Title

Dietary intake of 20 polyphenol subclasses in a cohort of UK women

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Short running title

Polyphenol intake of UK women

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Abstract

Background Establishing and linking the proposed health benefits of dietary polyphenols to their consumption requires measurement of polyphenol intake in appropriate samples and an understanding of factors that influence their intake in the general population.

Methods This study examined polyphenol intake estimated from 3 day and 7 day food diaries in a sample of 246 UK women aged 18-50 years. Estimation of the intake of 20 polyphenol subclasses commonly present in foods consumed by the sample studied was done using Phenol-Explorer® and USDA polyphenol databases. Women were potential participants in the Leeds Women's Wellbeing Study (LWW) (N= 143), a dietary intervention study aimed at overweight women (mean age: 37.2 ± 9.4 years; mean BMI: 30.8 ± 3.1 kg/m^2) and the Diet and Health Study (DH) (N = 103) which aimed to examine the relationship between polyphenol intake and cognitive function (mean age: 25.0 ± 9.0 years; mean BMI: 24.5 ± 4.6 kg/m^2).

Results The estimated intake of polyphenol subclasses was significantly difference between the two samples ($p<0.01$) with consumption of 1292 ± 844 and 808 ± 680 mg/day for the LWW and DH groups respectively. Flavanols and hydroxycinnamic acids were the most important contributors to the polyphenols consumed by both groups, owing to tea and coffee consumption. Other major polyphenol food sources included fruits, vegetables and processed foods.

Conclusion Older women consumed more polyphenol-containing foods and beverages, which was due to the higher coffee and tea consumption amongst the LWW participants.

Keywords Polyphenols. flavonoids. phenolic acids. food diary. Phenol-Explorer

Introduction

Dietary assessment is an important technique for estimating food intake. This process first requires a reliable collection of food intake data, followed by accurate and appropriate analysis of food intake using available comprehensive databases which provide details of the nutrient content of foods. Two polyphenol databases that are widely used in the estimation of polyphenol intake are the United States Department of Agriculture (USDA) [1] and the Phenol-Explorer® [2] databases.

Several studies have estimated polyphenol intake and their association with health benefits in various parts of the world. For example, a recent study identified an association between daily flavonoid and stilbene intake and lipid profiles amongst Chinese adults [3]. The emphasis in this study was on fruit, vegetables and nuts which are commonly consumed by the Chinese population. A study of Iranian adults reported a lower prevalence of metabolic syndrome in participants with higher dietary intake of selected polyphenols estimated using Phenol-Explorer® [4]. Another study from Spain which also used Phenol-Explorer® found a reduction in cardiovascular disease risk amongst participants with greater intake of dietary polyphenols [5]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study estimated intake of particular flavonoids (flavonols, flavanones and flavones), anthocyanins, phytoestrogens, lignans and phenolic acids in ten European countries using 24 hour dietary recall methods [6-10]. Within the EPIC study, the UK "health conscious" cohort, which includes fish eaters, vegans and lacto-ovo vegetarians, consumed higher amounts of flavanones [6], anthocyanins [7], and phytoestrogens [8], but lower total phenolic acids [10] as compared to the general population. However, no comparison could be made for total flavonoids because only total flavonoid intake data of the general population from EPIC participating countries were presented [11]. In the EPIC study, tea and fruit were the major flavonoid contributors for the UK sample [11] but non-flavonoid phenolics were not considered, nor was the impact of body weight and age on polyphenol source or consumption.

In this study, we took advantage of two existing samples, potential participants in the Leeds Women's Wellbeing Study (LWW) and the Diet and Health Study (DH), since both studies required potential participants to complete 3 or 7 day food diaries, but polyphenol intake was not emphasized and therefore these data provide incidental assessment of the polyphenol intake of UK women. The different study aims – LWW was a dietary intervention study targeted women who wanted to make dietary changes to improve their health and wellbeing and maintain a healthy body weight and DH examined the relationship between habitual polyphenol intake and cognitive function targeted young, healthy women – attracted different samples of women. Together these

studies allowed us to estimate the effect of age and BMI on the intake of a wide range of polyphenols in the UK population.

