyanins in the diet of the Australian population by age-group

% total anthocyanin intake per age group

Age (years)	2-3		4-8		9-13		14-18		19-30		31-50		51-70		71+	
n	464		789[1]		787		772[2]		1592		3565		2907		1277	
1.	Blackberry, raw	36.6	Blackberry, raw	65.2	Blackberry, raw	37.5	Cherry, raw	23.9	Grape, raw,[3]	15.4	Eggplant,[4]	8.1	Eggplant,[4]	23.1	Eggplant,[4]	26.4
2.	Raspberry, raw	12.8	Blueberry, raw	8.3	Blueberry, raw	12.7	Eggplant,[4]	15.7	Cherry, raw	8.5	Blackberry, raw	7.4	Grape, raw,[3]	9.1	Wine, red,	10.3
3.	Blueberry, raw	10.3	Grape, raw[3]	4.9	Grape, raw[3]	11.2	Grape, raw[3]	14.9	Cranberry, raw	8.2	Cherry, raw	5.7	Blackberry, raw	8.4	Cherry, raw	8.3
4.	Grape, raw[3]	8.6	Cherry, raw	2.6	Cherry, raw	7.3	Raspberry, purchased frozen	8.9	Eggplant,[4]	5.3	Blueberry, raw	5.1	Wine, red	7.8	Blueberry, raw	7.1
5.	Cabbage, red, raw	8.8	Raspberry, purchased frozen	2.4	Eggplant[4]	7.0	Blueberry, raw	8.5	Plum, unpeeled, raw	4.9	Raspberry, raw	4.9	Blueberry, raw	7.1	Blackberry, raw	5.1
6.	Cherry, raw	4.0	Plum, unpeeled, raw	2.4	Plum, unpeeled, raw	4.8	Cabbage, red, raw	5.9	Bean, black,[5]	4.5	Cranberry, raw	4.8	Cherry, raw	6.3	Plum, unpeeled, raw	4.9
7.	Raspberry, purchased frozen	3.4	Blueberry, purchased frozen	2.1	Raspberry, raw	3.2	Plum, unpeeled, raw	5.4	Raspberry, purchased frozen	3.9	Grape, black sultana, raw	4.6	Plum, unpeeled, raw	4.7	Raspberry, purchased frozen	4.7
8.	Plum, unpeeled, raw	3.2	Eggplant[4]	2.0	Raspberry, purchased frozen	2.6	Blueberry, purchased frozen	2.0	Blueberry, raw	3.8	Radish, peeled or unpeeled, raw	4.4	Raspberry, raw	2.9	Grape, Thompson,	3.4
9.	Eggplant[4]	2.7	Raspberry, raw	1.3	Apple, red skin, unpeeled, raw	1.6	Apple, red skin, unpeeled, raw	1.7	Wine, red	3.8	Raspberry, purchased frozen	4.3	Blueberry, purchased frozen	2.6	Radish, raw	3.2
10	Blueberry, purchased frozen	1.4	Apple, pink lady, unpeeled, raw	0.9	Pear, unpeeled, raw, not further defined	1.3	Pear, unpeeled, raw, not further defined	1.5	Raspberry, raw	3.2	Wine, red, sparkling	4.1	Cabbage, red, raw	2.4	Raspberry, raw	2.9
Sum		**91.8**		**92.1**		**89.2**		**88.5**		**61.6**		**53.5**		**74.4**		**76.3**

[1]n=2 excluded due to implausible dietary consumption data, [2]n=1 excluded due to implausible dietary consumption data, [3]Combination of Grape, raw, Grape, red, raw, and Grape, Thompson, seedless; [4]Eggplant, peeled or unpeeled, fresh or frozen, raw; [5]Bean, black, dried, boiled, microwaved or steamed, drained

Table 3: Association between anthocyanin intake and change in blood pressure in adults aged 50+yrs

