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Title page

Adherence to UK dietary guidelines is associated with higher dietary intakes of total and specific polyphenols compared to a traditional UK diet: further analysis of data from the CRESSIDA randomised controlled trial.

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Abstract

Adherence to dietary guidelines may result in higher intakes of polyphenols via increased consumption of fruits, vegetables and whole grains. We compared polyphenol dietary intakes and urinary excretion between two intervention groups in the CRESSIDA study: a 12-week, parallel-arm, randomised controlled trial (n=161; 64 M, 97 F; aged 40-70 y). One group adhered to UK dietary guidelines (DG) whereas the other group consumed a representative UK diet (control). We estimated polyphenol dietary intakes, using 4-day food diaries (4-DFD) and food frequency questionnaires (FFQ), and analysed twenty-four hour polyphenol urinary excretion by LC-MS/MS on a subset of participants (n=46 control; n=45 DG). A polyphenol food composition database for 4-DFD analysis was generated using Phenol-Explorer and USDA databases. Total polyphenol intakes by 4-DFD at endpoint (geometric means with 95% CI, adjusted for baseline and gender) were significantly higher in the DG group (1279 mg/d/10 MJ; 1158, 1412) compared to the control group (1084 mg/d/10 MJ; 980, 1197). The greater total polyphenol intakes in the DG group were attributed to higher intakes of anthocyanidins, proanthocyanidins and hydroxycinnamic acids, with the primary food sources being fruits, cereal products, nuts and seeds. FFQ estimates of flavonoid intakes also detected greater intakes in DG compared with the control group. Twenty-four hour urinary excretion showed consistency with 4-DFD in their ability to discriminate between dietary intervention groups for 6 out of 10 selected, individual polyphenols. In conclusion, following UK dietary guidelines increased total polyphenol intakes by approximately 20%, but not all polyphenol subclasses corresponded with this finding.

1 **Introduction**

2 Greater consumption of fruit and vegetables and whole grains is associated with reduced risk of chronic
3 diseases^(1, 2, 3, 4, 5). Increased intakes of different components present in these food groups, like fibre⁽⁶⁾,
4 micronutrients⁽⁷⁾ and polyphenols⁽⁸⁾, have been identified as being partly responsible for beneficial
5 effects. However, results from the UK National Diet and Nutrition Survey (NDNS) rolling
6 programme^(9, 10, 11) reveal that consumption of fruit and vegetables and whole grains is below
7 recommended intakes in at least 70% of the UK adult population^(9, 10, 11, 12). Dietary polyphenols are a
8 diverse range of phytochemicals containing 1 or more aromatic rings attached to a hydroxyl group. The
9 term “polyphenol” commonly encompasses phenolic acids, flavonoids, stilbenes and lignans, which are
10 derived from a wide range of plant foods including fruits, vegetables, and cereals. However, beverages
11 such as tea, coffee, red wine, and fruit juices represent the main dietary sources⁽¹³⁾. Epidemiological
12 studies have shown a negative relationship between consumption of polyphenols and cardiovascular
13 disease^(14, 15, 16, 17, 18), cancer^(19, 20, 21, 22) and type 2 diabetes^(18, 23, 24, 25, 26). Multiple mechanisms have
14 been identified that may contribute to any direct causal relationship between dietary polyphenols and
15 prevention of chronic diseases, including modulating inflammatory pathways, exerting effects on
16 oxidative signalling and enzyme activity, and regulation of gene expression⁽⁸⁾.

17 Previous reports in UK populations, using either 24 h recall^(27, 28, 29, 30, 31) or food diaries^(27, 28, 29, 30, 31),
18 suggest that habitual polyphenol intakes are in the range of 800-1600 mg/d, depending on the
19 population studied. However, the use of different dietary assessment methods, food polyphenol
20 composition databases, and gaps in polyphenol composition data for certain foods limit the reliability
21 of current intake estimates in various countries⁽³²⁾. Food diaries may provide a relatively accurate
22 estimate of polyphenol intake and are more suitable for dietary intervention studies with smaller study
23 populations, but they reflect short-term intakes rather than habitual consumption patterns, which could
24 be particularly misleading for seasonally available foods⁽³³⁾. Research groups have designed and
25 validated FFQs to estimate dietary flavonoid intake in different populations^(34, 35), which may provide
26 more reliable habitual intake estimations for specific populations. However, these remain unavoidably
27 susceptible to bias due to self-reporting errors, portion size quantification and estimation errors
28 resulting from the lack of data on polyphenol content in food⁽³⁶⁾. Whichever dietary assessment
29 method is selected, the resulting intake data will only be accurate if the polyphenol composition of
30 foods database is fit for purpose. At present, the most commonly used food polyphenol composition

31 databases are Phenol-Explorer^(37, 38, 39), which provides information on the content of 502 polyphenols
32 (of the 4 classes) in 459 food items including aglycones, glycosides, and esters, and the USDA
33 databases^(40, 41, 42) which includes 35 flavonoids (aglycones only) in 506 food items, respectively.
34 Urinary excretion of polyphenols has been shown to be a suitable biomarker for the intake of
35 polyphenols^(43, 44), fruit and vegetables^(45, 46), polyphenol-rich beverages^(47, 48) and polyphenol-rich
36 food^(49, 50). The presence of polyphenol metabolites in urine is closely related to the quantity consumed
37 and overall metabolism in the body. However, different polyphenols can produce common metabolites
38 and so the biomarker selected must reflect the specific intake of the parent polyphenol in question in
39 order to accurately estimate intakes of individual phenolic compounds⁽⁵¹⁾.

40 Dietary guidelines in the UK are population-based recommendations for maintenance of health and
41 wellbeing, and to reduce risk of chronic diseases. No dietary recommendations exist for polyphenols,
42 although the advantages and disadvantages of this approach have been debated^(52, 53, 54). A dietary
43 pattern consistent with current dietary guidelines could reasonably be assumed to be richer in
44 polyphenols than the average UK dietary pattern due to increased intakes of fruit and vegetables and
45 whole grains. However, not all fruits and vegetables are polyphenol-rich⁽⁵⁵⁾, and in fact the majority of
46 dietary polyphenols in the UK are provided by tea, coffee, and cocoa intakes⁽³⁰⁾. The aim of this study
47 was to compare dietary polyphenol intakes in a free-living study population randomised to either
48 following UK dietary guidelines, or consuming a representative, more traditional UK diet. The
49 hypothesis was that adherence to UK dietary guidelines would result in an increase in total dietary
50 polyphenol intakes compared with a diet that was more representative of the UK adult population. The
51 primary outcome variable was total polyphenol intake, adjusted for energy intake, assessed by 4-day
52 food diary. Secondary outcome variables included intakes of classes, subclasses and individual
53 phenolic acids/polyphenols. Data were also compared with FFQ⁽¹⁵⁾, and biomarkers of polyphenol
54 intake (24 h excretion of representative urinary metabolites).

