Lack of acute or chronic effects of epicatechin-rich and procyanidin-rich apple extracts on blood pressure and cardiometabolic biomarkers in adults with moderately elevated blood pressure: a randomized, placebo-controlled, cross-over trial

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Abbreviations

AI, Augmentation Index; ba_PWV, brachial-ankle pulse wave velocity; cf_PWV, carotid femoral pulse wave velocity; DPB, diastolic blood pressure; EC,

epicatechin (refers to (–)-EC unless stated otherwise); ET-1, endothelin-1; FMD, flow-mediated dilatation; NO, nitric oxide; PC, procyanidin; SBP, systolic blood pressure; TAG, triglycerides.

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ABSTRACT

Background: The reported effects of flavanol-rich foods such as cocoa, dark chocolate and apples on blood pressure and endothelial function may be due to the monomeric flavanols (mainly (-)-epicatechin (EC)), the oligomeric flavanols (procyanidins; PC) or other components. Reports of well controlled intervention studies that test the effects of isolated oligomeric flavanols on biomarkers of cardiovascular health are lacking.

Objective: We studied the acute and chronic effects of an EC-rich apple flavanol extract and isolated apple PCs on systolic BP and other cardiometabolic biomarkers.

Design: Forty-two healthy men and women with moderately elevated BP completed this randomized double-blind, placebo-controlled, four arm crossover trial. Participants ingested a single dose of an apple flavanol extract (70 mg monomeric flavanols, 65 mg PC), a double dose of this extract (140 mg monomeric flavanols, 130 mg PC), an apple PC extract (130 mg PC, 6.5 mg monomeric flavanols) or placebo capsules once daily for 4 weeks, in random order. Biomarkers of CVD risk and vascular function were measured before and 2h after ingestion of the first dose, and after 4 weeks intervention.

Results: Compared to the placebo, none of the isolated flavanol treatments significantly ($p<0.05$) changed SBP or DBP (peripheral and aortic), plasma NO reaction products or measures of arterial stiffness (cf_PWV, ba_PWV and AI) after 2 h or 4 weeks of intervention. There were no changes in plasma endogenous metabolite profiles, or circulating NO, endothelin-1, total-, HDL-, LDL-cholesterol, TAGs, fasting glucose, fructosamine and insulin after 4 weeks of intervention.

Conclusions: Our data suggest that in isolation, neither monomeric flavanols or PC affect BP, blood lipid profiles, endothelial function or glucose control in individuals with moderately elevated blood pressure. The reported benefits of consuming flavanol-rich cocoa, chocolate and apple products appears to be dependent on other components, which may work in combination with monomeric flavanols/PC.

INTRODUCTION

Monomeric flavanols such as epicatechin (EC) and their oligomeric derivatives (procyanidins; PC) are a sub-class of the flavonoids that are widely available as part of the human diet. Some epidemiological studies have reported an inverse correlation between the consumption of flavanol-rich foods and cardiovascular disease (CVD) (1-3). These observations are substantiated by several meta-analyses of human intervention trials demonstrating that acute and chronic ingestion of flavanol-rich foods and beverages such as dark chocolate, cocoa products and apples causes decreases in blood pressure (BP) in both healthy, normotensive individuals and hypertensive patients (4-6), reductions in serum insulin (7) and improvements in circulating lipid profiles (4, 8). Similarly, some studies have demonstrated significant reductions in fasting blood glucose (9) as well as improvements in markers of vascular function such as endothelial-derived nitric oxide (NO) (10-12), endothelin-1 (ET-1) (10) and pulse wave velocity (PWV) (13, 14), a measure of arterial stiffness and an emerging marker of CVD risk.

The main food sources studied for their BP-lowering properties are flavanol-rich cocoa products, tea and apples. Meta-analyses of randomized controlled trials (RCTs) reported that repeated daily ingestion of chocolate and cocoa beverages over 2-18 weeks significantly reduced systolic BP (SBP) and diastolic BP (DBP) by 5.9 mmHg and 3.3 mmHg (4) and by 4.5 and 2.5 mmHg (6).

Evidence as to whether or not the cardio-protective effects observed in intervention studies with cocoa/chocolate and other flavanol-rich foods/extracts are due to the EC monomer is inconsistent (12, 15-17). For example, Schroeter et al. published data from human dietary intervention studies and mouse aortic ring relaxations experiments with cocoa flavanols and isolated EC and concluded that EC

was responsible for the beneficial effects of cocoa flavanols on endothelial function (12), whereas other studies have reported no significant effects of a similar dose of isolated EC on endothelial function in humans (20). Even though PC have been shown to possess potent biological activity in vitro (17, 18), we are not aware of any studies that have assessed the effects of isolated PC (i.e. depleted of flavanol monomers) on blood pressure in humans.

The aim of this study was to investigate the effects of isolated flavanols on BP and other biomarkers of cardiometabolic health. To achieve this, we used (i) an EC-rich flavanol extract derived from flavanol-rich apples for which we have previously reported the bioavailability of EC and shown it to be similar to that for cocoa products (19), and (ii) an apple PC extract that was depleted of monomeric flavanols. The apple flavanol extracts are devoid of other bioactive substances found in cocoa extracts such as the methylxanthines. We report the effects of the EC-rich flavanol extract at two doses and the effects of a PC rich extract depleted of monomeric flavanols versus a placebo control. To assess the broader effects of flavanols on host metabolism, we also report, to our knowledge for the first time, the effects of the treatments on plasma metabolite profiles using a non-targeted LC-MS based platform that allows relative quantification of several hundred metabolites, the majority being host metabolites.

SUBJECTS AND METHODS

Study population

Forty-three, apparently healthy men and women aged 50+ years with a SBP between 120 and 159 mmHg at eligibility assessment were recruited in and around Norwich, UK. Office SBP was measured by a research nurse using an automated BP

monitor (Omron). The exclusion criteria were as follows: smoking; medical conditions such as gastrointestinal disease, diabetes, cancer, heart disease, stroke; HRT (unless stable on therapy for a period of at least 6 months); medications judged to affect the trial outcome such as blood pressure and lipid lowering therapy; some dietary supplements (e.g. fish oils); clinical results at eligibility assessment judged to affect the trial outcome or be indicative of a health problem. The study period was from August 2014 to March 2016. The trial was conducted in the Human Nutrition Unit at the Quadram Institute Bioscience, Norwich, UK (formerly Institute of Food Research) and all procedures were approved by both the Human Research Governance Committee of the Quadram Institute Bioscience and the Norfolk Research Ethics Committee. Each participant gave written informed consent prior to taking part in the trial. The trial is registered with clinicaltrials.gov (Ref: NCT02013856).

Study design

The study was a randomized, double-blind, placebo-controlled four arm crossover trial investigating the acute and chronic effects of apple derived flavanols on risk markers for CVD **(Figure 1)**. The four treatments were: (i) an apple extract delivering 70 mg monomeric flavanols and 65 mg PC (low dose), (ii) an apple extract delivering 140 mg monomeric flavanols and 130 mg PC (high dose), (iii) an apple extract depleted of monomeric flavanols but containing 130 mg PC, and (iv) a placebo control. Apple extracts and placebo (microcrystalline cellulose) were delivered in opaque, cellulose based capsules suitable for oral consumption and which release their contents within 15 minutes of reaching the stomach (K-caps vegetarian capsules; GoCap).

The primary outcome measure for this trial was SBP. Secondary outcome measures were biomarkers of endothelial function and cardiometabolic risk including blood lipids (total cholesterol, HDL/LDL cholesterol and TAG), glucose, insulin, fructosamine, ET-1, plasma NO reaction products, and measures of arterial stiffness (cf_PWV, ba_PWV and AI).

The apple extracts and placebo were encapsulated and bottled before the start of the trial, with all capsules having identical appearance. The encapsulated flavanols were shown to remain stable throughout the study by regular analysis of sample capsule contents for monomeric and oligomeric flavanols using a validated method (20). The four treatments were randomly allocated as A, B, C or D by a designated person not assigned to the trial. The order in which the participants ingested the treatments was determined by a computer generated (randomization.com) sequence of letters from A-D using a block randomisation approach. Participants meeting the eligibility criteria were allocated sequentially to a treatment sequence upon enrolment by the study manager. The participants, principal investigator, study manager, nurse, statistician and those assessing outcomes were blinded to the interventions; un-blinding was done once statistical analyses were complete.

Capsules (n=2) were ingested once daily (in the morning) for 28 d with a minimum two-week washout period between each of the treatments. To aid compliance, participants were provided with a capsule checklist and asked to record consumption of the capsules on each day. Compliance to treatment was assessed from the checklist and by counting the unused capsules returned at the end of the treatment period. For 24 h prior to the start of each treatment period and for the 28 d treatment period, participants were asked to exclude from their diets some food

sources that contribute significantly to total flavanol intake (e.g. dark chocolate/cocoa) and limit other food sources to levels that would support compliance (i.e. tea, berry fruits) or have known cardio-protective effects (e.g. oily fish). Foods on the limited list were completely excluded from the diet for 24 h prior to assessment days. A list of prohibited and limited foods was provided to the participants to aid compliance.

Participants were assessed at the start and end of each 28 d treatment period. To investigate the chronic effects of flavanols on risk markers for CVD the following measurements were made on day 1 at 0 h (baseline) and day 29 of each treatment period: blood pressure (peripheral and aortic); arterial stiffness (ba_PWV; cf_PWV; AI); fasting blood samples were collected for the analysis of plasma flavanols; circulating levels of lipids/lipoproteins, glucose, fructosamine, insulin, NO reaction products and ET-1. To investigate the acute effects of flavanols on risk markers for CVD, BP, arterial stiffness and NO reaction products were re-assessed 2 h after ingestion of the capsules on day 1. This time point was chosen to coincide with expected peak plasma concentrations of flavanols and participants remained fasted until completion of the 2-h measurements.

Apple extracts

The apple extracts were produced from a crude apple juice by a combination of alcoholic extraction and chromatographic separation to generate i) an epicatechin-rich extract containing around 30 % (*w/w*) of monomeric catechins (90% (–)-EC, 10% (+)-catechin) that retained some of the PC and ii) an oligomeric PC rich extract that was significantly depleted of monomeric catechins. Both materials were produced from the same single crop of apples grown in Herefordshire, UK. Extracts were

157 analysed for monomeric and oligomeric catechins by HPLC-based separation using
158 a HILIC stationary phase (Luna Hilic column 150 x 2.0 mm; 3µM) coupled with
159 fluorescence detection as described previously (20). The flavanol composition of the
160 three apple treatments is shown in **Table 1**. In addition, the apple extracts were
161 shown to contain only the (-)-enantiomer of EC and the (+)-enantiomer of catechin
162 using an HPLC column with a chiral stationary phase. The extracts contained minor
163 quantities of other apple polyphenols as follows: Epicatechin-rich extract (low dose) -
164 4.5 mg chlorogenic acid, 3.2 mg phloridzin, <1 mg quercetin glycosides; procyanidin-
165 rich extract – 11.8 mg chlorogenic acid, 10.3 mg phloridzin, 3.0 mg quercetin
166 glycosides.
167
168 **Blood pressure assessment**
169 Office BP was measured using an automated BP monitor (Omron M6).
170 Measurements were conducted in a quiet room after a 10 min period of rest with the
171 participant in a semi-supine position and the arm resting at heart level.
172 Measurements were conducted using the same designated arm (determined at
173 eligibility assessment) for the duration of the entire study. Four consecutive readings
174 were obtained at 5 min intervals, and the first reading discarded.
175 BP was also assessed using the validated Vicorder device (Smart Medical;
176 Gloucester, UK). Peripheral SBP (pSBP) and peripheral DBP (pDBP) measurements
177 were obtained in a similar manner to office BP. Aortic SBP (aSBP) and aortic DBP
178 (aDBP) were derived from the peripheral BP and brachial pulse wave analysis from
179 an in-built transfer function of the vicorder device. To minimize the effects of diurnal
180 variation in BP, baseline and day 29 measurements were always conducted at the
181 same time of day (07.30 – 08.00) for all participants.