BP parameters	Anthocyanin intake in all population (consumer population) Effect size (regression coefficient and 95% Confidence Interval) per unit (mg) increase					
	Model 1[1] n=4184 (3327)			***Model 2***[2] **n=4184 (3327)**		
	Regression coefficient	**95% CI**	***p value***	**Regression coefficient**	***95% CI***	***p value***
Systolic BP	-0.04 (-0.03)	-0.06, -0.01 (-0.05, -0.01)	0.001* (0.002)*	-0.04 (-0.03)	-0.06, -0.01 (-0.05, -0.01)	<0.01* (<0.01)*
Gender	-2.50 (-2.51)	-4.28, -0.72 (-4.45, -0.58)	0.01 (0.01)	-2.67 (-2.68)	-4.40, -0.94 (-4.62, -0.74)	0.003* (0.01)*
BMI				0.06 (0.1)	-0.002, 0.12 (-0.004, 0.1)	0.06 (0.07)
High BP diagnosis				-7.92 (-7.38)	-9.80, -6.05 (-9.63, -5.12)	<0.001* (<0.001)*
Smoking status				0.70 (1.06)	-0.06, 1.46 (0.11, 2.01)	0.07 (0.03)*
Physical activity				0.09 (0.15)	-0.61, 0.78 (-0.63, 0.92)	0.80 (0.7)
Diastolic BP	0.01 (0.01)	-0.01, 0.02 (-0.01, 0.02)	0.005* (0.03)	0.01 (0.01)	-0.01, 0.03 (-0.01, 0.02)	<0.01* (0.02)*
Gender	-1.56 (-1.31)	-2.51, -0.62 (-2.40, -0.22)	0.002 (0.02)	-1.52 (-1.30)	-2.49, -0.54 (-2.42, -0.18)	0.002* (0.02)*
BMI				0.06 (0.06)	0.03, 0.09 (0.02, 0.11)	<0.001* (0.01)*
High BP diagnosis				-0.77 (-0.54)	-1.76, 0.22 (-1.64, 0.55)	0.12 (0.33)
Smoking status				-0.24 (-0.19)	-0.64, 0.16 (-0.68, 0.30)	0.24 (0.44)
Physical activity				-0.09 (-0.04)	-0.53, 0.35 (-0.49, 0.42)	0.68 (0.88)

[1] Model 1 covariates = age (included as a class variable) and gender; [2]Model 2 covariates = age (included as a class variable), gender, BMI, hypertension diagnosis, Physical activity and smoking status; * Significant at p<0.05; values in bracket represent analysis for consumer population.

Table 4: Reported anthocyanin intake (mg/d) in population studies by country

Country (reference)	Sample size	Age/Gender[1]	Dietary assessment	Total anthocyanin intake (mg/d)
Australia (this study)	12,153	≥2yrs	2 x 24h DR (MSM method)	24.17
Australia (46)	10,851	≥2yrs.	24h DR	1.4
Australia (47)	79	≥49yrs	4-day WFR	7.0
China (62)	1393	35-75	FFQ	28[2]
Europe (55)		35-74yrs.	FFQ (GA²LEN)	
Denmark	268			7.5
Finland	122			5.9
Sweden	1,085			6.5
UK	139			9.8
Portugal	233			22.1
Belgium	107			10.5
Germany	305			5.5
The Netherlands Amsterdam	174			8.1
Poland	116			9.2
Europe (56)		35-74yrs.	24h DR	
Greece	2,687			31.82
Spain	3,220			31.58
Italy	3,953	35-74yrs./F (1 out of 5 centres)		42.79
France	4,735			37.42
Germany	4,415	35-74yrs./F		35.09
The Netherlands	3,980			22.56
UK	1,280	35-74yrs./F (1 out of 2 centres)		26.12
Denmark	3,917			28.21
Sweden	6,050			20.96
Norway	1,797	35-74yrs./F		26.56
Finland (63)	1950	42-60yrs./M	4-day food record	6.2
Finland (64)	2007	25-64yrs.	48h diet recall	47
France (65)	4942	45-60yrs.	≥6 24h diet recall	35
Spain (43)	40,683	35-64yrs.	Diet history questionnaire	18.88
United Kingdom (66)	1,997	18-76yrs./F	FFQ	18
USA (67)	8,809	>19yrs.	24h diet recall	3.1
USA (68)	5,420	≥20	24h diet recall	11.48

[1] gender specified when sample size is gender specific; WFR, weighted food record; 24hrDR, 24hr dietary recall; [2]excludes malvidin and petunidin