55 **Methods**

56 **Study design**

57 The Cardiovascular risk REduction Study: Supported by an Integrated Dietary Approach (CRESSIDA)
58 was a 12-week parallel-designed, randomised, controlled trial funded by the Food Standards
59 Agency/Department of Health (UK) (N02047), sponsored by King's College London. This study was
60 conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures

61 involving human subjects were approved by the St. Thomas' Hospital Research Ethics Committee
62 (10/H0802/24)⁽⁵⁶⁾. Written informed consent was obtained from all subjects. This trial was registered at
63 Current Controlled Trial (<http://www.controlled-trials.com/ISRCTN>) as ISRCTN92382106. The
64 primary aim of the original study⁽⁵⁶⁾ was to assess effects on CVD risk factors when following a diet
65 based on UK dietary guidelines (DG) in comparison to a representative UK diet (control). A sample
66 size of 78/group had 90% power to detect a 4 mm Hg effect of diet on daytime systolic ambulatory
67 blood pressure (alpha 0.05); this sample size was also large enough to detect a 5% change in the ratio
68 of total cholesterol (TC):HDL cholesterol and a 1% unit change in flow-mediated dilatation of the
69 brachial artery with sufficient statistical power. The results of these primary outcomes have been
70 published previously⁽⁵⁶⁾.

71 The data presented here comprises a secondary analysis of dietary intake data from both the DG and
72 control groups at baseline and endpoint of the 12-week dietary intervention period, to determine
73 whether adherence to UK dietary guidelines results in an increase in polyphenol intake.

74 **Participants**

75 Healthy men and women [BMI (in kg/m²) ≥ 18.5 and ≤ 35] were recruited from the London area
76 (August 2010–July 2012) by newspaper (London Metro) and electronic advertisement (King's College
77 London e-mail and website). Participants between the age of 40-70 y were chosen because risk
78 increases markedly with age and an upper age limit of 70 y was selected because absolute annual risk
79 of CVD exceeds 2% beyond that age in the majority of people, and a higher proportion are receiving
80 medication on daily basis. A full list of inclusion and exclusion criteria has been reported previously⁽⁵⁶⁾.
81 For randomisation a purpose-designed clinical database was used (MedSciNet AB, Stockholm,
82 Sweden), which undertook the minimisation randomisation, balancing the treatment arms for the
83 minimisation variables of gender, age and ethnicity⁽⁵⁶⁾. Participants received dietary advice in person at
84 baseline and at week 4, and by phone at week 6 and 8. Dietary assessment included two FFQ and two
85 four-day food diaries (4-DFD) administered at baseline and endpoint and two 24-h recalls administered
86 at weeks 4 and 8. Urine samples were collected at four time points; baseline, week 4, 8 and endpoint.

87 **Dietary advice**

88 Participants randomised to the DG group were advised to increase fruit and vegetables intake to 5
89 portions/day; whole grains intake to $>50\%$ of cereal intake; to consume 2 portions of fish per week (1
90 of which should be oily); to replace full-fat with reduced fat dairy products; to replace fats rich in

91 saturated fatty acids with spreads/oils low in saturated fatty acids and high in monounsaturated fatty
92 acids; to restrict salt intake to <6 g/d (<100 mmol/d) and to reduce intake of free sugars. The control
93 diet comprised a nutritionally balanced, traditional UK diet, formulated with familiar foods (full cream
94 milk, cheese, butter, meat and meat products, non-wholegrain cereals), reflecting typical UK intakes of
95 fruit and vegetables (3 portions/d), with a higher content of saturated fatty acids (14% energy),
96 unrestricted intakes of salt and sugar, and low intakes of oily fish. The study included provision of
97 spread, oil, whole grain pasta, rice and cereal bars, minimally processed wholegrain breakfast cereal
98 (oats, muesli, etc.), almonds and macadamia nuts and tinned oily fish to the intervention group and
99 spread, oil and refined cereals as pantry items to the control group. Participants of both groups were
100 instructed to refrain from taking nutritional supplements during the study⁽⁵⁷⁾.

101 **Four-day food diary (4-DFD)**

102 Dietary intake of polyphenols was quantified from 4-DFD, at baseline and endpoint of the 12-wk
103 intervention. A polyphenol food composition database was generated using Phenol-Explorer^(37, 38, 39)
104 and USDA databases^(40, 41, 42), and extended using polyphenol retention factors and recipes provided by
105 participants. If a recipe was not provided by a participant, a standard recipe was obtained from either
106 the UK food tables⁽⁵⁸⁾, a UK food industry recipe book⁽⁵⁹⁾, the BBC Good Food website⁽⁶⁰⁾ or additional
107 websites specialised in UK recipes and other countries' typical cuisines. In total 118 recipes were
108 obtained from participants, 93 from UK food tables, 20 from a UK food industry recipe book, 78 from
109 the BBC Good Food website and 94 from other websites. Polyphenol intake was estimated for four
110 classes of polyphenols: flavonoids, phenolic acids, lignans and stilbenes. An additional group of
111 polyphenols were included under the name "other polyphenols"; since Phenol-Explorer grouped a
112 series of compounds including alkylmethoxyphenols, alkylphenols, curcuminoids, furanocoumarins
113 and tyrosols. The flavonoid intake was analysed in eight subclasses: anthocyanins, dihydrochalcones,
114 flavanols (flavan-3-ols monomers and theaflavins), proanthocyanidins, flavanones, flavones, flavonols
115 and isoflavones, for each subclass a range of 2 to 11 of the most representative individual compounds
116 were selected. For phenolic acids subclass 2 groups were analysed: hydroxybenzoic acids and
117 hydroxycinnamic acids, for each group a range of 4 to 6 of the most representative individual
118 compounds were selected. For lignans subclass, 4 individual compounds were analysed: pinoresinol,
119 lariciresinol, secoisolariciresinol and matairesinol. For stilbenes subclass, resveratrol was selected and
120 analysed. A total of 52 individual polyphenols were analysed and 1141 food items were included in the
121 final database.

122 **Food frequency questionnaires (FFQ)**

123 Dietary intake of flavonoids was quantified from FFQ (EPIC-Norfolk FFQ v.6)⁽⁶¹⁾ at baseline and
124 endpoint of the 12-wk intervention. Flavonoid intake was estimated from 6 subclasses and most
125 representative compounds for each: flavanones (hesperetin, naringenin, eriodictyol), anthocyanins
126 (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin), flavones (apigenin, luteolin),
127 flavonols (quercetin, kaempferol, myricetin, isorhamnetin), flavan-3-ols (catechin, epicatechin,
128 gallic acid, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate) and polymers
129 (theaflavins, thearubigins and proanthocyanidins). In total 32 individual flavonoids were analysed
130 using a database created and kindly provided by Dr Amy Jennings and Prof Aedin Cassidy at
131 University of East Anglia⁽¹⁵⁾ and modified for the requirements of the FFQ analysis, so that the
132 flavonoid content of 130 food items were analysed. FFQ registers the frequency of consumption of
133 specific foods, of standard portion size, in a month. There were nine answer options which varied from
134 “never or less than once in a month” to “six or more per day”. To calculate the total intake of
135 subclasses of flavonoids, frequencies were converted to daily portions and multiplied by flavonoid
136 content in each specific food item; the results were summed for each subclass and for each participant.