Arterial stiffness assessments

Cf_PWV, ba_PWV and AI were measured with participants in the semi-supine position (30° angle) using the vicorder device. To determine cf_PWV, an inflatable sensor (30 mm) was placed over the right carotid region and a BP cuff placed around the upper right thigh to measure the carotid and femoral pressure pulse waves, respectively. Path length was determined by measuring the distance (in cm) between the supra-sternal notch and the mid-point of the thigh cuff. The carotid sensor and thigh cuff were inflated (~ 60 mmHg) and waveforms simultaneously recorded over 10-15 consecutive heartbeats. To determine ba_PWV, BP cuffs were placed around the ankle and the right upper arm. Path length was determined by measuring the distance between the supra-sternal notch and the mid-point of the ankle cuff. Both cuffs were inflated and pressure waveforms simultaneously recorded.

Biochemical markers for CVD

Whole blood was collected into EDTA, heparin and serum separating tubes (Becton-Dickenson). Samples collected into EDTA and heparin tubes were immediately centrifuged at 2500 x g for 10 mins. Samples collected in serum separating tubes were centrifuged after 30 minutes at 2000 x g for 10 mins. After centrifugation, plasma and serum were stored at -80 ºC until analysis.

Plasma ET-1 and insulin status were determined using commercially available ELISA kits (R&D systems) according to the manufacturer's instructions. Serum lipids (total/HDL/LDL cholesterol and TAG), glucose and fructosamine status were

determined using an automated clinical chemistry analyser (Randox; Daytona plus). The plasma concentration of NO reaction products (nitrate + nitrite + nitrosothiols) was determined using a Sievers 280i NO analyser (Sievers Instruments Inc) comprising of a purge vessel attachment, hot water bath to control the temperature of the reaction vessel and a chill bath to control the condenser. The 280i NOA reduces nitrite, nitrate and nitrosothiols to NO in the purge vessel which is then quantified according to the chemiluminescence signal released transiently within the instrument. Plasma samples were deproteinated with cold ethanol (1:2 dilutions) and centrifuged before analysis. Samples (20 µL) were injected onto the purge vessel and analysed in triplicate.

Plasma metabolite profiles

Identification of the plasma metabolites in d1 (baseline) and d29 samples was conducted by Metabolon USA (*www.metabolon.com*) using ultra-performance liquid chromatography (Waters ACQUITY)) and a high resolution mass spectrometer (Thermo Scientific) interfaced with a heated electrospray ionization source and orbitrap mass analyser operated at 35,000 mass resolution. A total of 671 relevant metabolites were identified. The classes of metabolites were: xenobiotics, lipids amino acids, nucleotides, peptides, carbohydrates, vitamins and catecholamines (**see on-line supplemental Table 3 for a list of all metabolites)**. Values were normalized in terms of raw area counts, each metabolite rescaled to set the median equal to 1, and any missing values imputed with the minimum observed value for each compound. The average percentage missing values was 10.2% overall; 32.1% for xenobiotics, 5.9% for non-xenobiotics, and 4.5% for the dataset used for multivariate analyses (see below).

232

233 **Analysis of epicatechin in plasma**

234 Plasma samples (200 µl) were mixed with 5 % aqueous trichloroacetic acid (200 µl)
235 and dimethylformamide (200 µl). Taxifolin (10 µl; 10µg/mL) was added as an internal
236 standard. Samples were vortexed mixed before centrifugation at 17,000 g for 15
237 min. Post centrifugation, samples were filtered (0.45 µm disposable filter) prior to
238 UPLC-MS analysis.

239 Samples were injected onto a Waters C18 column (100 mm × 2.1 mm; 1.7
240 µM) connected to an Agilent 1200 LC system with the eluent passing through a
241 diode array detector and a triple quadrupole mass spectrometer (Applied Biosystems
242 6490, Ontario, Canada) and eluted at a flow rate of 0.4 ml/min. Elution was achieved
243 using a gradient of increasing solvent B (acetonitrile and 0.1% acetic acid) from
244 solvent A (water and 0.1% acetic acid) as follows: T = 0 min, solvent B = 5%; t = 0.5
245 min, solvent B = 5%; t = 10 min, solvent B = 95%; t = 11 min, solvent B = 95%; t =
246 11.1 min, solvent B = 5% and t = 13 min, solvent B = 5%. The injection volume was
247 1 µL. Catechin/EC metabolites were quantified against a (-)-EC standard over the
248 range 10 – 1000 µg/mL.

249

250 **Statistical analysis**

251 The study was powered to detect a mean reduction in SBP of 4.1 mmHg at 90%
252 power and with a significance level of 0.05, and the pre-study power calculation was
253 based on SBP in a cohort of participants enrolled in a study conducted at University
254 College Cork, Ireland. The calculations supported the recruitment of 39 participants
255 in total. For the biomarkers of CVD risk, post – pre-differences between the four
256 treatments were analysed using a non-parametric repeated measures ANOVA after

257 excluding subjects with an incomplete set of differences. All data are presented as
258 means ± SD.
259 Plasma metabolite data were analysed using multivariate methods were used to
260 analyse the plasma metabolite data. For this, Xenobiotics and compounds for which
261 more than half the values were missing (110 & 11 compounds, respectively) were
262 removed, after checking the prevalence of missing values was not treatment specific.
263 The resulting dataset (8 measurements per volunteer x 550 compounds, logged
264 data) was analysed by Principal Component Analysis (PCA) (21) and by ANOVA-
265 simultaneous component analysis (ASCA) (22) with factors time, treatment, and an
266 interaction term. For ASCA a permutation test to each factor (x1000 permutations)
267 provided a p-value (H_0= the factor has no effect on the experimental outcome). A
268 second dataset was derived (ratio between values at day 29 and day 1, logged) and
269 analysed by PCA. One to fourteen scores were used as input for classification into 4
270 groups (high monomeric flavanol dose, low monomeric flavanol dose, placebo and
271 PC) by discriminant analysis (21). A permutation test (random attribution of each
272 volunteer's 4 measurements to 4 groups x1000 permutations) was used to generate
273 a p-value (probability of getting the classification success rate obtained with the real
274 groups if there was no structure to the data). All analyses were conducted in Matlab
275 2015a (The Mathworks, Cambridge, UK) except for ASCA which was carried out with
276 the PLS Toolbox (version 8.01; EigenVector Research) (23).
277
278 **RESULTS**
279
280 **Study population**

Of the forty-three participants randomized to treatment, forty two completed the trial (15 men and 27 women). One participant withdrew part way through the first test period citing difficulties with study time commitments as the reason for withdrawal. No serious adverse events were reported during the trial. **Figure 1** shows the flow of participants through the trial. Participant parameters at eligibility assessment were (mean ± SD, n=42): age 63 ± 7 years; BMI 25.9 ± 3.1 kg/m^2; SBP 137 ± 10; DBP 80 ± 7 mmHg. Compliance to treatments was high with overall compliance of 99.7% (capsules taken as a proportion of intended total) and individual compliance of ≥ 92%.

Absorption of epicatechin and metabolites

Plasma EC metabolites were not detected in the baseline samples collected prior to the start of any of the treatments. Mean plasma total EC metabolite concentrations 2 h after ingestion of the low and high dose EC treatments were 3.51 ± 2.2 and 9.07 ± 4.71, μmol/L, respectively. The major metabolites were EC-monoglucuronides, EC-monosulfates and methyl-EC-monosulfates. As expected, only trace amounts of EC metabolites were detected 2 h after ingestion of the PC treatment (mean plasma peak concentration, 0.01 μmol/L). EC metabolites were not detected in any of the samples collected after ingestion of the placebo treatment, demonstrating excellent adherence to the low flavanol diet by all participants.

Effects of treatments on SBP and other markers of CVD risk

Table 2 shows the mean values for the various biomarkers of CVD risk for all participants at the start of the trial. All values were within reported physiological ranges. At 2 h after the ingestion of different doses of apple flavanols, no significant

differences were observed in SBP, DBP (peripheral and aortic), plasma NO, cf_PWV, ba_PWV or AI between treatments and placebo control **(Table 3)**. Furthermore, we did not observe significant differences in BP, arterial stiffness or the plasma biomarkers ET-1, NO reaction products, total cholesterol, HDL/LDL cholesterol, triglycerides, glucose, fructosamine or insulin after 4 weeks' intervention (**Table 4**). Post-hoc analysis of the primary outcome data (sBP; SD of differences = 5.69 mm Hg) showed that the study had a power of 80% to detect a difference of 2.53 mm Hg ($P<0.05$) between placebo and any one of the other treatments.

Effects of treatments on metabolic profiles

Inter-individual differences in metabolite profiles were large relative to differences between profiles of a given individual measured for different treatments and time points **(**see PCA scores plot of individual metabolite profiles, **Figure 2 A and supplemental Figure 1 A).** The change in profiles between the two time-points was, however, of a similar scale between individuals (see PCA scores plot for ratioed data, (d29 vs d1), **Figure 2 B and supplemental Figure 1 B**). However, the overall success rate of classification of the profiles into the four treatment groups was no higher than would be expected by chance ($p >0.1$). Moreover, in the multivariate analysis of variance ASCA neither time, treatment, or their interaction, was found to be significant ($p=0.91$, $p=1$, $p=1$ respectively). This suggested that none of the treatments altered the metabolite profiles in a statistically significant (i.e. consistent) way.

DISCUSSION

The main finding of this study was that, compared to a placebo control, there were no significant effects of consuming an EC-rich flavanol extract (at doses of 70 or 140 mg monomeric flavanols/d) or a monomeric flavanol-depleted PC-rich extract either 2 h after a single dose (acute) or after daily consumption for 28 days on SBP or any of the other biomarkers of cardiometabolic risk. The appearance of EC phase-2 conjugates in plasma 2 h after ingestion of the EC-rich flavanol extracts demonstrates that the EC was effectively absorbed resulting in mean peak plasma concentrations in the 3-10 μmol/L range, which is in keeping with previous reports (19). Since the isolated PC treatment provided a dose of procyanidin equivalent to that for the high dose EC-rich flavanol extract, these data show that neither monomeric flavanols nor PC significantly affected any of the cardiometabolic biomarkers. Further, they confirm the findings of a previously published report which indicated that PC are not a source of monomeric flavanols (i.e. EC and catechin are not released during fermentation by the gut microbiota) (24).

Oligomeric PC have been shown to exert biological activity in vitro (17, 18) but their bioavailability is very limited, and only nM concentrations of dimers and occasionally trimers have been reported to appear in peripheral blood. As far as we are aware, the current report is the first RCT describing the effects of isolated PC on biomarkers for CVD. The finding of no significant effects on any of the biomarkers assessed is consistent with the limited bioavailability of PC, although it is possible that PC can exert effects in the body via the absorption of gut microbiota-derived metabolites such as hydroxyphenyl-γ-valerolactones, phenylvaleric acids and hydroxyphenylacetic acids which may be more extensively absorbed (25, 26). Although the data presented here do not suggest that these gut microbiota-derived metabolites were effective in changing the biomarkers assessed in this study, further

research of their biological activities is warranted. As a group, the microbiota-derived ring fission products represent the most substantial form of EC metabolites in humans, accounting for 78% of the radiolabel absorbed and excreted in urine over the 48 hours following ingestion of a single dose of [2-^{14}C]-(–)-EC compared to 22% for epicatechin phase-2 conjugates (26).