Materials and methods

Participants and study design

This investigation employed a cross sectional design where habitual polyphenol intake was assessed using food diaries. The diaries were collected from two different studies, namely the Leeds Women's Wellbeing Study (LWW) (NHS ethics reference number: 10/H1305/6) and the Diet and Health Study (DH) (Ref No: 12-0020). The LWW data were collected between 20/04/2010 and 10/08/2011 while DH study was collected between 01/06/2012 and 30/06/2013. Both studies were conducted in the Human Appetite Research Unit (HARU) at the Institute of Psychological Sciences, University of Leeds. LWW study was intended to facilitate weight loss through two approaches; healthy eating advice alone or healthy eating with extra advice to increase fibre intake to a minimum of 25 g/day amongst overweight and obese women. Data taken from the LWW study were socio-demographic information and 7 day food diaries collected during the screening phase of the study. The inclusion criteria for the DH study were; women aged 18 to 50 years, not pregnant, non-smoker, normal body mass index (BMI) and above (≥18.5 kg/m2) and English as their first language.

Dietary assessment

Food intake was assessed using a self-completed food diary. 7-Day food diaries were collected from LWW participants during the screening phase prior to entering a weight loss intervention trial. For the DH study, a 3-day food diary in which all food consumed for 2 weekdays and 1 weekend day was given to participants during their first visit and was returned on their second visit at least one week later, so that the diary was completed between visit one and visit two. Participants were encouraged to record their food intake using household measures and to include the food packaging within the diary where possible. The participants were informed how to fill in the food diary and were shown examples of good dietary recording from example diaries. Food intake data from the food diaries were analysed using WinDiets®. This software comprised of two food databases; namely UK Food Tables 2008 and USA Food Tables 2008. The data were inputted in gram (g) of

foods consumed by the participants. To facilitate the approximation of portion size, the latest food portion guideline book for selected UK foods was used in the study [12]. Basal metabolic rate (BMR) was calculated using Schofield equations [13]. The BMR value was used to verify accuracy of dietary recording of the participants and was divided by energy intake (EI/BMR) to identify incidences of underreporting. Underreporting is assumed when the EI/BMR is less than <1.14, normal is in the range 1.14 to 2.4 and over reporting is >2.4 [14].

Estimation of polyphenol intake

Foods which did not contain any polyphenols such as meat-based products were omitted from the estimation of polyphenols. Ingredients of processed foods such as canned foods and pre-packaged meals were checked for polyphenol-containing ingredients. Foods that contained more than 1 mg per serving of any polyphenol were identified using the Phenol-Explorer® database [2] when possible, and in combination with the USDA database [1] on selected flavonoids to enable examination of the polyphenol content of as many foods as possible. Only ingredients with a polyphenol content of ≥ 1 mg per serving were included in the calculation of polyphenol intake. Data for polyphenol content obtained from Phenol-Explorer® was selected from mean content obtained from methods involving chromatography. Missing data from fruit, such as citrus fruits and sultanas, were estimated based on tangerine and raisin data from USDA and Phenol-Explorer® respectively. For other food groups which are mainly comprised of processed foods, the estimation was made according to the percentages of ingredients in the food products. Data for thearubugins from the USDA database was added to the existing data in Phenol-Explorer® because this compound is a major contributor to the flavanol content of tea [15]. Data for proanthocyanidins obtained from Phenol-Explorer® in the form of dimers and trimers were added together and presented in the flavanols group. Twenty polyphenol subclasses were selected for the estimation on the basis that these compounds are commonly present in foods consumed by the sample studied. The cut off used for foods to be included in the polyphenol estimation was based on a previous study which referred to foods that contributed less than 1 mg/day as minor contributors to polyphenol intake [16]. Thus, foods that contained less than 1 mg of polyphenols as consumed in a usual portion were excluded from the analysis. Polyphenol intake was presented based on average intake per day.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS, version 19). All data were examined for outliers using boxplots and the normality assumptions were checked for each inferential analysis. There was no significant deviation from normality and sample size was sufficient to retain all data with no outliers excluded from the analysis. Data from continuous variables are presented as mean ± standard deviation. Percentages are used for categorical variables. Polyphenol intake data from LWW and DH samples were combined to provide a better estimation and representativity of the polyphenol intake amongst UK women. The results are presented in two ways; namely, a comparison between study groups (LWW vs DH) to identify differences between 3 days and 7 days food recording and between beverage consumption groups. Chi-squared tests were used to identify the association between two categorical variables. Independent t-tests and analysis of variance (ANOVA) models were used to test differences in polyphenol intakes between study group and beverage consumption group. In order to examine the influence of age and BMI, these continuous variables were included in an ANCOVA with study group as a between-subjects factor and polyphenol intake as the dependent variable. Data which were not normally distributed were analysed using non-parametric tests of differences between groups; namely Mann-Whitney U-test for two groups and Kruskall-Wallis test for more than two groups. In all analyses, p values of <0.05 or <0.01 were considered statistically significant.