137 **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)**

138 Twenty-four hour urine collections were made as previously described⁽⁵⁶⁾, using boric acid as
139 preservative⁽⁶²⁾ and aliquots stored at -80 °C until analysis. Completeness of urine collection was
140 measured using recovery of para-aminobenzoic acid according to a standard protocol⁽⁶³⁾. LC-MS/MS
141 analysis was only performed on endpoint urine samples, collected at week 12 of the intervention to
142 coincide with dietary assessment by 4-DFD. A previously published protocol for polyphenol analysis in
143 urine⁽⁴⁷⁾ was adapted and developed for the quantification of ten aglycone metabolites of polyphenols.
144 A subsample of the CRESSIDA study was selected for the analysis; the selection was based on the fruit
145 and vegetables (F&V) intake reported in the endpoint FFQ, subsample included participants in the
146 control group reporting the lowest intakes of F&V, and participants in the intervention group reporting
147 the highest intakes of F&V were analysed. Quantification of endpoint samples allowed the comparison
148 between groups at the end of the dietary intervention; the aim was to detect greater amounts of
149 polyphenols excreted in urine by participants who reported consuming the most polyphenols by FFQ
150 compared to participants who reported consuming the least, and to determine agreement with food
151 diary data. One or two of the most representative aglycones of different polyphenol subclasses were
152 selected for quantification. Ten phenolic compounds were analysed; phloretin (dihydrochalcones),

153 epicatechin (flavan-3-ols), hesperetin and eriodictyol (flavanones), luteolin (flavones), quercetin
154 (flavonols), daidzein (isoflavones), gallic and vanillic acid (phenolic acids) and enterolactone as a
155 product of microbial metabolism of lignans in colon. The phenolic compounds selected for
156 quantification have been suggested as suitable biomarkers of polyphenol intake⁽⁵⁵⁾ with a high recovery
157 and good correlation with fruit and vegetable intakes when estimated by FFQ, 24-h recalls or food
158 diaries⁽⁵⁰⁾. The major food sources of the ten phenolic compounds are fruit, vegetables and wholegrain
159 products, or represent the main metabolites of parent glucosides present in the previously mentioned
160 foods.

161 Purified standards phloretin, epicatechin, eriodictyol, hesperetin, luteolin, daidzein, gallic and vanillic
162 acids were obtained from Santa Cruz Biotechnology, Germany and quercetin, enterolactone and
163 catechin-2, 3, 4-¹³C₃ from Sigma-Aldrich, UK. Stock solutions were prepared for all the purified
164 phenolic compounds, two milligrams of each of the ten purified compounds were diluted in one
165 millilitre of methanol or dimethyl sulfoxide according to supplier specifications and one milligram of
166 Catechin-2, 3, 4-¹³C₃ (internal standard) in one millilitre of methanol. Stock solutions were stored at -40
167 °C or -80 °C (internal standard). Enzyme β-glucuronidase/sulfatase (0.05 g) type H-5 from *Helix*
168 *Pomatia* (Sigma-Aldrich, UK) was diluted in one millilitre of 0.2% sodium chloride solution to create a
169 working solution of 50,000 units per millilitre; the enzyme solution was stored at -40 °C. Urine
170 samples (250 µl) were processed for hydrolysis of glucuronide and sulphated metabolites using a
171 modified version of Ito *et al.* ⁽⁴⁷⁾. Urine samples were acidified with acetic acid (20 µl, 0.58 M) and
172 incubated with 1300 units of β-glucuronidase/sulfatase and 300 ng of internal standard at 37 °C, 120
173 rpm for 1.5 h. A liquid-liquid extraction with ethyl acetate was performed twice (400 µl and 300 µl).
174 The two organic layers were pooled and evaporated to dryness under N₂ then stored at -80 °C for 1-4
175 days until their reconstitution with 100 µl of 40% methanol immediately before injection. Analysis of
176 urine samples was performed on a HPLC system Hewlett-Packard series 1100 binary pump, coupled to
177 a triple quadrupole mass spectrometer, Micromass Quattro LC (Micromass, Limited), operating in
178 negative electrospray ionization (ES-) mode, equipped with a Zorbax SB-C18 column (2.1 x 50mm,
179 3.5mm, Agilent). Ionization and fragmentation were optimized for each polyphenol by direct infusion
180 of a standard solution and specific values for collision energy parameters were identified for each
181 polyphenol. Peak for each polyphenol identity was established by the parent and daughter ion pair peak
182 and retention time. Solvents A (water with 0.1% v/v formic acid) and B (acetonitrile with 0.1% v/v
183 formic acid) were run in a 95/5% proportion at a flow rate of 0.2 ml/min with the following gradient:

184 0–1 min, 5% solvent B; 1–4 min, increase solvent B from 5% to 10%; 4–5 min, increase solvent B
185 from 10% to 90%, 5–5.2 min, decrease solvent B from 90% to 5%, 5.2–15 min isocratic for 9.8 min.
186 Peak areas were plotted against the internal standard response. A good linearity (r^2 0.970–0.990) was
187 observed for all the polyphenols quantified except for luteolin ($r^2= 0.922$). Samples were run in the
188 same batch and chromatograms were processed automatically by MassLynx Mass Spectrometry
189 Software (MASSLYNX™ version 3.5) using the same processing integrate parameters, peak-to-peak
190 amplitude and peak detection. Intra-assay coefficients of variability were phloretin 7.5%, epicatechin
191 6%, hesperetin 21.9%, eriodictyol 9.6%, luteolin 9.8%, quercetin 10.8%, daidzein 3.1%, gallic acid
192 21.4%, vanillic acid 14.4% and enterolactone 5.1%. Final quantities of phenolics compounds were
193 estimated after adjustment by urinary volume. Calibration curves were prepared by spiking HPLC-
194 grade water with 2.5, 5, 12.5, 25, 50, 100, 150, 200 and 250 μ l of mixed polyphenols working solution
195 and 100 μ l of internal standard working solution, the calibration curve range was 5–500 ng/ml. Spiked
196 HPLC-grade water samples were treated with enzyme and extracted with ethyl acetate as were the
197 urine samples, spiked samples were injected in duplicate at each concentration level. Limits of
198 quantification were established using the spiked samples for calibrations curves; minimum detected
199 values were 5 ng/ml for phloretin, epicatechin, eriodictyol, hesperetin, luteolin, daidzein, gallic acid
200 and vanillic acid and 10 ng/ml for quercetin and enterolactone.

201 **Statistical analysis**

202 Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) v.21. Four-
203 day food diary intake data⁽⁶⁴⁾ were expressed as weight of intake (g) per day per 10 MJ energy intake,
204 in order to adjust for variability in total food intake between individuals over the 4 days, whereas FFQ
205 data were expressed simply as weight of intake (g) per day. Normality of data distribution was
206 evaluated visually by inspection of histograms and normal Q-Q plots. Non-normally distributed
207 variables were natural log transformed prior to statistical analysis by parametric methods, or analysed
208 by non-parametric methods where log transformation failed to yield a normal distribution. Independent
209 sample t-tests were conducted to compare groups at baseline in order to verify that the polyphenol
210 intakes of each treatment group were similar before the dietary intervention. Where data were normally
211 distributed, one-way analysis of variance with gender as a fixed factor and baseline value as covariate
212 (ANCOVA) was conducted to find differences between groups at endpoint. Where data could not be
213 transformed to a normal distribution, a Mann-Whitney U test was conducted to find differences

214 between groups at endpoint. Correlation analyses on urinary and dietary polyphenols were conducted
215 by two-tailed Spearman's correlations.