Despite the lack of clinical effects of monomeric and oligomeric flavanols in this report, the BP-lowering effects of flavanol-rich foods and extracts has been reported for a significant number of human intervention trials, most commonly after consumption of cocoa and cocoa based products over various durations in both healthy and non-healthy populations. Several meta-analyses of these trials report significant mean reductions in SBP as follows; 5.9 ($p = 0.0003$) (4), 4.5 ($p = <0.001$) (6), 2.8 ($p = 0.005$) (27) and 4.7 ($p = 0.002$) mmHg (5). The largest net reduction in office SBP observed in our trial was 0.39 mmHg after 28 d ingestion of the apple extract containing a 70 mg dose of monomeric flavanols; this falls well short of the BP responses reported in meta-analyses, was not significant, and there was not an increased response at the higher dose. Aside from the differences in study design (e.g. hypertensive vs normotensive, dose and duration of intervention, food matrix etc.) which makes direct comparisons in systematic reviews challenging, it has been hypothesised that the ‘open label’ approach to the treatments in many of these trials (e.g. flavanol-rich dark chocolate vs flavanol poor white chocolate) has increased the potential for the observed reductions in BP to be an ‘exaggerated’ placebo effect. This is supported by the lack of effect observed in several trials after ingestion of a cocoa beverage, in which the control drink has been better able to facilitate a blinded approach to the treatment, and that the effects of cocoa intervention trials on BP

were weak and non-significant when sub-grouped to take account of the blinding of participants to the treatment (-0.71 mmHg, p = 0.54) (27).

Ingestion of flavanol-rich foods and beverages has also been reported to cause improvements in other biomarkers of CVD risk. For example, meta-analyses of human intervention trials after cocoa and green tea supplementation have shown significant reductions in serum insulin (7) and LDL cholesterol (4, 28, 29). In the study reported here, the largest reduction in LDL cholesterol was 0.08 mmol/L after 28 d supplementation with the apple flavanol extract containing 70 mg of monomeric flavanols. This effect size is 2-3-fold less than the overall reductions observed in the reported meta-analyses of RCT's, and is not significant. Similarly, in some trials cocoa ingestion has been shown to decrease plasma ET-1 and increase plasma NO reaction products (10, 30) and in a dose dependant manner (30). We previously reported a significant increase in plasma NO 6 h after ingestion of apple purees containing 25 and 100 mg EC but not after 14 d ingestion (31). However, the magnitude of change in NO reaction products was similar for both doses of EC/flavanols, and there was not an associated effect on ET-1. Likewise, 4 weeks' ingestion of a cocoa beverage containing as little as 64 mg EC/d has been reported to significantly reduce cf_PWV by 0.4 m/s (13) which is double the reduction of 0.2 m/s observed for the equivalent duration and dose of epicatechin ingested by participants in the study reported here.

There is a contradiction between an increasing number of reports describing increases in flow mediated dilatation, reductions in BP and changes in other biomarkers caused by consumption of flavanol-rich complex foods and beverages, and in particular cocoa and cocoa products, and the more recent reports that show no significant effects of purified EC (19, 20, this report) and purified PC (this report) on these vascular biomarkers. Two alternative paradigms can be considered: Either (i)

404 the physiological effects of consuming cocoa / dark chocolate are caused by other
405 components in cocoa and are not dependent on the flavanols or (ii) the flavanols are
406 involved in causing these effects but only in combination with other components in
407 cocoa. Cocoa contains other compounds such as sugar, fats and bioactive
408 phytochemicals including the methylxanthines theobromine and caffeine, all of which
409 have the potential to affect the physiological responses to consumption of cocoa
410 products. Theobromine for example, is present in chocolate at concentrations up to
411 1.4% by weight (32) and there is evidence that it has biological activity (33, 34), and
412 the effects of caffeine are widely reported and include increasing FMD and BP and
413 reducing insulin sensitivity (35, 36). Further, a recent report provides evidence from
414 carefully controlled dietary intervention trials to support the supposition that cocoa
415 flavanols work in combination with other cocoa components to affect BP, endothelial
416 function and other vascular biomarkers (37). Sansone and colleagues show that cocoa
417 flavanols alone significantly increased flow mediated dilatation (FMD) of the brachial
418 artery but did not significantly decrease BP 2 h after consumption of the test drinks,
419 whereas when administered in combination with methylxanthines, both FMD and DBP
420 were improved, and the FMD increase was significantly higher than with cocoa
421 flavanols alone. Methylxanthines alone did not significantly affect FMD, DBP or SBP.
422 These recent findings (lack of effects of isolated monomeric and oligomeric PC on BP
423 (15, 16) this report), and a recent report showing that cocoa flavanols were only
424 effective in reducing BP when administered in combination with methylxanthines (37)
425 serves as a reminder that foods are complex and contain numerous compounds,
426 therefore, ascribing a physiological response to consumption of a complex food like
427 cocoa to just one compound or class of compounds is somewhat naive. Although it
428 could be argued that not using a complex food such as apples or cocoa as the

treatment was a weakness in the trial design, the appropriate placebo food products (apples and cocoa that are similar except they lack the flavanols) are not available.

CONCLUSION

Acute and chronic consumption of isolated apple monomeric flavanols and PC oligomers had no significant effect on BP, various other cardiometabolic biomarkers or the concentrations of circulating host metabolites in pre-hypertensive adults. These data do not support the notion that in isolation, monomeric flavanols and/or PC can significantly affect blood pressure and other biomarkers of cardiometabolic risk.

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Table 1. *Mass (mg) of monomers and oligomers in apple flavanol treatments*[1]

Analyte	High dose	Low dose	PC
(-)-Epicatechin	125.9	62.9	6.5
(+)-Catechin	14.0	7.0	
dp2	80.0	40.0	10.3
dp3	31.6	15.8	14.3
dp4	11.4	5.7	21.9
dp5	4.2	2.1	23.7
dp6	2.3	1.2	19.1
dp7	1.2	0.6	12.7
dp8	0.0	0.0	10.3
dp9	0.0	0.0	10.4
dp10	0.0	0.0	7.4
Total procyanidins	130.7	65.4	130.1
Total flavanols	270.6	135.3	136.5

[1] The apple flavanol treatments were High dose = 140 mg monomers + 130 mg PC; Low dose = 70 mg monomers + 65 mg PC; PC = 130 mg PC. (–)-EC was the predominant monomer in the monomer rich extract (90% (–)-EC, 10% (+)-catechin). dp = degree of polymerisation, e.g. dp2 refers to an epicatechin dimer.

Table 2. *Subject characteristics and biomarkers of CVD risk at baseline (n=42)*

Measurement variable	Baseline value
Gender ratio (M/F)	15/27
BMI (kg/m^2)	25.9 ± 3.1[1]
Age (yr)	63.0 ± 7.0
Office SBP (mmHg)	121 ± 9[2]
Office DBP (mmHg)	69 ± 7
Vicorder pSBP (mmHg)	128 ± 10.8
Vicorder pDBP (mmHg)	66 ± 5.4
Vicorder aSBP (mmHg)	126 ± 11
Vicorder aDBP (mmHg)	66 ± 6
Cf_PWV (m/s)	8.2 ± 1.3
Ba_PWV (m/s)	14.4 ± 1.9
AI (%)	26.8 ± 6.4
ET-1 (pg/mL)	1.1 ± 0.4
NO reaction products (µmol/L)	35.9 ± 17.0
Insulin (pmol/L)	39.8 ± 17.1
Glucose (mmol/L)	5.3 ± 0.5
Fructosamine (µmol/L)	252.2 ± 47.1
Total cholesterol (mmol/L)	6.2 ± 1.0
HDL_Cholesterol (mmol/L)	1.68 ± 0.52
LDL_Cholesterol (mmol/L)	3.91 ± 0.92
Triglycerides (mmol/L)	1.50 ± 0.95

[1]Data are presented as mean ± SD (all such values)
[2]SBP measured at baseline was lower than that measured at eligibility assessment. This was expected because BP rises during the morning and baseline measurements were recorded earlier in the day.

Table 3. *Changes in biomarkers of CVD risk, 2 h after ingestion of treatments containing either a low dose of monomeric apple flavanols, a high dose of monomeric apple flavanols, oligomeric procyanidins or placebo*[1]

Measurement variable	n	Low dose	High dose	Procyanidins	Placebo	P-values[2]
Office SBP (mmHg)	42	6.14 ± 5.49	7.08 ± 6.11	7.79 ± 7.81	7.15 ± 6.05	0.206
Office DBP (mmHg)	42	3.03 ± 2.79	3.94 ± 3.92	2.91 ± 3.24	3.58 ± 2.74	0.444
Vicorder pSBP (mmHg)	42	6.81 ± 7.12	7.36 ± 6.75	7.17 ± 6.53	6.13 ± 6.88	0.754
Vicorder pDBP (mmHg)	42	1.91 ± 3.66	2.71 ± 3.51	1.81 ± 3.30	2.12 ± 3.28	0.849
Vicorder aSBP (mmHg)	42	6.27 ± 7.03	6.92 ± 6.67	6.82 ± 6.61	5.86 ± 6.66	0.780
Vicorder aDBP (mmHg)	42	1.76 ± 3.64	3.36 ± 5.16	1.46 ± 3.66	2.23 ± 3.31	0.381
Cf_PWV (m/s)	42	-0.04 ± 0.57	0.11 ± 0.72	-0.15 ± 1.77	0.18 ± 0.70	0.531
Ba_PWV (m/s)	42	0.35 ± 0.50	0.34 ± 0.60	0.43 ± 0.66	0.31 ± 0.68	0.869
AI (%)	42	-0.33 ± 2.92	-1.19 ± 3.26	-0.95 ± 2.39	-1.04 ± 2.85	0.351
NO reaction products (μM)	34	-4.98 ± 5.49	-4.67 ± 6.88	-3.78 ± 4.69	-5.73 ± 5.13	0.461

[1] Low dose = 70 mg monomers + 65 mg procyanidins; High dose = 140 mg monomers + 130 mg procyanidins; procyanidins = 6.5 mg monomers + 130 mg procyanidins. Data are mean ± SD (all such values)

[2] P values were calculated from the post – pre-differences between the four treatments using repeated measures ANOVA after excluding subjects with an incomplete set of differences. (see on-line supporting material for actual values; supplemental Table 1)

Table 4. *Changes in biomarkers of CVD risk, 29 d after ingestion of treatments containing either a low dose of monomeric apple flavanols, a high dose of monomeric apple flavanols, oligomeric procyanidins or placebo*[1]

Measurement variable	n	Low dose	High dose	Procyanidins	Placebo	p-value[2]
Office SBP (mmHg)	42	-0.39 ± 6.11	1.83 ± 6.62	2.54 ± 7.02	1.60 ± 5.04	0.076
Office DBP (mmHg)	42	-0.19 ± 3.94	1.06 ± 4.43	0.77 ± 3.90	0.55 ± 3.57	0.501
Vicorder pSBP (mmHg)	42	-1.36 ± 7.14	0.83 ± 7.72	2.07 ± 6.58	1.61 ± 7.50	0.283
Vicorder pDBP (mmHg)	42	-0.61 ± 3.41	1.04 ± 4.43	0.81 ± 4.12	0.93 ± 3.01	0.536
Vicorder aSBP (mmHg)	41	-1.44 ± 7.26	0.74 ± 7.71	2.44 ± 6.51	1.93 ± 7.33	0.155
Vicorder aDBP (mmHg)	41	-0.54 ± 3.40	1.34 ± 5.11	0.68 ± 3.93	1.09 ± 3.03	0.565
Cf_PWV (m/s)	42	-0.20 ± 0.67	-0.03 ± 0.53	-0.25 ± 1.74	0.03 ± 0.68	0.791
Ba_PWV (m/s)	41	-0.02 ± 0.63	0.30 ± 0.73	0.17 ± 0.82	0.15 ± 0.60	0.057
AI (%)	41	0.00 ± 2.35	-0.07 ± 2.88	0.24 ± 2.30	0.28 ± 2.60	0.786
NO reaction products (μM)	34	2.40 ± 24.38	9.17 ± 30.19	4.36 ± 19.08	5.03 ± 18.94	0.234
Endothelin-1 (pg/mL)	36	-0.01 ± 0.38	0.04 ± 0.38	0.09 ± 0.32	0.05 ± 0.29	0.289
Insulin (pmol/L)	36	-2.40 ± 23.05	-5.14 ± 13.02	-4.28 ± 12.38	-1.57 ± 11.65	0.339
Glucose (mmol/L)	34	-0.06 ± 0.31	-0.16 ± 0.30	-0.09 ± 0.25	0.01 ± 0.40	0.142
Fructosamine (μmol/L)	33	-1.20 ± 16.79	2.03 ± 14.83	2.71 ± 12.66	2.57 ± 25.33	0.944
Total cholesterol (mmol/L)	34	-0.13 ± 0.51	-0.01 ± 0.60	-0.03 ± 0.62	-0.05 ± 0.67	0.836
LDL cholesterol (mmol/L)	34	-0.08 ± 0.53	-0.03 ± 0.46	-0.04 ± 0.56	-0.02 ± 0.53	0.971
HDL cholesterol (mmol/L)	34	-0.03 ± 0.14	0.03 ± 0.18	0.02 ± 0.16	0.01 ± 0.25	0.674
Triglycerides (mmol/L)	34	-0.17 ± 0.49	-0.08 ± 0.37	-0.10 ± 0.43	0.06 ± 0.37	0.067

[1] Low dose = 70 mg monomers + 65 mg procyanidins; High dose = 140 mg monomers + 130 mg procyanidins; procyanidins = 6.5 mg monomers + 130 mg procyanidins. Data are mean ± SD.