Results

Table 1 presents the characteristics of participants according to the two study samples (LWW and DH). There was a significant difference in age and BMI between the two study groups ($p<0.01$). LWW participants were older and heavier than DH participants since the former were recruited specifically because they were overweight and intended to participate in a weight loss intervention. Furthermore, higher BMI is associated with increasing age [17]. More of the DH participants performed regular exercise ($p<0.05$) and were students ($p<0.01$) than the LWW participants. There was no significant difference between the two study samples in the frequency of participants in the EI/BMR categories. Under reporters were more frequent amongst DH participants (45.1%) as compared to LWW participants (38.5%) and none over reported energy intake.

Table 2 represents the polyphenol food sources and the polyphenols contained in each food, commonly consumed by the participants. In this study, coffee and tea were the major beverages consumed. Various types of tea including black, green, camomile, and fruit tea were consumed by the participants. Onion, potato and tomato were the most important vegetables contributing to polyphenol intake. Commonly consumed fruits were

bananas and apples, while processed foods, such as milk chocolate, baked beans, hummus, ready to cook sauces and soups, were the most important sources of polyphenol intake.

A comparison between the LWW and the DH studies was made for specific polyphenol intake (Table 3) based on the average intake per day derived from the 3 day and 7 day diaries respectively. Overall, the intake of polyphenols for the LWW group was higher than DH except for dihydrochalcones and lignans. These differences might be due to the higher coffee and tea consumption and the greater diversity of food sources consumed by the LWW participants. The daily intake of all major polyphenol groups was significantly different between the two studies ($p<0.01$), whereby LWW participants' intakes were higher. Moreover, the daily intake of polyphenol subclasses also showed a significant difference between the two studies ($p<0.01$) with mean intakes of 1292 ± 844 and 808 ± 680 mg/day for LWW and DH participants respectively. This finding can be explained by higher energy (kcal) intake of LWW participants in addition to positive association found between energy (kcal) intake and polyphenol subclasses intake ($r = 0.237$, $p<0.0001$). Moreover, when age and BMI were included as covariates in an ANCOVA to compare polyphenol intake between LWW and DH participants, both age ($F1, 242 =117.18$, $p<0.001$) and BMI ($F1, 242= 4.203$, $p<0.05$) were significant covariates and were positively related to total polyphenol intake per day but the difference in polyphenol intake between the LWW and DH samples was no longer significant suggesting that differences can be accounted for by age and BMI.

In order to identify the contribution of polyphenol sources other than coffee and tea, a comparison was made between intake of total polyphenol subclasses (Total dataset) and intake excluding coffee and tea (NoCorT dataset) (Table 4). The average intakes of polyphenol subclasses were 1089 ± 814 and 213 ± 129 mg/day for Total and NoCorT datasets respectively. Clearly some polyphenols are only present in coffee or tea, or in fruit and vegetables, but others are present in more than one group. The relative percentages of NoCorT to Total datasets were calculated to identify the contribution of coffee and tea polyphenols to polyphenol intake. A value of 100% indicates that all polyphenols are derived from fruit and vegetable sources, whereas a value of 0% indicates that beverages provide all the polyphenols in the category. The alkylmethoxyphenol and flavanol content of the diets came almost entirely from coffee and tea intake. Hydroxybenzoic acids and hydroxycinnamic acids were also mainly derived from the beverage sources. Overall, the intake of polyphenols after the addition of total flavonoids, total phenolic acids and total all other polyphenols was ~5-fold greater for the Total dataset as compared to the dataset excluding coffee and tea (NoCorT). The major polyphenol food sources for participants who did not consume coffee and tea mainly came from vegetables (e.g. onions, potatoes,