216 **Results**

217 A total of 165 healthy men and women aged 40–70 years were recruited, and 162 completed the
218 CRESSIDA study (64 M, 97 F) (**Figure 1**). Dietary intake of polyphenols was quantified from 322 x 4-
219 DFD (n=161) and from 322 FFQ (n=161) (EPIC-Norfolk FFQ v.6)⁽⁶¹⁾ at baseline and endpoint of the
220 12-wk intervention, one participant randomised to the DG group did not complete the collection of
221 food diaries therefore was eliminated from the analysis. Urinary excretion of polyphenols was analysed
222 in a subsample (n=91) of the CRESSIDA study, 45 samples from participants in the control group
223 reporting the lowest intakes of F&V, and 46 samples from participants in the intervention group
224 reporting the highest intakes of F&V.

225 Characteristics of the whole CRESSIDA study population at baseline are shown in **Table 1**. Mean BMI
226 was significantly higher in the control group. Intakes of wholegrain cereals, fruits and vegetables and
227 dietary fibre were higher in the study population at baseline than those reported in a larger,
228 representative UK population sample ^(11,56). As reported previously, at endpoint urinary potassium
229 excretion was 9 mmol/d greater in the DG group indicating higher consumption of fruit and vegetables.
230 Reported wholegrain cereal intake was 81 g/d at endpoint in the DG group (mainly wheat, oats and
231 rice) compared with 32 g/d in the control group, as confirmed by higher plasma alkylresorcinol
232 concentrations, reflecting intakes of whole grains mainly from wheat, barley, and rye ⁽⁵⁶⁾.

233 **Four-day food diary**

234 Median total polyphenol intake at baseline in the whole study population (with lower and upper limits
235 of IQR) was 1183 mg/d (745, 1613), or 1282 mg/d per 10 MJ energy intake (896, 1838). As shown in
236 **Table 2**, baseline intakes of total polyphenols were significantly higher in the DG group compared to
237 the control group due to greater intakes of total flavonoids, the main contributor being total
238 proanthocyanidins.

239 At endpoint, total polyphenol intakes per 10 MJ energy intake, adjusted for gender and baseline
240 polyphenol intake, were higher in the DG group than in the control group (**Table 2**). Analysis of
241 intakes of polyphenol classes at endpoint (unadjusted for gender and baseline as non-parametric
242 statistical analysis was necessary) demonstrated that the DG group had higher intakes of total

243 flavonoids (although also higher in DG at baseline), total lignans, phenolic acids, stilbenes, and “other
244 polyphenols” compared to the control group. For the major flavonoid subclasses, the DG group
245 reported higher intakes of anthocyanidins, proanthocyanidins (although these were also higher at
246 baseline), and isoflavones, but not dihydrochalcones, flavanols, flavones or flavonols. For the major
247 phenolic acids subclasses, the DG group reported higher intakes of hydroxycinnamic acids, but the
248 trend for higher intakes of hydroxybenzoic acids was not statistically significant.

249 For individual (poly)phenols, there were no differences between groups at baseline, but at endpoint the
250 group following the DG diet reported higher intakes of a number of anthocyanidins including cyanidin,
251 malvidin, peonidin and petunidin ($P<0.05$) compared to the control group. Furthermore, intakes of
252 individual flavanones (naringenin, eriodictyol, and hesperetin, $P<0.05$), a flavone (luteolin, $P<0.05$),
253 and isoflavones (daidzein and genistein, $P<0.01$) were higher in the DG group compared to the control
254 group at endpoint. With regards to lignans, phenolic acids and stilbenes classes, there were higher
255 intakes of secoisolariciresinol ($P<0.05$), matairesinol ($P<0.001$), protocatechuic acid ($P<0.01$), vanillic
256 acid ($P<0.001$), 4-hydroxybenzoic acid ($P<0.001$), syringic acid ($P<0.001$), p-coumaric acid ($P<0.001$)
257 and ferulic acid ($P<0.05$) at endpoint in the DG group compared to the control group. There were no
258 differences between groups in individual flavonols (including kaempferol, quercetin, myricetin and
259 isorhamnetin) nor flavanols (including individual catechins and theaflavins).

260 The main sources of total polyphenols at endpoint in both groups were tea, coffee & wine (control;
261 61%, DG; 59%), fruits (control; 10%, DG; 17%) and fruit juices (control; 8%, DG; 4%) (**Figure 2**).
262 Main sources of flavonoids in control and DG groups were tea, coffee & wine (control; 55%, DG;
263 55%), fruits (control; 14%, DG; 21%) and fruit juices (control; 10%, DG; 5%). Main sources of the
264 flavanols subclass were tea, coffee & wine (control; 64%, DG; 65%), fruits (control; 13%, DG; 19%)
265 and chocolates (control; 11%, DG; 4%). There was an increase in the percentage of polyphenols
266 sourced by fruits, cereal products (breakfast cereal and cereal bar) and nuts & seeds (see **Figure 3** for
267 further details) in the group following the DG diet compared to baseline. There was a decrease in
268 percentage of polyphenols derived from less recommended food options as fruit juices, chocolates and
269 biscuits (**Figure 3**).

270 **Food frequency questionnaire**

271 There were no differences in total flavonoids and flavonoid subclass estimated intakes between groups
272 at baseline. At endpoint the DG group reported higher intakes of total flavonoids, total anthocyanidins,

273 total flavones, total flavanols, and total proanthocyanidins (**Table 3**). Flavanone and flavonol intakes
274 did not differ between groups at endpoint. Differences between individual polyphenol components
275 revealed higher reported intakes of cyanidin, delphinidin, petunidin and peonidin ($P<0.001$), malvidin
276 and pelargonidin ($P<0.005$), catechin ($P<0.01$), and epicatechin-3-O-gallate, epigallocatechin-3-O-
277 gallate, myricetin and luteolin ($P<0.05$) in the DG group (data not shown) compared to control.

278 **Urinary polyphenol excretion**

279 The ten aglycones quantified in urine were either metabolites of parent glucuronide/sulphated
280 compounds formed in human tissues, or metabolites produced by the gut microbiota, e.g. enterolactone
281 from lignans present in fibre-rich food. At endpoint there was a greater 24 h urinary excretion of
282 phloretin, eriodictyol, hesperetin, luteolin, quercetin, gallic acid, vanillic acid and enterolactone in the
283 DG group compared to the control group (**Table 4**), and a non-significant tendency towards a similar
284 response for daidzein excretion. Daidzein was not detected in 37 participants of the 91 subsample,
285 which could contribute to the lack of significance in the results given that values observed are
286 considerably higher in the intervention group. The only polyphenol that was excreted in similar
287 amounts in both dietary groups was epicatechin.