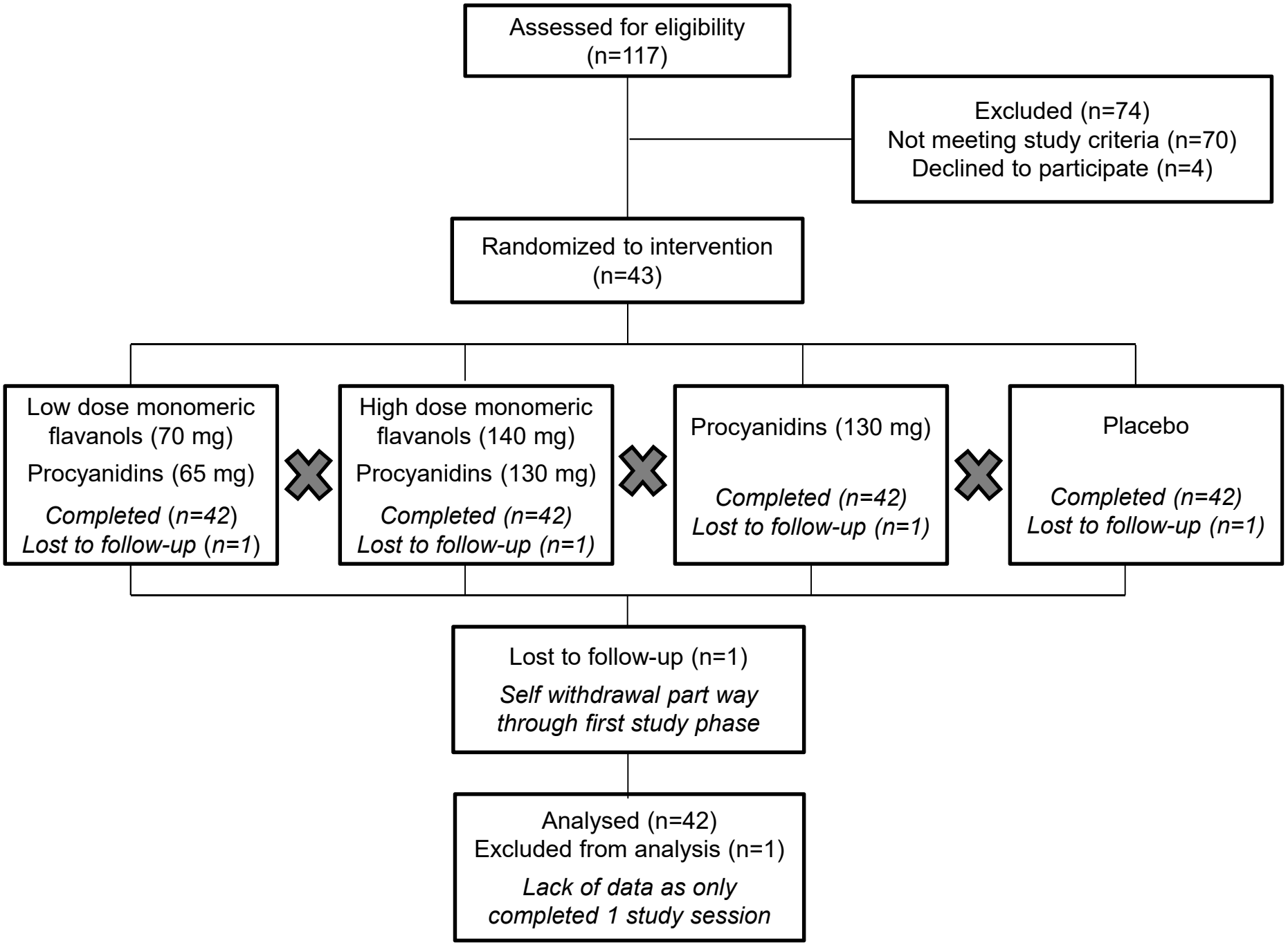
[2] P values were calculated form the post–pre-differences between the four treatments using repeated measures ANOVA after excluding subjects with an incomplete set of differences. (See on-line supporting material for actual values; supplemental Table 2)

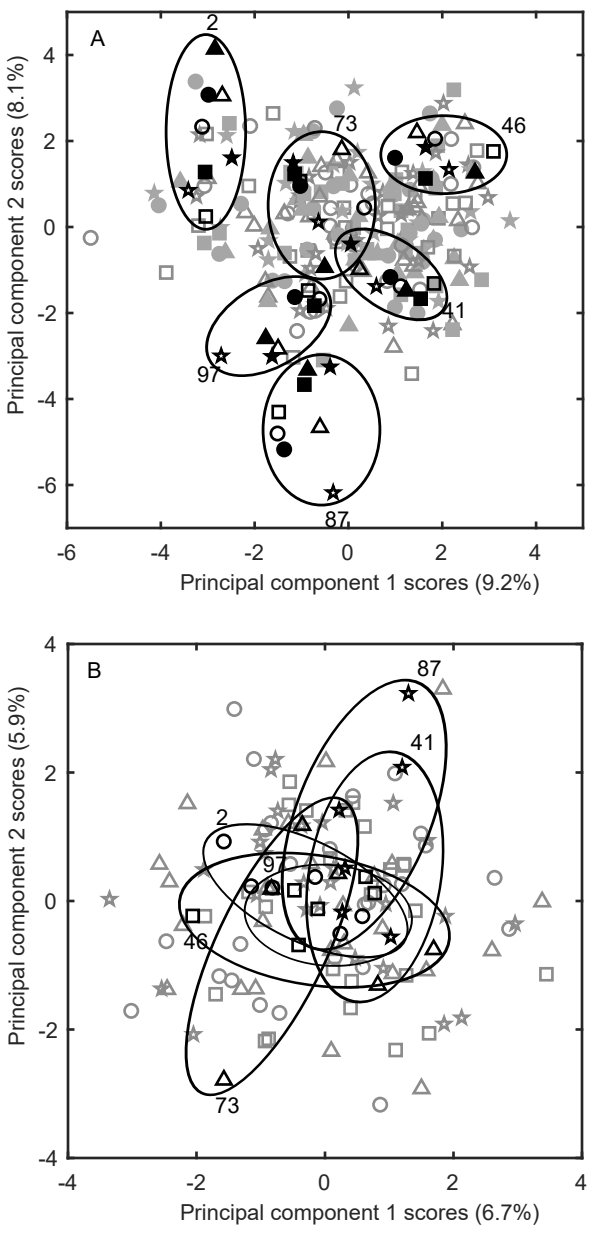
Figure Legends

Figure 1. Flow of participants through trial. Of the 117 participants assessed for eligibility, 74 did not meet the inclusion criteria. Eligible participants (n=43) were allocated sequentially to a treatment sequence order that was pre-determined using a block randomization approach. 1 participant dropped out of the trial part way through the first treatment phase.

Figure 2. Principal component analysis (PCA) for the metabolomic data for all participants and treatments, for both time points (A; n=32 x 4 treatments x 2-time points (day 1, day 29)) and for the ratio of post-to-pre-treatment (B; n=32 x 4 treatments).

Scores on the first two principal components are shown and the percentage variance accounted for by each component indicated on each axis. The circle represents low dose monomeric flavanol treatment, the square the high dose monomeric flavanol treatment, the triangle the placebo, and the star the procyanidin treatment. For (A), open symbols represent day 1 and closed symbols day 29 of the treatments. Six individuals are highlighted; the volunteer number is given and an ellipse shows the spread of scores for each participant) (See on-line supporting material for scores on the third and fourth principle components; supplemental Figure 1).


Assessed for eligibility
(n=117)
Excluded (n=74)
Not meeting study criteria (n=70)
Declined to participate (n=4)
Randomized to intervention
(n=43)
Low dose monomeric flavanols (70 mg)
Procyanidins (65 mg)
Completed (n=42)
Lost to follow-up (n=1)
High dose monomeric flavanols (140 mg)
Procyanidins (130 mg)
Completed (n=42)
Lost to follow-up (n=1)
Procyanidins (130 mg)
Completed (n=42)
Lost to follow-up (n=1)
Placebo
Completed (n=42)
Lost to follow-up (n=1)
Lost to follow-up (n=1)
Self withdrawal part way through first study phase
Analysed (n=42)
Excluded from analysis (n=1)
Lack of data as only completed 1 study session


A
2
73
46
41
97
87
Principal component 2 scores (8.1%)
Principal component 1 scores (9.2%)
B
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2
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73
Principal component 2 scores (5.9%)
Principal component 1 scores (6.7%)

On line supporting material

Supplemental Table 1. *Differences in biomarkers of CVD risk 2h after ingestion of treatments containing either a low dose of monomeric apple flavanols, a high dose of monomeric apple flavanols, oligomeric procyanidins or placebo*

Measurement variable	n	Low dose		High dose		Procyanidins		Placebo	
		0h	2h	0h	2h	0h	2h	0h	2h
Office SBP (mmHg)	42	121 ± 10	127 ± 11	120 ± 10	127 ± 11	120 ± 9	128 ± 13	121 ± 9	128 ± 9
Office DBP (mmHg)	42	69 ± 7	72 ± 7	68 ± 7	72 ± 7	68 ± 6	71 ± 7	69 ± 7	73 ± 7
Vicorder pSBP (mmHg)	42	128 ± 11	134 ± 13	126 ± 10	134 ± 12	126 ± 10	133 ± 12	127 ± 9	133 ± 10
Vicorder pDBP (mmHg)	42	66 ± 5	68 ± 6	66 ± 6	68 ± 6	66 ± 5	68 ± 6	66 ± 5	68 ± 5
Vicorder aSBP (mmHg)	41	126 ± 11	132 ± 13	124 ± 10	131 ± 12	124 ± 10	131 ± 12	124 ± 9	130 ± 10
Vicorder aDBP (mmHg)	41	65 ± 8	67 ± 9	65 ± 6	69 ± 6	65 ± 8	67 ± 8	66 ± 5	68 ± 5
Cf_PWV (m/s)	42	8.2 ± 1.4	8.2 ± 1.4	8.1 ± 1.3	8.2 ± 1.4	8.3 ± 1.8	8.1 ± 1.5	8.1 ± 1.2	8.3 ± 1.5
Ba_PWV (m/s)	42	14.4 ± 2.0	14.7 ± 2.0	14.3 ± 1.9	14.6 ± 2.1	14.2 ± 1.9	14.7 ± 1.9	14.5 ± 2.2	14.8 ± 2.2
AI (%)	42	27 ± 6	26 ± 6	28 ± 7	26 ± 6	27 ± 6	26 ± 7	27 ± 6	26 ± 5
Nitric oxide (μM)	34	40 ± 21	31 ± 12	37 ± 20	32 ± 16	34 ± 14	30 ± 12	36 ± 14	31 ± 10