broccoli, beans), fruits (e.g: strawberries, blueberries, apples), wholemeal bread, chocolate and chocolate drink. These foods were frequently consumed by the participants, however, no quantification was made to determine the percentage of contribution of the foods to the intake of polyphenol subclasses.

Discussion

This study focused on the habitual polyphenol intake of women in the UK. Women have an important role in food selection and consumption within the family [18]. In addition, women reportedly perceive themselves to be more conscious about food, more likely to read nutritional labels, practise healthy eating and be more knowledgeable about health and nutrition as compared to men [19]. The higher number of under reporters in the DH study may be related to age and practising certain dietary restrictions for weight maintenance. A previous study has suggested that young women tend to perceive themselves as overweight, thus efforts to lose weight are becoming more common [20]. However, this might also reflect underreporting of actual intake rather than lower intake per se. Underestimation of 37 % was previously reported in a study that used food recording as tool for measurement of total energy intake when compared to the doubly labelled water method [21]. In an effort to minimise underreporting, participants were advised to be honest about their intake, especially with respect to the intake of foods which might be perceived as "unhealthy" such as confectionery or snacks.

A comparison of polyphenol subclasses intake was made between under and normal reporters, and no significant difference was found. This finding can partly be explained by the perception that coffee and tea drinking are not considered unhealthy habits therefore, participants are more likely to have reported their consumption honestly. Furthermore, as tea and coffee dominate as sources of polyphenol subclasses intake but contribute few, if any, calories there would be little impact on energy intake. Furthermore, flavonoids and phenolic acids which are widely present in fruit and vegetables would be less likely to be under reported by the participants because these foods are considered healthy. Moreover, participants from the DH study were informed that the objectives of the study were to examine the effects of polyphenols and the major sources of polyphenols were briefly explained in the participant information sheet which should encourage rather than discourage reporting of these foods. Knowing the purpose of a study can encourage socially desirable responses. DH participants were expected to over report their polyphenol intake as compared to LWW participants. However, the opposite finding was demonstrated in this study. In relation to food intake, this is often reflected by over reporting of foods perceived to be healthy and underreporting of foods perceived to be unhealthy. Previous research has reported that participants believed that the consumption of foods perceived to be 'good' in

219 larger quantities would promote less weight gain [22]. To overcome this problem, surreptitious recording of
220 food intake or disguising the purpose of the study is recommended so that emphasis is drawn away from the
221 particular food groups under study.

222 In this study, it was apparent that there were more participants from both groups who consumed both
223 coffee and tea (39 %) or consumed tea only (32.5 %) than just coffee (11.8 %) or neither tea nor coffee (16.7
224 %). The average volume of coffee and tea consumed was 160 ± 239 and 328 ± 377 ml/day respectively. Higher
225 daily tea consumption (814 ± 450 ml/day) was reported from a longitudinal study amongst men in South Wales
226 [23]. The men were older than the current sample and were mainly working class in an industrial town, where
227 tea would be a routine part of their daily lives. Thus they represent a very different group to the average UK
228 population and to the samples considered in our study. From our data, consumers of both coffee and tea were
229 shown to drink more tea than coffee in terms of volume consumed daily. A similar finding was reported in a
230 study amongst Scottish adults, whereby high tea consumers were likely to drink less coffee [24].