288 **Discussion**

289 The aim of this secondary analysis of dietary intake data from the CRESSIDA trial was to investigate
290 whether following advice to adhere to UK dietary guidelines increased polyphenol intakes. Baseline
291 polyphenol/flavonoid intakes from 4-DFD and FFQ were in alignment with previous reports in UK
292 populations using 24 h recall^(27, 28, 29, 30, 31, 65, 66, 67) or FFQ⁽¹⁵⁾. The hypothesis was supported by the
293 finding that total estimated polyphenol intakes were approximately 200 mg per 10 MJ energy intake
294 higher at endpoint in the DG group compared to the control group. However, estimates of total
295 polyphenol intakes are only as accurate as the polyphenol composition data, and rely on the sum of the
296 components, which may lead to underestimates if complete composition profile data are unavailable for
297 individual foods. Therefore, total polyphenol intake data should be interpreted in the context of the
298 individual polyphenol intakes. Estimated dietary intakes by 4-DFD showed that the DG group had
299 higher intakes of individual polyphenols/phenolic acids where main food sources were fruits and
300 vegetables (cyanidin, malvidin, peonidin, petunidin, protocatechuic acid, naringenin, eriodictyol,
301 hesperetin and syringic acid), nuts and seeds (secoisolariciresinol and matairesinol) and soy products
302 (daidzein, genistein and other isoflavonoids) compared to the control group. In agreement with the 4-

303 DFD results, FFQ estimates of total flavonoids, proanthocyanidins and anthocyanidins were higher in
304 the DG group compared to the control group. Ten representative aglycone polyphenols and phenolic
305 acids (from glucuronidated and sulphated metabolites) were measured in 24 h urine samples collected
306 concurrently with 4-DFD as objective biomarkers of (poly)phenol intakes, in order to determine the
307 accuracy of the dietary intake estimates. The ability to discriminate between the dietary group
308 subsamples was consistent with 4-DFD, except for epicatechin, daidzein and quercetin where there
309 were no significant differences in urinary excretion in contrast with higher estimated intakes in the DG
310 group by 4-DFD. Both the 4-DFD and urinary biomarker methods agreed that intakes of
311 phlorizin/phloretin, eriodictyol, hesperidin, luteolin, vanillic acid and lignans/enterolactone were
312 greater in the DG group.

313 The ability to discriminate between dietary intervention groups supports the utility of 4-DFD and
314 urinary metabolites for assessment of dihydrochalcones, flavanones, flavones, hydroxybenzoic acids
315 and lignan intakes over short-term periods. Differing findings may be due to incomplete phenolic
316 composition data, the well-known inaccuracies of self-reported dietary assessment methodology^(68, 69),
317 and the inter-individual variability in absorption and metabolism^(8, 70) or incomplete deconjugation of
318 sulphated/glucuronidated metabolites⁽⁷¹⁾ reflected in the urinary biomarkers. Urine polyphenol
319 concentrations may also be the sum product of endogenous metabolism and dietary intakes. For
320 example, gallic acid is a common metabolite of black tea theaflavins and thearubigins^(72, 73), therefore
321 its urinary excretion could reflect the consumption of such beverage combined with foods providing
322 gallic acid *per se*. Vanillic acid can be found in high concentrations in foods consumed in small
323 quantities such as herbs; also in moderate concentration in dates, olives and cranberries and in low
324 concentrations in foods more frequently consumed like oats and rice⁽³⁹⁾. It is also a common metabolite
325 of anthocyanins^(74, 75), and so variability in urinary vanillic acid excretion could reflect varying intakes
326 of both vanillic acid and anthocyanin-rich foods, therefore it is likely that the increased urinary
327 excretion at endpoint mainly represents intake of foods rich in anthocyanins rather than vanillic acid.
328 Vanillic acid is also reported to be present in urine after consumption of black tea⁽⁷⁶⁾ and dark
329 chocolate⁽⁷⁷⁾; however, intakes of black tea and dark chocolate were not higher in the DG group at
330 endpoint, and therefore are unlikely to account for the increased urinary excretion and reported intakes
331 in the 4-DFD. Enterolactone is the main colonic metabolite of lignans, which are phytoestrogenic
332 compounds present in high concentrations in vegetables, cereals and grain products, seeds, nuts and
333 berries and other fruits^(65, 78). These urinary excretion data are in agreement with previous
334 epidemiological studies⁽⁷⁹⁾, supplementation studies where known doses of polyphenols were

335 administrated^(44, 80), in RCTs aiming to increase flavonoid-rich fruit and vegetables intake⁽⁸¹⁾ and with
336 population under free-living conditions⁽⁴³⁾. Polyphenol urinary excretion has shown to be an useful
337 biomarker of fruit and vegetables intakes, capable of detecting even small changes in studies under
338 controlled diets^(45, 82, 83).

339 Following dietary guidelines did lead to increases in specific polyphenols that have been associated
340 with health benefits (e.g. anthocyanins, flavanones, isoflavones, phenolic acids and lignans^(84, 85, 86, 87) .
341 This suggests that consuming at least 5 portions F&V per day and >50 % of cereal intake as wholegrain
342 will lead to an enrichment in dietary polyphenol profiles above and beyond the large amounts of
343 polyphenols supplied by beverages. In particular, estimated anthocyanidin intakes were augmented by
344 following dietary guidelines which may confer a reduction in risk of T2D⁽²⁵⁾, and acute reductions in
345 postprandial glycaemia^(88, 89, 90,91). The dietary intake results reported here are consistent with the theory
346 that greater intakes of specific polyphenols may contribute to the protective effects of a diet rich in
347 fruits and vegetables and wholegrain cereals, alongside greater dietary intakes of nutrients and other
348 non-nutrient bioactives, e.g. potassium, vitamin C, soluble fibre, carotenoids and glucosinolates.
349 However, our results confirm^(29, 30, 31) that food groups that are recommended by UK government as
350 part of the Eatwell Guide – fruits and vegetables, wholegrain cereals, beans, pulses, nuts and seeds -
351 contribute a much smaller proportion of daily polyphenol intakes in a UK population compared to
352 commonly consumed beverages, even when that population is following dietary guidelines. Analysis of
353 dietary data showed no group differences in (poly)phenol intakes (by 4-DFD) where main food sources
354 were not targets for UK dietary guidelines such as tea (catechins, theaflavins, proanthocyanidins,
355 quercetin) and wine (stilbenes, proanthocyanidins). However, the sources of these polyphenols may
356 have differed between groups; for example, the DG group reported lower intakes of stilbenes from
357 wine and increased intakes of stilbenes from fruit.

358 The relative impacts of total and specific (poly)phenol intakes on risk of CVD and type 2
359 diabetes requires investigation. Clearly there is only limited scope for increasing total polyphenol
360 intake by following dietary guidelines – an approximate increase of 200 mg per day per 10 MJ energy
361 intake. The extent to which this is relevant to cardio-metabolic health is currently unclear due to the
362 lack of robust evidence for the relative impact of different polyphenol subclasses on risk factors for
363 CVD and type 2 diabetes. Although the evidence is currently insufficient, it is plausible to hypothesize
364 that in the future there may be consensus that tea and coffee (poly)phenols^(92, 93) may have some of the
365 strongest cardio-metabolic protective effects amongst all dietary phenolics, so that due to the large

366 amounts of tea and coffee consumed at a population level, the gain to health in consuming at least 5
367 portions/day of fruits and vegetables and choosing wholegrain sources of starchy carbohydrates is
368 likely to be due to other nutritional factors. Conversely, future advances in nutritional science may one
369 day demonstrate that certain polyphenols specific to fruits, vegetables or wholegrain cereals have
370 particularly potent bioactivity in preventing inflammation, atherosclerosis or insulin resistance, which
371 would bring about a departure from the prevailing approach of considering all plant chemicals that
372 contain a similar chemical structure in the same way. There has been some debate around whether
373 establishing dietary reference intakes (DRI) for polyphenols could be beneficial; with suggested
374 approaches including recommendations for polyphenol-rich F&V (5-a-day)⁽⁵²⁾, establishing a specific
375 daily dose for a given effect⁽⁵⁴⁾ or establishing values to improve health or prevent disease risk in
376 different life stages⁽⁵³⁾. Further research is required to provide robust interventional evidence for any
377 refinement to current “5-a-day” guidelines, since the relative health impact of a multitude of other non-
378 nutrient bioactives and the complex array of plant cell wall polysaccharides, resistant starch and
379 oligosaccharides (collectively known as fibre) contained in low-polyphenol plant-based foods is only
380 partially understood. Furthermore, in the light of the fact that NDNS survey data suggest that the UK
381 population are not meeting current recommendations for F&V then it would be futile, and possibly
382 counter-productive, to add further complexity to existing public health dietary advice.

