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RESEARCH ARTICLE

Impact of processing on the bioavailability and vascular effects of blueberry (poly)phenols

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Scope: Blueberries are a rich source of flavonoids and phenolic acids. Currently, little information is available regarding the impact of processing on the bioavailability and the bioactivity of blueberry (poly)phenols.

Methods and results: In a randomized, controlled crossover trial, ten healthy volunteers consumed (a) blueberry-containing baked products, (b) an unprocessed blueberry drink containing the same amount of freeze-dried blueberry powder as used in the baked products, and (c) matched control baked products. Endothelial function was measured as flow-mediated dilation (FMD) and plasma samples taken at baseline and at 1, 2, 4, and 6 h postconsumption. Although processing did not significantly change the total (poly)phenolic amount, the processed products contained significantly less anthocyanins (−42%), more chlorogenic acid (23%), no flavanol nonamers or decamers, and significantly more flavanol dimers and trimers (36% and 28%, respectively). FMD increased after 1, 2, and 6 h consumption of the baked products to a similar degree as the unprocessed blueberries, despite significant differences in the levels of individual plasma metabolites. No changes were observed after the consumption of the control product.

Conclusion: Careful processing can preserve important biological activities of blueberries despite changing the blueberry (poly)phenol composition and plasma metabolite profile.

Keywords:
Blueberry / Endothelial function / (Poly)phenols / Processing / Vascular function

1 Introduction

In recent years, blueberries have received much attention due to their potential positive roles in human health and disease prevention. Increasing evidence suggests that the high polyphenol and phenolic acid content of blueberries may be responsible for their beneficial effects [1–8]. Blueberries are a well-known source of flavonoids and phenolic compounds. The major group of (poly)phenols present in blueberries are anthocyanins, followed by procyanidins, flavonols, and chlorogenic acids [9–12]. We have recently provided the first evidence to link improvements in endothelial function after acute consumption of blueberry in healthy individuals to (poly)phenols occurring in blood. In a biphasic pattern, the vascular effects observed at 1–2 h were intake-dependent and correlated with increases in plasma metabolites possibly derived from the metabolism and/or breakdown of anthocyanins and chlorogenic acid (5-*O*-caffeoylquinic acid), whereas the effects observed at 6 h were linked to gut microbial metabolites [6]. This suggests that the potential health effects of (poly)phenol-rich food depends on the amount and composition of the phenolic compounds. Both factors are greatly influenced by food processing [13, 14].

In order to exert a health benefit, the compound of interest needs to withstand food processing, be released from the food matrix postingestion, be bioaccessible in the gastrointestinal tract, undergo metabolism, and then the biologically active compound/metabolite needs to reach the target

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Abbreviations: AUC, area under the curve; **FMD**, flow-mediated dilation

tissue of action [15]. Therefore, the changes that take place with processing are key factors that may profoundly affect the health benefits related to (poly)phenol consumption. To date, studies have investigated how processing affects the phenolic composition of blueberries, but very limited information exists regarding the effects of processing on their nutritional quality and vascular health effects (biological activity). This information is needed for processors who desire to retain or possibly boost levels of polyphenols in their products, as well as consumers who wish to incorporate higher levels of bioactive compounds into their diet in order to savor health benefits [10]. In the current study, we aim to address this by investigating the effects of acute consumption of (a) processed blueberry-rich baked products in comparison with (b) the same amount of unprocessed blueberries or (c) blueberry (poly)phenol free baked control products, on endothelial function measured as flow-mediated dilation (FMD) and on the absorption and metabolism of blueberry (poly)phenols, in a randomized controlled human intervention trial with ten healthy male individuals.

2 Materials and methods

2.1 Materials

All individual flavonoid and phenolic acid standards were obtained from Sigma–Aldrich Co. Ltd. (Poole, UK) or Extrasynthese (Genay, France). Water, methanol, acetic acid, and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Loughborough, UK). HPLC columns were from Hichrom (Reading, UK). β-glucuronidase and sulfatase (Helix pomatia, Type H1) was purchased from Sigma–Aldrich Co. Ltd.. Oasis HLB SPE cartridges were purchased from Waters (Elstree, UK). Unless otherwise stated, all chemicals and reagents were obtained from Sigma–Aldrich Co Ltd. or Fisher Scientific.