Data are presented as mean ± SD

On line supporting material

Supplemental Table 2. *Differences in biomarkers of CVD risk, 29 d after ingestion of treatments containing either a low dose of monomeric apple flavanols, a high dose of monomeric apple flavanols, oligomeric procyanidins or placebo*

Measurement variable	n	Low dose		High dose		Procyanidins		Placebo	
		0h	d29	0h	d29	0h	d29	0h	d29
Office SBP (mmHg)	42	121 ± 10	121 ± 10	120 ± 10	122 ± 10	120 ± 9	123 ± 11	121 ± 9	123 ± 9
Office DBP (mmHg)	42	69 ± 7	69 ± 7	68 ± 7	69 ± 7	68 ± 6	69 ± 7	69 ± 7	73 ± 8
Vicorder pSBP (mmHg)	42	128 ± 11	126 ± 10	126 ± 10	127 ± 11	126 ± 10	128 ± 11	127 ± 9	128 ± 10
Vicorder pDBP (mmHg)	42	66 ± 5	66 ± 6	66 ± 6	67 ± 6	66 ± 5	67 ± 6	66 ± 5	67 ± 5
Vicorder aSBP (mmHg)	41	126 ± 11	124 ± 10	124 ± 10	125 ± 11	124 ± 10	126 ± 11	124 ± 9	126 ± 10
Vicorder aDBP (mmHg)	41	65 ± 8	65 ± 9	65 ± 6	67 ± 6	65 ± 8	66 ± 8	66 ± 5	67 ± 5
Cf_PWV (m/s)	42	8.2 ± 1.4	8.0 ± 1.4	8.1 ± 1.3	8.1 ± 1.4	8.3 ± 1.8	8.0 ± 1.4	8.1 ± 1.2	8.1 ± 1.4
Ba_PWV (m/s)	41	14.4 ± 2.0	14.3 ± 2.1	14.3 ± 1.9	14.6 ± 2.3	14.2 ± 1.9	14.4 ± 2.1	14.5 ± 2.2	14.4 ± 2.0
AI (%)	41	27 ± 6	27 ± 7	28 ± 7	27 ± 6	27 ± 6	27 ± 6	27 ± 6	27 ± 5
Nitric oxide (µM)	34	40 ± 21	38 ± 20	37 ± 20	44 ± 31	34 ± 14	42 ± 36	36 ± 14	43 ± 24
Endothelin-1 (pg/mL)	36	1.11 ± 0.40	1.09 ± 0.42	1.08 ± 0.42	1.10 ± 0.43	1.12 ± 0.61	1.12 ± 0.42	1.08 ± 0.39	1.13 ± 0.38
Insulin (pmol/L)	36	41.0 ± 24.0	37.8 ± 20.9	40.9 ± 20.5	35.4 ± 17.7	40.0 ± 19.8	35.8 ± 18.3	40.4 ± 21.5	38.4 ± 18.8
Glucose (mmol/L)	34	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.4	5.3 ± 0.4	5.2 ± 0.4	5.3 ± 0.5	5.3 ± 0.5
Fructosamine (µmol/L)	33	254.5 ± 35.7	254.1 ± 37.1	253.9 ± 37.4	256.2 ± 38.0	254.0 ± 34.1	259.3 ± 37.8	253.6 ± 50.7	259.8 ± 40.6
Total cholesterol (mmol/L)	34	6.2 ± 1.0	6.1 ± 1.1	6.2 ± 0.9	6.2 ± 1.0	6.1 ± 1.0	6.1 ± 1.0	6.2 ± 1.0	6.2 ± 1.1
LDL cholesterol (mmol/L)	34	3.9 ± 0.9	3.8 ± 0.9	3.9 ± 0.9	3.9 ± 0.9	3.9 ± 0.9	3.8 ± 0.9	3.9 ± 1.0	3.9 ± 0.9
HDL cholesterol (mmol/L)	34	1.7 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
Triglycerides (mmol/L)	34	1.5 ± 0.7	1.4 ± 0.7	1.4 ± 0.6	1.3 ± 0.5	1.5 ± 0.8	1.3 ± 0.6	1.4 ± 0.9	1.4 ± 0.5

Data are presented as mean ± SD

ONLINE SUPPLEMENTAL MATERIAL

List of compounds observed in the Metabolon metabolomics analysis

BIOCHEMICAL	SUPER_PATHWAY	SUB_PATHWAY
1,2-dilinoleoyl-GPC (18:2/18:2)	Lipid	Phosphatidylcholine (PC)
1,2-dipalmitoyl-GPC (16:0/16:0)	Lipid	Phosphatidylcholine (PC)
1,3,7-trimethylurate	Xenobiotics	Xanthine Metabolism
1,3-dimethylurate	Xenobiotics	Xanthine Metabolism
1,5-anhydroglucitol (1,5-AG)	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
1,7-dimethylurate	Xenobiotics	Xanthine Metabolism
1-(1-enyl-oleoyl)-2-linoleoyl-GPE (P-18:1/18:2)*	Lipid	Lysoplasmalogen
1-(1-enyl-oleoyl)-GPE (P-18:1)*	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)*	Lipid	Plasmalogen
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	Lipid	Lysoplasmalogen
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	Lipid	Lysoplasmalogen
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	Lipid	Plasmalogen
1-(1-enyl-stearoyl)-GPE (P-18:0)*	Lipid	Lysoplasmalogen
1-arachidonoyl-GPC (20:4n6)*	Lipid	Lysophospholipid
1-arachidonoyl-GPE (20:4n6)*	Lipid	Lysophospholipid
1-arachidonoyl-GPI (20:4)*	Lipid	Lysophospholipid
1-lignoceroyl-GPC (24:0)	Lipid	Lysophospholipid
1-linolenoyl-GPC (18:3)*	Lipid	Lysophospholipid
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	Lipid	Phosphatidylcholine (PC)
1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*	Lipid	Phosphatidylcholine (PC)
1-linoleoyl-GPC (18:2)	Lipid	Lysophospholipid
1-linoleoyl-GPE (18:2)*	Lipid	Lysophospholipid
1-linoleoyl-GPI (18:2)*	Lipid	Lysophospholipid
1-methylguanidine	Amino Acid	Guanidino and Acetamido Metabolism
1-methylhistidine	Amino Acid	Histidine Metabolism
1-methylimidazoleacetate	Amino Acid	Histidine Metabolism
1-methylnicotinamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
1-methylurate	Xenobiotics	Xanthine Metabolism
1-methylxanthine	Xenobiotics	Xanthine Metabolism
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)*	Lipid	Phosphatidylethanolamine (PE)
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	Lipid	Phosphatidylethanolamine (PE)
1-oleoyl-2-linoleoyl-GPI (18:1/18:2)*	Lipid	Phosphatidylinositol (PI)
1-oleoyl-GPC (18:1)	Lipid	Lysophospholipid
1-oleoyl-GPE (18:1)	Lipid	Lysophospholipid
1-oleoyl-GPI (18:1)*	Lipid	Lysophospholipid
1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*	Lipid	Phosphatidylcholine (PC)
1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	Lipid	Phosphatidylcholine (PC)
1-palmitoleoyl-GPC (16:1)*	Lipid	Lysophospholipid
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	Lipid	Phosphatidylethanolamine (PE)
1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)*	Lipid	Phosphatidylinositol (PI)
1-palmitoyl-2-gamma-linolenoyl-GPC (16:0/18:3n6)*	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	Lipid	Phosphatidylethanolamine (PE)
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	Lipid	Phosphatidylethanolamine (PE)
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*	Lipid	Phosphatidylinositol (PI)
1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-GPC (16:0)	Lipid	Lysophospholipid
1-palmitoyl-GPE (16:0)	Lipid	Lysophospholipid
1-palmitoyl-GPI (16:0)	Lipid	Lysophospholipid
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Lipid	Phosphatidylcholine (PC)
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Lipid	Phosphatidylethanolamine (PE)
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	Lipid	Phosphatidylinositol (PI)
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	Lipid	Phosphatidylserine (PS)
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	Lipid	Phosphatidylcholine (PC)
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	Lipid	Phosphatidylethanolamine (PE)
1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	Lipid	Phosphatidylinositol (PI)
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	Lipid	Phosphatidylcholine (PC)
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	Lipid	Phosphatidylethanolamine (PE)
1-stearoyl-2-oleoyl-GPI (18:0/18:1)*	Lipid	Phosphatidylinositol (PI)
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	Lipid	Phosphatidylserine (PS)
1-stearoyl-GPC (18:0)	Lipid	Lysophospholipid
1-stearoyl-GPE (18:0)	Lipid	Lysophospholipid
1-stearoyl-GPI (18:0)	Lipid	Lysophospholipid
10-undecenoate (11:1n1)	Lipid	Medium Chain Fatty Acid
13-HODE + 9-HODE	Lipid	Fatty Acid, Monohydroxy

16a-hydroxy DHEA 3-sulfate	Lipid	Steroid
18-hydroxycorticosterone	Lipid	Steroid
2'-deoxyuridine	Nucleotide	Pyrimidine Metabolism, Uracil containing
2'-O-methylcytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing
2'-O-methyluridine	Nucleotide	Pyrimidine Metabolism, Uracil containing
2,3-dihydroxy-2-methylbutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2,3-dihydroxyisovalerate	Xenobiotics	Food Component/Plant
2-acetamidophenol sulfate	Xenobiotics	Drug
2-aminoadipate	Amino Acid	Lysine Metabolism
2-aminobutyrate	Amino Acid	Glutathione Metabolism
2-aminoheptanoate	Lipid	Fatty Acid, Amino
2-aminooctanoate	Lipid	Fatty Acid, Amino
2-aminophenol sulfate	Xenobiotics	Chemical
2-hydroxy-3-methylvalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2-hydroxyacetaminophen sulfate*	Xenobiotics	Drug
2-hydroxybutyrate/2-hydroxyisobutyrate	Amino Acid	Glutathione Metabolism
2-hydroxyglutarate	Lipid	Fatty Acid, Dicarboxylate
2-hydroxyhippurate (salicylurate)	Xenobiotics	Benzoate Metabolism
2-hydroxyibuprofen	Xenobiotics	Drug
2-hydroxyoctanoate	Lipid	Fatty Acid, Monohydroxy
2-hydroxyphenylacetate	Amino Acid	Tyrosine Metabolism
2-methoxyacetaminophen glucuronide*	Xenobiotics	Drug
2-methoxyacetaminophen sulfate*	Xenobiotics	Drug
2-methoxyresorcinol sulfate	Xenobiotics	Chemical
2-methylbutyrylcarnitine (C5)	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2-methylbutyrylglycine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
2-oxindole-3-acetate	Xenobiotics	Food Component/Plant
2-oxoarginine*	Amino Acid	Urea cycle; Arginine and Proline Metabolism
2-palmitoyl-GPC (16:0)*	Lipid	Lysophospholipid
2-piperidinone	Xenobiotics	Food Component/Plant
2-stearoyl-GPE (18:0)*	Lipid	Lysophospholipid
21-hydroxypregnenolone disulfate	Lipid	Steroid
3,7-dimethylurate	Xenobiotics	Xanthine Metabolism
3-(3-hydroxyphenyl)propionate	Xenobiotics	Benzoate Metabolism
3-(4-hydroxyphenyl)lactate	Amino Acid	Tyrosine Metabolism
3-(cystein-S-yl)acetaminophen*	Xenobiotics	Drug
3-(N-acetyl-L-cystein-S-yl) acetaminophen	Xenobiotics	Drug
3-aminoisobutyrate	Nucleotide	Pyrimidine Metabolism, Thymine containing
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	Lipid	Fatty Acid, Dicarboxylate
3-hydroxy-2-ethylpropionate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxy-3-methylglutarate	Lipid	Mevalonate Metabolism
3-hydroxybutyrate (BHBA)	Lipid	Ketone Bodies
3-hydroxybutyrylcarnitine (1)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
3-hydroxybutyrylcarnitine (2)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
3-hydroxyhexanoate	Lipid	Fatty Acid, Monohydroxy
3-hydroxyhippurate	Xenobiotics	Benzoate Metabolism
3-hydroxyisobutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-hydroxyoctanoate	Lipid	Fatty Acid, Monohydroxy
3-hydroxypyridine sulfate	Xenobiotics	Chemical
3-hydroxyquinine	Xenobiotics	Drug
3-indoxyl sulfate	Amino Acid	Tryptophan Metabolism
3-methoxycatechol sulfate (1)	Xenobiotics	Benzoate Metabolism
3-methoxytyrosine	Amino Acid	Tyrosine Metabolism
3-methyl catechol sulfate (1)	Xenobiotics	Benzoate Metabolism
3-methyl-2-oxobutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-methyl-2-oxovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-methyladipate	Lipid	Fatty Acid, Dicarboxylate
3-methylcytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing
3-methylglutaconate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-methylglutarylcarnitine (2)	Amino Acid	Leucine, Isoleucine and Valine Metabolism
3-methylhistidine	Amino Acid	Histidine Metabolism
3-methylxanthine	Xenobiotics	Xanthine Metabolism
3-phenylpropionate (hydrocinnamate)	Xenobiotics	Benzoate Metabolism
3-ureidopropionate	Nucleotide	Pyrimidine Metabolism, Uracil containing
3beta-hydroxy-5-cholestenoate	Lipid	Sterol
4-acetamidobutanoate	Amino Acid	Polyamine Metabolism
4-acetamidophenol	Xenobiotics	Drug
4-acetamidophenylglucuronide	Xenobiotics	Drug
4-acetaminophen sulfate	Xenobiotics	Drug
4-allylphenol sulfate	Xenobiotics	Food Component/Plant
4-aminophenol sulfate (2)	Xenobiotics	Drug
4-cholesten-3-one	Lipid	Sterol
4-ethylphenylsulfate	Xenobiotics	Benzoate Metabolism
4-guanidinobutanoate	Amino Acid	Guanidino and Acetamido Metabolism
4-hydroxychlorothalonil	Xenobiotics	Chemical
4-hydroxycinnamate sulfate	Amino Acid	Tyrosine Metabolism
4-hydroxycoumarin	Xenobiotics	Drug
4-hydroxyglutamate	Amino Acid	Glutamate Metabolism
4-hydroxyhippurate	Xenobiotics	Benzoate Metabolism
4-hydroxyphenylacetylglutamine	Peptide	Acetylated Peptides
4-methyl-2-oxopentanoate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
4-methylcatechol sulfate	Xenobiotics	Benzoate Metabolism