231 The determination of major polyphenol food sources can be made by assessing the amount of
232 polyphenols present in food and the quantity of food consumed [25]. In addition, the determination of
233 polyphenol food sources relies on two aspects. Firstly, whether the foods have a high polyphenol content, so
234 even if a small amount is consumed the contribution to polyphenol intake is significant. Secondly, some foods
235 are consumed in large quantities however, because of their low polyphenol content, their contribution to intake
236 of individual subclasses is not significant. An example of the first situation is spinach and onions which have a
237 high polyphenol content, while the second is pineapple and cabbage which have a low polyphenol content.
238 Conversely, coffee and tea fulfil both aspects whereby these beverages are consumed in high amounts and have
239 a high polyphenol content and this is why they dominate the Total dataset.

240 The total polyphenol intake as reported from other studies ranges from 800 to 1200 mg/day [5, 16, 26,
241 27]. The value of total polyphenols estimated in this study by summing 20 polyphenols is within a reasonable
242 range when compared to the other studies. The main polyphenol food sources for the studies with total
243 polyphenol intake above 1 g per day are beverages such as coffee, tea and fruit juices as reported by study from
244 France and Poland [16, 27]. The other polyphenol food sources include fruit, vegetables, legumes and cereal
245 products. The disparity between all these studies in the estimation of total polyphenols can partly be explained
246 by the different number of polyphenol subclasses included in the estimation of polyphenol intake. The different

databases used to estimate polyphenol intake also can contribute to the differences in total polyphenol estimation between countries.

In terms of food intake, data from the food diaries demonstrates that the major polyphenol food sources consumed by the studied samples, such as tea, coffee, potatoes and apples are similar to those reported from previous studies [16, 27]. An Australian study also identified black and green tea as major flavonoid food sources along with wine, apples and oranges [28]. A recent study has estimated the total flavonoid intake amongst the non-Mediterranean countries in Europe including Germany, the Netherlands, UK, Sweden and Norway [11]. This study reported two major contributors to flavonoid intake of the non-Mediterranean countries namely tea and fruits, with the UK population showing the highest intake of total flavonoids (average of 549 mg/d in men and 502 mg/d in women). Tea was also the major contributor to flavonoid intake in our study. An implication of this is the possibility that health promotion to increase the serving size of fruit and vegetables as a good source of polyphenol foods can also emphasize the point that these two food sources are also significant contributors to polyphenol intake.

In addition, the inclusion of thearubigins in the estimation of flavanols was demonstrated to be an important approach for a better estimation of polyphenol content in tea. The importance of this compound was reported by the EPIC study which focused on thearubigin intake in several European countries [29]. This study has reported that the UK general population were the highest tea consumers, with 48 % of total flavonoids being contributed by thearubigins. However, there is a possible limitation in the usage of data on thearubigins from USDA.

The current study was limited by its small sample size with a large age range (18 – 50 years). In addition, being health-conscious might be a possible motivating factor for the participants to volunteer for these two studies, and might influence the foods consumed (or reported) by the participants during the dietary recording. Thus, the representativeness of this sample to the general female population of the UK may be somewhat limited. Finally, there is a substantial lack of available information on the polyphenol content of processed foods. Food recording can possibly cause some alterations in the habitual food intake of the participants. However, to deal with this possibility, participants were encouraged to bring all food packaging along with them in case they had difficulties in explaining the food portion size. The estimation of certain foods was made based on the percentage of polyphenol-containing ingredients in the food products.

The average polyphenol intake of the whole sample, estimated from 20 polyphenol subclasses present in commonly consumed foods, exceeded 1 g per day. The intake of polyphenol subclasses was higher amongst LWW participants, whilst DH participants had 37.5% lower polyphenol subclasses intake than the LWW participants, despite being aware of the polyphenol focus of the study. In addition, 56% of LWW participants consumed more than 1 g polyphenols/day compared to DH (36 %). These effects can be explained by the significant differences in age and BMI between the two study samples which account for the difference in polyphenol intake. The major polyphenol food sources of the women studied in this study were tea and coffee, thus women who did not consume tea or coffee had much lower average polyphenol intake.

Future studies should be longitudinal in design, and include samples which vary in socio-economic status, age and BMI. In addition, the effect of food processing on the polyphenol content of foods should be taken into consideration in subsequent research in order to better estimate polyphenol intake.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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