383 The validation of adequate biomarkers of dietary intake, of exposure to dietary components and
384 of compliance to dietary interventions has been a focus of investigation for many years⁽⁹⁴⁾ and the
385 identification of polyphenol intake biomarkers is no exception^(83, 95). Assessment of compliance in
386 dietary intervention studies with fruits and vegetables has been done by validated biomarkers⁽⁹⁶⁾ such as
387 vitamin C, carotenoids and potassium, however polyphenol excretion in urine may be used to assess
388 flavonoid-rich dietary interventions⁽⁸¹⁾ or specific fruit and vegetable intakes, for example phloretin for
389 apple intake and naringenin and hesperetin for citrus fruit^(95, 97). Given the importance of accurate
390 assessment of polyphenol intake in order to link it with beneficial effects on health⁽⁹⁸⁾, the ability to
391 detect the amount consumed represents a challenge in epidemiological and clinical studies.

392 The bespoke food composition table developed for the 4-DFD polyphenol analysis in the
393 present study included the estimation of 52 individual compounds and contained 1141 foods
394 representative of an average UK diet as well as some of the most commonly eaten food from non-
395 traditional UK cuisine as Turkish, Indian, Italian, Chinese, Japanese, and others. This food composition
396 table could be used as a basis for future studies analysing diet patterns in the UK population, comparing

397 polyphenols intake and/or linking intake with health benefits, although it would require a significant
398 staff cost commitment to maintain and update with new recipes and new versions of Phenol Explorer
399 over time. Although an onerous process, the creation of a bespoke food composition table for
400 polyphenol analysis in a specific population seems to be the most effective way to obtain an accurate
401 estimation of polyphenol intake. Although the FFQ is a valid and reliable dietary assessment tool for
402 estimation of habitual intake in populations, it has limitations in the way that it can collect information,
403 since it is based on uniform estimated portion sizes and is semi-quantitative. Dietary intake of total
404 flavonoid intake, six flavonoid subclasses and 32 individual flavonoids (aglycones) were estimated by
405 FFQ (EPIC-Norfolk) using methodology previously applied in large cohort studies^(15, 17, 23, 25, 26, 99). The
406 EPIC-Norfolk FFQ, although validated for whole foods intake⁽¹⁰⁰⁾, has not been validated for flavonoid
407 intake. Methodological barriers of this approach in regard to flavonoid estimation are particularly
408 limiting in smaller study cohorts: some foods are grouped incongruously with regards to their flavonoid
409 composition such as “strawberries, raspberries and kiwi”, or “peaches, plums and apricots” and “wine”
410 (no separation of red, white and rosé). Some important flavonoid sources are missing such as
411 blueberries, soy beverages, and lemon, among others. The inadequacy of this method could lead to
412 under- or overestimation of flavonoid intakes. Studies have used food diaries for estimation of flavanol
413 intake in European populations⁽²⁹⁾, isoflavone and lignan intakes⁽⁶⁶⁾ and 20 subclasses of
414 polyphenols⁽³¹⁾ in a UK population, each one creating their own food composition table for polyphenol
415 analysis, however they have not included objective biomarkers for corroborating agreement between
416 methods. More specialised questionnaires for intake estimation have been developed for individual
417 compounds like quercetin and naringenin⁽¹⁰¹⁾, flavonoid subclasses such as flavonols and flavones in a
418 Chinese population⁽¹⁰²⁾, complete flavonoid class in Australian population⁽³⁴⁾ and Flemish
419 population⁽¹⁰³⁾. In summary, although the FFQ results reported here were in broad agreement with the
420 4-DFD regarding the discernment of differences in total flavonoid intakes in a dietary intervention
421 study, future research in this area could include the development of a specific FFQ, validated in a UK
422 population, for estimation of phenolic intakes including all classes and main subclasses as were
423 estimated by 4-DFD in this study.

424 Limitations in the present study include the hydrolysis of urinary polyphenols by
425 glucuronidase/sulphatase enzymes prior to analysis, which could compromise the stability of
426 polyphenols that are to be quantified^(104, 105). Glucuronidase/sulphatase enzymes may fail to completely
427 hydrolyse all sulphated and methylated-sulphated metabolites, which may have been compounded by
428 the addition of boric acid as a preservative to prevent yeast growth, and therefore urinary excretion of

429 the corresponding aglycone could be underestimated⁽⁷¹⁾. Furthermore, our approach required
430 preselection of polyphenols to be analysed which means that many other polyphenols present in 24 h
431 urine that may have also functioned as biomarkers of intake were not detected and quantified. Although
432 reported anthocyanidin intakes were greater in the DG group, we did not attempt to analyse
433 anthocyanins in urine as they have low recovery as parent compounds and only weak correlations with
434 dose ingested, necessitating the use of stable isotopes to accurately measure the excretion of
435 anthocyanin metabolites⁽⁴⁴⁾. The 4-DFD proved to be an accurate approach for estimate of short-term
436 polyphenol intake, however, serious consideration must be made of the potential researcher burden and
437 cost if using these methods in larger scale studies, particularly where a simple indicator of compliance
438 to dietary advice is required.

439 Strengths of our study include the fact that the data collected in this study enabled a comparison of two
440 dietary assessment methods with objective biomarkers of (poly)phenol dietary intake collected
441 concurrently towards the end of the dietary intervention period. To date, this 3-way comparison has
442 only been reported previously in a cross-sectional⁽¹⁰⁶⁾ study using a crude estimate of total urinary
443 polyphenol excretion by the Folin-Ciocalteu assay. To the best of our knowledge this is the first study
444 to use the 3 way comparison on data obtained from an RCT allowing comparison of change from
445 baseline for individuals advised to consume a diet consistent with dietary guidelines.

446 In conclusion, participants following advice to adhere to UK dietary guidelines consumed a greater
447 amount of total polyphenols than a control group consuming a representative UK diet. In particular,
448 intakes of individual polyphenols that are mainly sourced from fruits and vegetables, nuts, seeds and
449 soy products were increased in the DG group. The results of this study advance the field of polyphenol
450 research as they demonstrate that a diet consistent with dietary guidelines is also moderately richer in
451 polyphenols compared to a typical UK dietary pattern, despite the relatively high baseline intakes
452 derived from commonly consumed beverages such as tea and coffee. In addition to increased intakes of
453 dietary fibre and certain micronutrients, replacement of saturated fatty acids with unsaturated fatty
454 acids, and lower salt and free sugars, following dietary guidelines will also increase polyphenol intakes
455 which may contribute to the overall reduction in risk factors for cardiometabolic diseases.