4-vinylphenol sulfate	Xenobiotics	Benzoate Metabolism
5,6-dihydrothymine	Nucleotide	Pyrimidine Metabolism, Thymine containing
5,6-dihydrouracil	Nucleotide	Pyrimidine Metabolism, Uracil containing
5-(galactosylhydroxy)-L-lysine	Amino Acid	Lysine Metabolism
5-acetylamino-6-amino-3-methyluracil	Xenobiotics	Xanthine Metabolism
5-acetylamino-6-formylamino-3-methyluracil	Xenobiotics	Xanthine Metabolism
5-bromotryptophan	Amino Acid	Tryptophan Metabolism
5-hydroxyhexanoate	Lipid	Fatty Acid, Monohydroxy
5-hydroxylysine	Amino Acid	Lysine Metabolism
5-methylthioadenosine (MTA)	Amino Acid	Polyamine Metabolism
5-methyluridine (ribothymidine)	Nucleotide	Pyrimidine Metabolism, Uracil containing
5-oxoproline	Amino Acid	Glutathione Metabolism
5alpha-androstan-3alpha,17beta-diol disulfate	Lipid	Steroid
5alpha-androstan-3alpha,17beta-diol monosulfate (1)	Lipid	Steroid
5alpha-androstan-3alpha,17beta-diol monosulfate (2)	Lipid	Steroid
5alpha-androstan-3beta,17alpha-diol disulfate	Lipid	Steroid
5alpha-androstan-3beta,17beta-diol disulfate	Lipid	Steroid
5alpha-androstan-3beta,17beta-diol monosulfate (2)	Lipid	Steroid
5alpha-pregnan-3beta,20alpha-diol disulfate	Lipid	Steroid
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Lipid	Steroid
5alpha-pregnan-3beta,20beta-diol monosulfate (1)	Lipid	Steroid
6-hydroxyindole sulfate	Xenobiotics	Chemical
6-oxopiperidine-2-carboxylate	Amino Acid	Lysine Metabolism
7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	Lipid	Sterol
7-methylguanine	Nucleotide	Purine Metabolism, Guanine containing
7-methylurate	Xenobiotics	Xanthine Metabolism
7-methylxanthine	Xenobiotics	Xanthine Metabolism
acesulfame	Xenobiotics	Food Component/Plant
acetylcarnitine (C2)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
acisoga	Amino Acid	Polyamine Metabolism
aconitate [cis or trans]	Energy	TCA Cycle
adenine	Nucleotide	Purine Metabolism, Adenine containing
adenosine 3',5'-cyclic monophosphate (cAMP)	Nucleotide	Purine Metabolism, Adenine containing
adenosine 5'-monophosphate (AMP)	Nucleotide	Purine Metabolism, Adenine containing
adipoylcarnitine (C6-DC)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
adrenate (22:4n6)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
adrenoylcarnitine (C22:4)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
alanine	Amino Acid	Alanine and Aspartate Metabolism
allantoin	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
alliin	Xenobiotics	Food Component/Plant
alpha-hydroxyisocaproate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
alpha-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
alpha-ketobutyrate	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
alpha-ketoglutarate	Energy	TCA Cycle
alpha-tocopherol	Cofactors and Vitamins	Tocopherol Metabolism
andro steroid monosulfate (1)*	Lipid	Steroid
androstenediol (3alpha, 17alpha) monsulfate (2)	Lipid	Steroid
androstenediol (3alpha, 17alpha) monsulfate (3)	Lipid	Steroid
androstenediol (3beta,17beta) disulfate (1)	Lipid	Steroid
androstenediol (3beta,17beta) disulfate (2)	Lipid	Steroid
androstenediol (3beta,17beta) monosulfate (1)	Lipid	Steroid
androstenediol (3beta,17beta) monosulfate (2)	Lipid	Steroid
androsterone sulfate	Lipid	Steroid
arabinose	Carbohydrate	Pentose Metabolism
arabitol/xylitol	Carbohydrate	Pentose Metabolism
arabonate/xylonate	Carbohydrate	Pentose Metabolism
arachidonate (20:4n6)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
arachidonoylcarnitine (C20:4)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
arachidonoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)
arachidoylcarnitine (C20)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
argininate*	Amino Acid	Urea cycle; Arginine and Proline Metabolism
arginine	Amino Acid	Urea cycle; Arginine and Proline Metabolism
asparagine	Amino Acid	Alanine and Aspartate Metabolism
aspartate	Amino Acid	Alanine and Aspartate Metabolism
behenoyl dihydrosphingomyelin (d18:0/22:0)*	Lipid	Sphingolipid Metabolism
behenoyl sphingomyelin (d18:1/22:0)*	Lipid	Sphingolipid Metabolism
behenoylcarnitine (C22)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
benzoate	Xenobiotics	Benzoate Metabolism
benzoylcarnitine*	Xenobiotics	Chemical
beta-citrylglutamate	Amino Acid	Glutamate Metabolism
beta-cryptoxanthin	Xenobiotics	Food Component/Plant
beta-guanidinopropanoate	Xenobiotics	Food Component/Plant
beta-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
beta-sitosterol	Lipid	Sterol
betaine	Amino Acid	Glycine, Serine and Threonine Metabolism
betonicine	Xenobiotics	Food Component/Plant
bilirubin (E,E)*	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
bilirubin (E,Z or Z,E)*	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
bilirubin (Z,Z)	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
biliverdin	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
butyrylcarnitine (C4)	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)
C-glycosyltryptophan	Amino Acid	Tryptophan Metabolism

caffeic acid sulfate	Xenobiotics	Xanthine Metabolism
caffeine	Xenobiotics	Xanthine Metabolism
campesterol	Lipid	Sterol
caproate (6:0)	Lipid	Medium Chain Fatty Acid
caprylate (8:0)	Lipid	Medium Chain Fatty Acid
carboxyethyl-GABA	Amino Acid	Glutamate Metabolism
carboxyibuprofen	Xenobiotics	Drug
carnitine	Lipid	Carnitine Metabolism
carotene diol (1)	Xenobiotics	Food Component/Plant
carotene diol (2)	Xenobiotics	Food Component/Plant
carotene diol (3)	Xenobiotics	Food Component/Plant
catechol sulfate	Xenobiotics	Benzoate Metabolism
ceramide (d18:1/14:0, d16:1/16:0)*	Lipid	Ceramides
ceramide (d18:1/17:0, d17:1/18:0)*	Lipid	Ceramides
ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)*	Lipid	Ceramides
cerotoylcarnitine (C26)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
cetirizine	Xenobiotics	Drug
chenodeoxycholate	Lipid	Primary Bile Acid Metabolism
chiro-inositol	Lipid	Inositol Metabolism
cholate	Lipid	Primary Bile Acid Metabolism
cholesterol	Lipid	Sterol
choline	Lipid	Phospholipid Metabolism
choline phosphate	Lipid	Phospholipid Metabolism
cinnamoylglycine	Xenobiotics	Food Component/Plant
cis-4-decenoylcarnitine (C10:1)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
citrate	Energy	TCA Cycle
citrulline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
cortisol	Lipid	Steroid
cortisone	Lipid	Steroid
creatine	Amino Acid	Creatine Metabolism
creatinine	Amino Acid	Creatine Metabolism
cys-gly, oxidized	Amino Acid	Glutathione Metabolism
cystathionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
cysteine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
cysteine s-sulfate	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
cysteine sulfinic acid	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
cysteine-glutathione disulfide	Amino Acid	Glutathione Metabolism
cysteinylglycine	Amino Acid	Glutathione Metabolism
cystine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
cytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing
decanoylcarnitine (C10)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
dehydroisoandrosterone sulfate (DHEA-S)	Lipid	Steroid
deoxycarnitine	Lipid	Carnitine Metabolism
deoxycholate	Lipid	Secondary Bile Acid Metabolism
desmethylnaproxen	Xenobiotics	Drug
desmethylnaproxen sulfate	Xenobiotics	Drug
diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [1]*	Lipid	Diacylglycerol
diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	Lipid	Diacylglycerol
diacylglycerol (14:0/18:1, 16:0/16:1) [1]*	Lipid	Diacylglycerol
diacylglycerol (14:0/18:1, 16:0/16:1) [2]*	Lipid	Diacylglycerol
diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	Lipid	Diacylglycerol
dihomo-linolenate (20:3n3 or n6)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
dihomo-linolenoyl-choline	Lipid	Fatty Acid Metabolism (Acyl Choline)
dihomo-linolenoylcarnitine (20:3n3 or 6)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
dihomo-linoleoylcarnitine (C20:2)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
dihydroorotate	Nucleotide	Pyrimidine Metabolism, Orotate containing
dimethyl sulfone	Xenobiotics	Chemical
dimethylarginine (SDMA + ADMA)	Amino Acid	Urea cycle; Arginine and Proline Metabolism
dimethylglycine	Amino Acid	Glycine, Serine and Threonine Metabolism
docosahexaenoate (DHA; 22:6n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
docosahexaenoylcarnitine (C22:6)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
docosahexaenoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)
docosapentaenoate (n3 DPA; 22:5n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
docosapentaenoylcarnitine (C22:5n3)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
docosatrienoate (22:3n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
dodecanedioate	Lipid	Fatty Acid, Dicarboxylate
dopamine 3-O-sulfate	Amino Acid	Tyrosine Metabolism
DSGEGDFXAEGGGVR*	Peptide	Fibrinogen Cleavage Peptide
ectoine	Xenobiotics	Chemical
EDTA	Xenobiotics	Chemical
eicosapentaenoate (EPA; 20:5n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
eicosapentaenoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)
eicosenoate (20:1)	Lipid	Long Chain Fatty Acid
eicosenoylcarnitine (C20:1)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
epiandrosterone sulfate	Lipid	Steroid
ergothioneine	Xenobiotics	Food Component/Plant
erythritol	Xenobiotics	Food Component/Plant
erythronate*	Carbohydrate	Aminosugar Metabolism
ethyl glucuronide	Xenobiotics	Chemical
ethyl paraben sulfate	Xenobiotics	Chemical
ethylmalonate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
etiocholanolone glucuronide	Lipid	Steroid