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Conflict of Interest

W.L.H. has current collaboration with Lucozade Ribena Suntory and DIANA Food SAS. T.A.B.S., D.P.R., J.D and M.L.C.A. reported no conflicts of interest.

Authorship

T.A.B.S. was principal investigator of the CRESSIDA study. T.A.B.S. and W.L.H. devised the CRESSIDA study, D.P.R. and J.D. recruited subjects into the study and supported the dietary intervention in the CRESSIDA study. M.L.C.A. and W.L.H. conceived the idea for secondary analysis of polyphenols intake data, analysed data, performed statistical analysis. M.L.C.A. wrote drafts of the manuscripts, which were edited by W.L.H. The manuscript was read, commented on, and approved by all authors.

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Figure legends

Figure 1 Flow of participants through the CRESSIDA study

Figure 2 Main polyphenol food sources in control and dietary guidelines groups, at endpoint, of the CRESSIDA study as estimated by four-day food diary

Figure 3 Percentage changes from baseline in main food group sources of polyphenols in dietary guidelines group as estimated by four-day food diary

Table 1 Baseline characteristics of the CRESSIDA study population by randomised group

Characteristic	Control (n=82)		Dietary Guidelines (n=79)		Difference between groups (<i>P</i> * value)
	Mean	SD	Mean	SD	
Gender (n)					
Female	50		47		
Male	32		32		
Age (years)	52	8.0	53	8.0	0.717
BMI (kg/m ²)	26.8	3.9	25.4	3.7	0.019
Waist circumference (cm)					
Female/male	91.9/98.2	10.0/12.2	87.1/97.3	12.2/9.3	0.093
SBP (mmHg)	122.0	11.5	121.6	14.3	0.848
DBP (mmHg)	73.9	7.1	73.8	8.0	0.930
Glucose (mmol/L)	5.0	0.4	5.3	0.5	0.990
Total cholesterol (mmol/L)	5.3	0.9	5.4	1.1	0.983
TC:HDL cholesterol ratio	3.6	1.0	3.5	1.0	0.262

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

*Statistical comparisons between groups at baseline by independent T-test or independent samples Mann-Whitney U-test.

Table 2 Daily intake of polyphenols estimated by four-day food diary and adjusted for energy intake following dietary guidelines and control diets.

Polyphenols (mg/d/10 MJ) Classes and subclasses	Baseline (n=161)		Endpoint (n=161)				Differences between groups at endpoint (P^{\ddagger})
			Control (n=82)		Dietary Guidelines (n=79)		
Total polyphenols*	1202 [†]	(1093, 1323)	1084	(980, 1197)	1279	(1158, 1412)	0.023 [§]
Flavonoids	840 [†]	(536, 1263)	802	(456, 1109)	882	(610, 1401)	0.014
<i>Anthocyanidins</i>	55	(14, 130)	30	(14, 108)	76	(25, 144)	0.032
<i>Dihydrochalcones</i>	1.6	(0.0, 3.8)	2	(0, 4)	2	(1, 6)	0.297
<i>Flavanols</i>	349	(114, 526)	311	(104, 498)	394	(128, 665)	0.159
<i>Proanthocyanidins</i>	231 [†]	(117, 339)	195	(115, 324)	249	(169, 381)	0.020
<i>Flavanones</i>	31	(4, 64)	24	(2, 58)	33	(8, 84)	0.096
<i>Flavones</i>	6	(3, 12)	5	(1, 12)	5	(2, 12)	0.603
<i>Flavonols</i>	80	(51, 112)	80	(50, 105)	83	(58, 127)	0.125
<i>Isoflavones</i>	0.3	(0.1, 9.9)	0.1	(0.1, 0.4)	0.5	(0.1, 65.0)	0.002
Lignans	0.5	(0.2, 5.0)	0.3	(0.1, 0.4)	0.5	(0.2, 6.0)	<0.001
Phenolic acids	289	(185, 569)	221	(132, 493)	344	(209, 522)	0.012
<i>Hydroxybenzoic acids</i>	90	(35, 143)	75	(32, 125)	100	(55, 170)	0.067
<i>Hydroxycinnamic acids</i>	149	(88, 450)	119	(59, 378)	172	(99, 444)	0.018
Stilbenes	0.09	(0.02, 0.71)	0.09	(0.02, 0.94)	0.26	(0.07, 1.25)	0.042
Other polyphenols	21	(11, 41)	13	(6, 30)	37	(20, 58)	<0.001

All values are medians (with lower and upper limits of IQR), except where * denotes values are geometric means (95% CI), adjusted for baseline and gender at endpoint.

†Statistically significant differences between groups at baseline by independent T-test (total polyphenols) or independent samples Mann-Whitney U-test (all other comparisons); intakes were higher in the DG group than the control group, $P < 0.05$.

‡Statistical comparisons between groups at endpoint by Mann-Whitney U-test, except where § denotes statistical comparisons between groups at endpoint adjusted for baseline and gender by ANCOVA.

Table 3 Median daily intake of flavonoids estimated by food frequency questionnaire in dietary guidelines and control groups

Flavonoids (mg/d) Class and subclasses	Baseline (n=161)		Endpoint (n=161)				Differences between groups at endpoint (P^*)
			Control (82)		Dietary Guidelines (79)		
Total flavonoids	661	(417, 867)	539	(350, 862)	715	(571, 906)	0.005
<i>Anthocyanidins</i>	19	(10, 35)	16	(9, 31)	32	(19, 50)	<0.001
<i>Flavones</i>	2.4	(1.6, 3.5)	2.7	(1.5, 3.7)	3.2	(2.0, 4.4)	0.013
<i>Flavonols</i>	35	(23, 47)	39	(21, 54)	39	(31, 50)	0.199
<i>Flavanols</i>	90	(40, 143)	85	(35, 146)	97	(80, 143)	0.048
<i>Flavanones</i>	23	(8, 42)	25	(10, 43)	27	(8, 49)	0.352
<i>Proanthocyanidins</i>	265	(184, 379)	232	(184, 371)	290	(229, 385)	0.021

Values are medians (lower and upper limits of the IQR). There were no significant differences between groups at baseline.

* Statistical comparisons between groups at endpoint by Mann-Whitney U-test.

Table 4 Comparison of urinary phenolic excretion method and polyphenol intake estimated by 4-day food diary in their ability to discriminate between subsample populations either adhering to dietary guidelines or a control diet.

Polyphenols	Urinary excretion [†] (μmol/d)					Dietary intake (mg/d/10 MJ)				
	Control (n=46)		Dietary Guidelines (n=45)		Difference between groups (<i>P</i>)	Control (n=46)		Dietary Guidelines (n=45)		Difference between groups (<i>P</i>)
Phloretin [‡]	0.09	(0.01, 0.28)	0.40*	(0.18, 0.97)	<0.001	1.2	(0.0, 3.5)	3.1*	(1.1, 5.9)	0.006
Epicatechin	0.45	(0.24, 0.60)	0.44	(0.21, 0.70)	0.943	30	(15, 50)	40*	(29, 67)	0.013
Eriodictyol	0.25	(0.09, 0.43)	0.51*	(0.20, 0.86)	0.011	0.00	(0.00, 0.02)	0.02*	(0.01, 0.06)	<0.001
Hesperetin	0.20	(0.00, 2.03)	1.38*	(0.09, 3.51)	0.010	8	(1, 23)	17*	(4, 35)	0.019
Luteolin	1.8	(0.9, 2.9)	2.7*	(1.5, 3.7)	0.017	0.5	(0.3, -8)	1.5*	(0.9, 3.3)	<0.001
Quercetin	4.7	(1.5, 7.4)	6.6	(2.4, 12.8)	0.105	50	(27, 66)	59*	(41, 85)	0.028
Daidzein	0.02	(0.00, 0.40)	0.11	(0.00, 0.80)	0.074	0.04	(0.04, 0.10)	3.5*	(0.02, 21.2)	0.006
Gallic acid	0.7	(0.2, 2.0)	1.4	(0.5, 3.6)	0.053	62	(18, 107)	90	(23, 153)	0.134
Vanillic acid	1.4	(0.2, 2.6)	3.0*	(0.9, 5.9)	0.023	0.2	(0.1, 0.2)	0.4*	(0.2, 0.6)	<0.001
Lignan/ Enterolactone [§]	5.7	(2.1, 10.6)	10.1*	(3.8, 22.6)	0.010	0.2	(0.1, 0.4)	0.6*	(0.3, 6.1)	<0.001

Excretion of polyphenols in 24 h urine and dietary intake of the direct dietary precursor estimated by 4-DFD at endpoint in a subsample of the CRESSIDA study population selected for the lowest (control group) and highest (dietary guidelines group) fruit and vegetables intake using self-reported data from food frequency questionnaires (n=91).

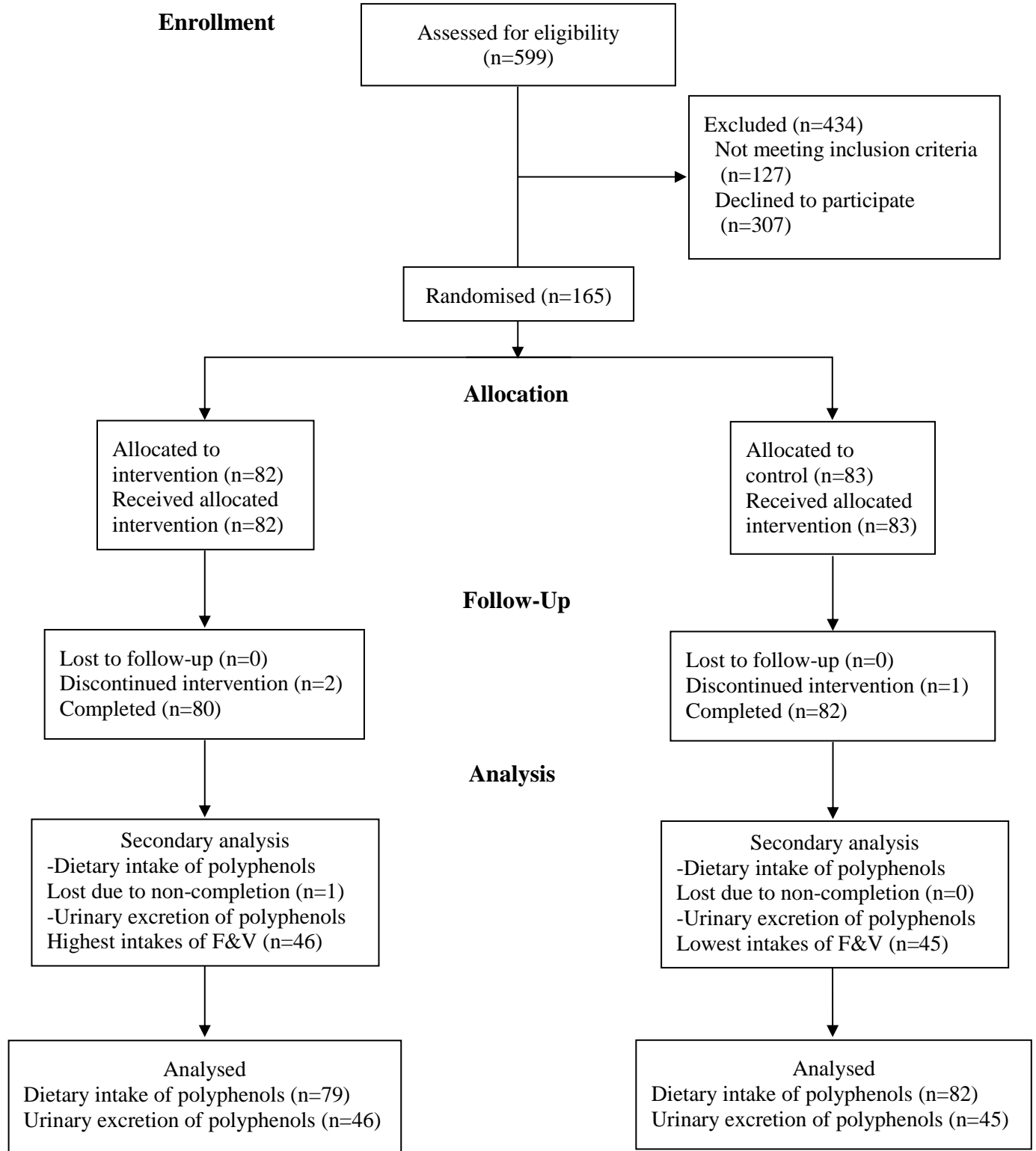
All values are median (lower and upper limits of IQR). All between group comparisons were carried out by independent samples Mann-Whitney U Test.

[†] Values adjusted for 24 h urine volume.

[‡] Sum of phloretin and phlorizin

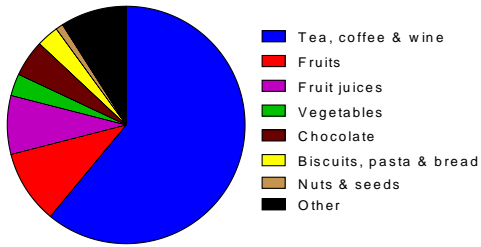
[§] Intake of lignans was used as direct dietary precursor of enterolactone.

*Statistically significant differences between groups at endpoint, *P*<0.05.

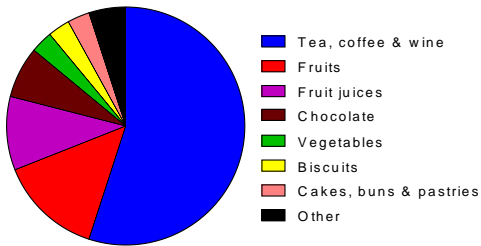


Control diet

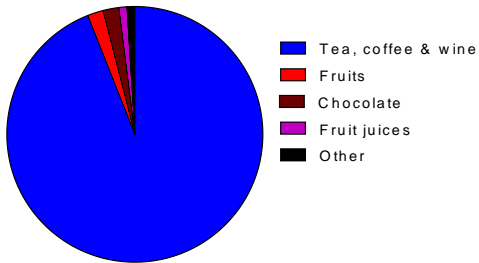
Total polyphenols



Flavonoids



Flavanols



Dietary guideline diet

Total polyphenols



Flavonoids



Flavanols