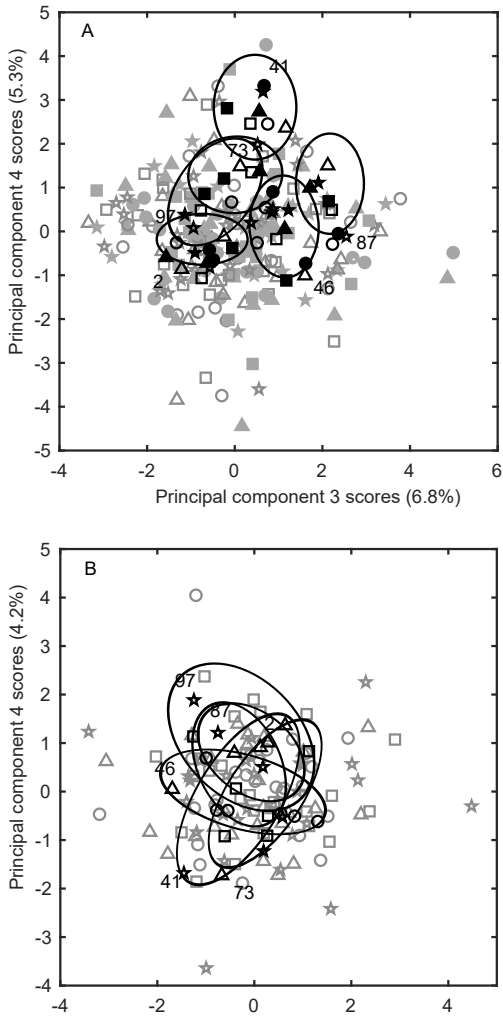
eugenol sulfate	Xenobiotics	Food Component/Plant
formiminoglutamate	Amino Acid	Histidine Metabolism
fructose	Carbohydrate	Fructose, Mannose and Galactose Metabolism
gamma-carboxyglutamate	Amino Acid	Glutamate Metabolism
gamma-CEHC	Cofactors and Vitamins	Tocopherol Metabolism
gamma-glutamyl-2-aminobutyrate	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamyl-alpha-lysine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamyl-epsilon-lysine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylalanine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylglutamate	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylglutamine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylglycine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylhistidine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylisoleucine*	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylleucine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylmethionine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylphenylalanine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylthreonine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamyltryptophan	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamyltyrosine	Peptide	Gamma-glutamyl Amino Acid
gamma-glutamylvaline	Peptide	Gamma-glutamyl Amino Acid
gamma-tocopherol/beta-tocopherol	Cofactors and Vitamins	Tocopherol Metabolism
gentisate	Amino Acid	Tyrosine Metabolism
gluconate	Xenobiotics	Food Component/Plant
glucose	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
glucuronate	Carbohydrate	Aminosugar Metabolism
glutamate	Amino Acid	Glutamate Metabolism
glutamine	Amino Acid	Glutamate Metabolism
glutarylcarnitine (C5-DC)	Amino Acid	Lysine Metabolism
glycerate	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
glycerol	Lipid	Glycerolipid Metabolism
glycerol 3-phosphate	Lipid	Glycerolipid Metabolism
glycerophosphoethanolamine	Lipid	Phospholipid Metabolism
glycerophosphoglycerol	Lipid	Glycerolipid Metabolism
glycerophosphoinositol*	Lipid	Phospholipid Metabolism
glycerophosphorylcholine (GPC)	Lipid	Phospholipid Metabolism
glycine	Amino Acid	Glycine, Serine and Threonine Metabolism
glycochenodeoxycholate	Lipid	Primary Bile Acid Metabolism
glycochenodeoxycholate glucuronide (1)	Lipid	Primary Bile Acid Metabolism
glycochenodeoxycholate sulfate	Lipid	Primary Bile Acid Metabolism
glycocholate	Lipid	Primary Bile Acid Metabolism
glycocholenate sulfate*	Lipid	Secondary Bile Acid Metabolism
glycodeoxycholate	Lipid	Secondary Bile Acid Metabolism
glycodeoxycholate sulfate	Lipid	Secondary Bile Acid Metabolism
glycolithocholate sulfate*	Lipid	Secondary Bile Acid Metabolism
glycosyl ceramide (d18:1/20:0, d16:1/22:0)*	Lipid	Ceramides
glycosyl ceramide (d18:1/23:1, d17:1/24:1)*	Lipid	Ceramides
glycosyl-N-behenoyl-sphingadienine (d18:2/22:0)*	Lipid	Sphingolipid Metabolism
glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Lipid	Sphingolipid Metabolism
glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Lipid	Sphingolipid Metabolism
glycoursodeoxycholate	Lipid	Secondary Bile Acid Metabolism
guanidinoacetate	Amino Acid	Creatine Metabolism
guanosine	Nucleotide	Purine Metabolism, Guanine containing
gulonate*	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism
heptanoate (7:0)	Lipid	Medium Chain Fatty Acid
hexanoylcarnitine (C6)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
hexanoylglutamine	Lipid	Fatty Acid Metabolism (Acyl Glutamine)
hippurate	Xenobiotics	Benzoate Metabolism
histidine	Amino Acid	Histidine Metabolism
homoarginine	Amino Acid	Urea cycle; Arginine and Proline Metabolism
homocitrulline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
homostachydrine*	Xenobiotics	Food Component/Plant
hydantoin-5-propionic acid	Amino Acid	Histidine Metabolism
hydroquinone sulfate	Xenobiotics	Drug
hyocholate	Lipid	Secondary Bile Acid Metabolism
hypotaurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
hypoxanthine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
ibuprofen	Xenobiotics	Drug
ibuprofen acyl glucuronide	Xenobiotics	Drug
imidazole lactate	Amino Acid	Histidine Metabolism
imidazole propionate	Amino Acid	Histidine Metabolism
iminodiacetate (IDA)	Xenobiotics	Chemical
indoleacetate	Amino Acid	Tryptophan Metabolism
indoleacetylglutamine	Amino Acid	Tryptophan Metabolism
indolelactate	Amino Acid	Tryptophan Metabolism
indolepropionate	Amino Acid	Tryptophan Metabolism
indolin-2-one	Xenobiotics	Food Component/Plant
inosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
isobutyrylcarnitine (C4)	Amino Acid	Leucine, Isoleucine and Valine Metabolism
isobutyrylglycine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
isoleucine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
isoursodeoxycholate	Lipid	Secondary Bile Acid Metabolism

isovalerylcarnitine (C5)	Amino Acid	Leucine, Isoleucine and Valine Metabolism
isovalerylglycine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
kynurenate	Amino Acid	Tryptophan Metabolism
kynurenine	Amino Acid	Tryptophan Metabolism
L-urobilin	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism
lactate	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)*	Lipid	Sphingolipid Metabolism
lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Lipid	Sphingolipid Metabolism
lanthionine	Xenobiotics	Chemical
laurylcarnitine (C12)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
leucine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
lignoceroyl sphingomyelin (d18:1/24:0)	Lipid	Sphingolipid Metabolism
lignoceroylcarnitine (C24)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
linoleate (18:2n6)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
linolenate [alpha or gamma; (18:3n3 or 6)]	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
linolenoylcarnitine (C18:3)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1]*	Lipid	Diacylglycerol
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*	Lipid	Diacylglycerol
linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]*	Lipid	Diacylglycerol
linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]*	Lipid	Diacylglycerol
linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]*	Lipid	Diacylglycerol
linoleoylcarnitine (C18:2)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
linoleoylcholine*	Lipid	Fatty Acid Metabolism (Acyl Choline)
lysine	Amino Acid	Lysine Metabolism
malate	Energy	TCA Cycle
maleate	Lipid	Fatty Acid, Dicarboxylate
malonate	Lipid	Fatty Acid Synthesis
maltotriose	Carbohydrate	Glycogen Metabolism
mannitol/sorbitol	Carbohydrate	Fructose, Mannose and Galactose Metabolism
mannose	Carbohydrate	Fructose, Mannose and Galactose Metabolism
margaroylcarnitine*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
methionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
methionine sulfone	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
methionine sulfoxide	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
methyl glucopyranoside (alpha + beta)	Xenobiotics	Food Component/Plant
methyl indole-3-acetate	Xenobiotics	Food Component/Plant
methyl-4-hydroxybenzoate sulfate	Xenobiotics	Benzoate Metabolism
methylsuccinate	Amino Acid	Leucine, Isoleucine and Valine Metabolism
myo-inositol	Lipid	Inositol Metabolism
myristoleoylcarnitine (C14:1)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
myristoyl dihydrosphingomyelin (d18:0/14:0)*	Lipid	Sphingolipid Metabolism
myristoylcarnitine (C14)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
N('1)-acetylspermidine	Amino Acid	Polyamine Metabolism
N-(2-furoyl)glycine	Xenobiotics	Food Component/Plant
N-acetyl-1-methylhistidine*	Amino Acid	Histidine Metabolism
N-acetyl-3-methylhistidine*	Amino Acid	Histidine Metabolism
N-acetyl-aspartyl-glutamate (NAAG)	Amino Acid	Glutamate Metabolism
N-acetyl-beta-alanine	Nucleotide	Pyrimidine Metabolism, Uracil containing
N-acetylalanine	Amino Acid	Alanine and Aspartate Metabolism
N-acetylalliin	Xenobiotics	Food Component/Plant
N-acetylarginine	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-acetylaspartate (NAA)	Amino Acid	Alanine and Aspartate Metabolism
N-acetylcarnosine	Peptide	Dipeptide Derivative
N-acetylcitrulline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-acetylglucosamine/N-acetylgalactosamine	Carbohydrate	Aminosugar Metabolism
N-acetylglucosaminylasparagine	Carbohydrate	Aminosugar Metabolism
N-acetylglutamate	Amino Acid	Glutamate Metabolism
N-acetylglutamine	Amino Acid	Glutamate Metabolism
N-acetylglycine	Amino Acid	Glycine, Serine and Threonine Metabolism
N-acetylhistidine	Amino Acid	Histidine Metabolism
N-acetylkynurenine (2)	Amino Acid	Tryptophan Metabolism
N-acetylleucine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
N-acetylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetylneuraminate	Carbohydrate	Aminosugar Metabolism
N-acetylphenylalanine	Amino Acid	Phenylalanine Metabolism
N-acetylproline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-acetylputrescine	Amino Acid	Polyamine Metabolism
N-acetylserine	Amino Acid	Glycine, Serine and Threonine Metabolism
N-acetyltaurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-acetylthreonine	Amino Acid	Glycine, Serine and Threonine Metabolism
N-acetyltryptophan	Amino Acid	Tryptophan Metabolism
N-acetyltyrosine	Amino Acid	Tyrosine Metabolism
N-acetylvaline	Amino Acid	Leucine, Isoleucine and Valine Metabolism
N-behenoyl-sphingadienine (d18:2/22:0)*	Lipid	Sphingolipid Metabolism
N-delta-acetylornithine	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-formylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-methylproline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N-methyltaurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
N-nervonoyl-hexadecasphingosine (d16:1/24:1)*	Lipid	Sphingolipid Metabolism
N-nervonoyl-sphingadiene (d18:2/24:1)*	Lipid	Sphingolipid Metabolism
N-palmitoyl-sphinganine (d18:0/16:0)	Lipid	Sphingolipid Metabolism
N-palmitoyl-sphingosine (d18:1/16:0)	Lipid	Sphingolipid Metabolism

N-stearoyl-sphingosine (d18:1/18:0)*	Lipid	Sphingolipid Metabolism
N-trimethyl 5-aminovalerate	Amino Acid	Lysine Metabolism
N1-Methyl-2-pyridone-5-carboxamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
N1-Methyl-4-pyridone-3-carboxamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
N1-methyladenosine	Nucleotide	Purine Metabolism, Adenine containing
N1-methylinosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
N2,N2-dimethylguanosine	Nucleotide	Purine Metabolism, Guanine containing
N2,N5-diacetylornithine	Amino Acid	Urea cycle; Arginine and Proline Metabolism
N6,N6,N6-trimethyllysine	Amino Acid	Lysine Metabolism
N6-acetyllysine	Amino Acid	Lysine Metabolism
N6-carbamoylthreonyladenosine	Nucleotide	Purine Metabolism, Adenine containing
N6-carboxymethyllysine	Carbohydrate	Advanced Glycation End-product
N6-methyladenosine	Nucleotide	Purine Metabolism, Adenine containing
N6-succinyladenosine	Nucleotide	Purine Metabolism, Adenine containing
naproxen	Xenobiotics	Drug
nervonate (24:1n9)*	Lipid	Long Chain Fatty Acid
nervonoylcarnitine (C24:1)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
nicotinamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
O-acetylhomoserine	Amino Acid	Glycine, Serine and Threonine Metabolism
O-methylcatechol sulfate	Xenobiotics	Benzoate Metabolism
O-sulfo-L-tyrosine	Xenobiotics	Chemical
octanoylcarnitine (C8)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
oleate/vaccenate (18:1)	Lipid	Long Chain Fatty Acid
oleoyl ethanolamide	Lipid	Endocannabinoid
oleoyl-arachidonoyl-glycerol (18:1/20:4) [1]*	Lipid	Diacylglycerol
oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	Lipid	Diacylglycerol
oleoyl-linolenoyl-glycerol (18:1/18:3) [2]*	Lipid	Diacylglycerol
oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	Lipid	Diacylglycerol
oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	Lipid	Diacylglycerol
oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	Lipid	Diacylglycerol
oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	Lipid	Diacylglycerol
oleoylcarnitine (C18:1)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
oleoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)
ornithine	Amino Acid	Urea cycle; Arginine and Proline Metabolism
orotate	Nucleotide	Pyrimidine Metabolism, Orotate containing
orotidine	Nucleotide	Pyrimidine Metabolism, Orotate containing
oxalate (ethanedioate)	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism
p-cresol sulfate	Xenobiotics	Benzoate Metabolism
p-cresol-glucuronide*	Amino Acid	Tyrosine Metabolism
palmitoleoyl-arachidonoyl-glycerol (16:1/20:4) [2]*	Lipid	Diacylglycerol
palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]*	Lipid	Diacylglycerol
palmitoleoyl-oleoyl-glycerol (16:1/18:1) [1]*	Lipid	Diacylglycerol
palmitoleoylcarnitine (C16:1)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
palmitoloelycholine	Lipid	Fatty Acid Metabolism (Acyl Choline)
palmitoyl dihydrosphingomyelin (d18:0/16:0)*	Lipid	Sphingolipid Metabolism
palmitoyl sphingomyelin (d18:1/16:0)	Lipid	Sphingolipid Metabolism
palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]*	Lipid	Diacylglycerol
palmitoyl-linoleoyl-glycerol (16:0/18:2) [1]*	Lipid	Diacylglycerol
palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]*	Lipid	Diacylglycerol
palmitoyl-myristoyl-glycerol (16:0/14:0) [2]	Lipid	Diacylglycerol
palmitoyl-oleoyl-glycerol (16:0/18:1) [1]*	Lipid	Diacylglycerol
palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	Lipid	Diacylglycerol
palmitoyl-palmitoyl-glycerol (16:0/16:0) [1]*	Lipid	Diacylglycerol
palmitoyl-palmitoyl-glycerol (16:0/16:0) [2]*	Lipid	Diacylglycerol
palmitoylcarnitine (C16)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
palmitoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)
pantothenate	Cofactors and Vitamins	Pantothenate and CoA Metabolism
paraxanthine	Xenobiotics	Xanthine Metabolism
perfluorooctanesulfonic acid (PFOS)	Xenobiotics	Chemical
phenol sulfate	Amino Acid	Tyrosine Metabolism
phenylacetylcarnitine	Peptide	Acetylated Peptides
phenylacetylglutamate	Peptide	Acetylated Peptides
phenylacetylglutamine	Peptide	Acetylated Peptides
phenylalanine	Amino Acid	Phenylalanine Metabolism
phenyllactate (PLA)	Amino Acid	Phenylalanine Metabolism
phenylpyruvate	Amino Acid	Phenylalanine Metabolism
phosphate	Energy	Oxidative Phosphorylation
phosphoethanolamine	Lipid	Phospholipid Metabolism
picolinate	Amino Acid	Tryptophan Metabolism
pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
pipecolate	Amino Acid	Lysine Metabolism
piperine	Xenobiotics	Food Component/Plant
pregn steroid monosulfate*	Lipid	Steroid
pregnanediol-3-glucuronide	Lipid	Steroid
pregnen-diol disulfate*	Lipid	Steroid
pregnenolone sulfate	Lipid	Steroid
pro-hydroxy-pro	Amino Acid	Urea cycle; Arginine and Proline Metabolism
proline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
prolylglycine	Peptide	Dipeptide
propionylcarnitine (C3)	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)
propionylglycine	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)
propyl 4-hydroxybenzoate sulfate	Xenobiotics	Benzoate Metabolism

pseudouridine	Nucleotide	Pyrimidine Metabolism, Uracil containing
pyridoxate	Cofactors and Vitamins	Vitamin B6 Metabolism
pyroglutamine*	Amino Acid	Glutamate Metabolism
pyrraline	Xenobiotics	Food Component/Plant
pyruvate	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism
quinate	Xenobiotics	Food Component/Plant
quinine	Xenobiotics	Drug
quinolinate	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
retinal	Xenobiotics	Food Component/Plant
retinol (Vitamin A)	Cofactors and Vitamins	Vitamin A Metabolism
ribitol	Carbohydrate	Pentose Metabolism
ribonate	Carbohydrate	Pentose Metabolism
S-1-pyrroline-5-carboxylate	Amino Acid	Glutamate Metabolism
S-allylcysteine	Xenobiotics	Food Component/Plant
S-methylcysteine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
S-methylcysteine sulfoxide	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
S-methylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
saccharin	Xenobiotics	Food Component/Plant
salicylate	Xenobiotics	Drug
salicyluric glucuronide*	Xenobiotics	Drug
sarcosine	Amino Acid	Glycine, Serine and Threonine Metabolism
serine	Amino Acid	Glycine, Serine and Threonine Metabolism
serotonin	Amino Acid	Tryptophan Metabolism
sphinganine	Lipid	Sphingolipid Metabolism
sphinganine-1-phosphate	Lipid	Sphingolipid Metabolism
sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d17:2/16:0, d18:2/15:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:0/18:0, d19:0/17:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:0/20:0, d16:0/22:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/14:0, d16:1/16:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/18:1, d18:2/18:0)	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/19:0, d19:1/18:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/20:0, d16:1/22:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/20:1, d18:2/20:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/24:1, d18:2/24:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/14:0, d18:1/14:1)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/16:0, d18:1/16:1)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/18:1)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/21:0, d16:2/23:0)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/23:1)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/24:1, d18:1/24:2)*	Lipid	Sphingolipid Metabolism
sphingomyelin (d18:2/24:2)*	Lipid	Sphingolipid Metabolism
sphingosine	Lipid	Sphingolipid Metabolism
sphingosine 1-phosphate	Lipid	Sphingolipid Metabolism
stachydrine	Xenobiotics	Food Component/Plant
stearate (18:0)	Lipid	Long Chain Fatty Acid
stearidonate (18:4n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
stearoyl ethanolamide	Lipid	Endocannabinoid
stearoyl sphingomyelin (d18:1/18:0)	Lipid	Sphingolipid Metabolism
stearoyl-arachidonoyl-glycerol (18:0/20:4) [1]*	Lipid	Diacylglycerol
stearoyl-arachidonoyl-glycerol (18:0/20:4) [2]*	Lipid	Diacylglycerol
stearoylcarnitine (C18)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
stearoylcholine*	Lipid	Fatty Acid Metabolism (Acyl Choline)
suberoylcarnitine (C8-DC)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
succinate	Energy	TCA Cycle
succinimide	Xenobiotics	Chemical
succinylcarnitine (C4-DC)	Energy	TCA Cycle
sulfate*	Xenobiotics	Chemical
tartarate	Xenobiotics	Food Component/Plant
tartronate (hydroxymalonate)	Xenobiotics	Bacterial/Fungal
taurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism
taurochenodeoxycholate	Lipid	Primary Bile Acid Metabolism
taurocholenate sulfate	Lipid	Secondary Bile Acid Metabolism
taurolithocholate 3-sulfate	Lipid	Secondary Bile Acid Metabolism
theanine	Xenobiotics	Food Component/Plant
theobromine	Xenobiotics	Xanthine Metabolism
theophylline	Xenobiotics	Xanthine Metabolism
thioproline	Xenobiotics	Chemical
threonate	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism
threonine	Amino Acid	Glycine, Serine and Threonine Metabolism
thymol sulfate	Xenobiotics	Food Component/Plant
thyroxine	Amino Acid	Tyrosine Metabolism
tiglylcarnitine (C5:1-DC)	Amino Acid	Leucine, Isoleucine and Valine Metabolism
trans-4-hydroxyproline	Amino Acid	Urea cycle; Arginine and Proline Metabolism
trans-urocanate	Amino Acid	Histidine Metabolism

tricosanoyl sphingomyelin (d18:1/23:0)*	Lipid	Sphingolipid Metabolism
trigonelline (N'-methylnicotinate)	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism
trimethylamine N-oxide	Lipid	Phospholipid Metabolism
tryptophan	Amino Acid	Tryptophan Metabolism
tryptophan betaine	Amino Acid	Tryptophan Metabolism
tyramine O-sulfate	Amino Acid	Tyrosine Metabolism
tyrosine	Amino Acid	Tyrosine Metabolism
umbelliferone sulfate	Xenobiotics	Food Component/Plant
uracil	Nucleotide	Pyrimidine Metabolism, Uracil containing
urate	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
urea	Amino Acid	Urea cycle; Arginine and Proline Metabolism
uridine	Nucleotide	Pyrimidine Metabolism, Uracil containing
ursodeoxycholate	Lipid	Secondary Bile Acid Metabolism
valine	Amino Acid	Leucine, Isoleucine and Valine Metabolism
vanillactate	Amino Acid	Tyrosine Metabolism
vanillylmandelate (VMA)	Amino Acid	Tyrosine Metabolism
xanthine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing
xanthurenate	Amino Acid	Tryptophan Metabolism
ximenoylcarnitine (C26:1)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
xylose	Carbohydrate	Pentose Metabolism



Supplemental Figure 1. Principal component analysis (PCA) for the metabolomic data for all volunteers and treatments, for both time-points (A; n=32 x 4 treatments x 2 time-points (day 1, day 29)), and for the ratio of post- to pre-treatment (B; n=32 x 4 treatments).
Scores on the third and fourth principal components are shown and the percentage variance accounted for by each component indicated on each axis. The circle represents the low dose monomeric flavonols treatment, the square the high dose monomeric flavanols, the triangle the placebo, and the star the oligomeric procyanidins. For (A), open symbols represent day 1 and closed symbols day 29 of the treatments. Six individuals are highlighted; the volunteer number is given and an ellipse shows the spread of scores for each of these volunteers.