2.2 Intervention study subjects

Ten healthy male volunteers were recruited from the University of Reading and surrounding area. Volunteers were assessed prior to the start of the trial for good health and were selected according to the following inclusion criteria: a signed consent form, age of 18–40 years inclusive, good general health assessed by their responses to a standard medical questionnaire and blood results (normal liver enzymes, hemoglobin, hematocrit, and leukocyte counts), absence of diabetes mellitus, hypertension (>150/90 mm/Hg), and anemia. Exclusion criteria: individuals taking anti-inflammatory, antibiotics, or blood pressure lowering medication within a 2-month period prior to the study. Volunteers were instructed not to alter their usual dietary or fluid intake. Those selected for the study were asked to refrain from the following for 24 h prior to, and during, the study: consumption of polyphenol-rich foods including fruits, vegetables, cocoa, chocolate, coffee, tea, and wine, participating in vigorous exercise (>3 × 20 min/wk) and consuming more than 168 g of alcohol (any form) per week. Written informed consent was obtained from all subjects prior to their participation in the study.

2.3 Blueberry-containing test materials

Freeze-dried wild blueberry powder (*Vaccinium angustifolium* Aiton) was kindly supplied by the Wild Blueberry Association of North America (WBANA, Maine, US). Freeze-dried wild blueberry powder was stored at −20°C. Volunteers consumed either three blueberry powder rich baked products (containing a total of 34 g freeze-dried blueberry powder, equivalent to 240 g of fresh blueberry, treatment named as "blueberry bun"), three macronutrient- and micronutrient-matched control products (referred to as "control bun"), or a blueberry drink containing the same amount of freeze-dried blueberry powder as the blueberry buns (34 g) dissolved in 500 mL of water. Blueberry powder was analyzed for flavonoid and phenolic acid content as previously described [11]. The composition of the blueberry-containing processed food is as follows: 40 g of dough and 20 g of filling. The weight of each bun was 60 g. The dough was made with the following ingredients (% w/w): strong white flour (46.4), freeze-dried blueberry powder (12.4), eggs (5.9), butter (5.9), yeast (1.1), salt (0.5), skimmed milk powder (4.6), sugar (7.7), and water. For the filling freeze-dried blueberry powder (31.8), sugar (22.2), salt (0.1), evaporated milk (12.6), corn flour (4.6), and eggs (28.7) were used. Details of the processing stages has been previously described [14].

2.4 Study design

The randomized controlled trial was designed to investigate the effects of processing on the vascular effects and bioavailability of blueberry (poly)phenols and was a randomized, crossover, controlled intervention trial, where volunteers were asked to consume blueberry baked products (3 pieces/buns), a blueberry drink containing the same amount and type of blueberry as the baked products, or three control baked products matched for micro and macronutrient composition of the blueberry baked products (3 pieces/buns) Intervention days were separated by 1 wk washout period. On arrival at the Nutrition Unit, subjects rested for 30 min in a quiet, temperature-controlled room before they were cannulated and blood samples were collected in the fasted state and at 0, 1, 2, 4, and 6 h after consumption of each intervention. FMD of the brachial artery was the primary outcome, and secondary outcomes included peripheral blood pressure and plasma (poly)phenol metabolites. Vascular measurements were conducted prior to consumption of each intervention drink and at 1, 2, 4, and 6 h postconsumption. Systolic and diastolic blood pressure was measured using an Omron MX2

automatic digital upper arm blood pressure monitor (Omron Healthcare UK Ltd., Milton Keynes, UK). All subjects completed a 2-day diet diary of their habitual dietary intake during the day before and the study day to make sure they complied with the low polyphenol diet. Blood samples were drawn into EDTA-containing vials, centrifuged immediately (1700 × *g*; 10 min; 4°C), and plasma collected, supplemented with 2% formic acid and immediately stored at −80°C until analysis. For biochemical analysis, blood samples were collected in lithium/heparin tubes and centrifuged (1700 × *g*; 10 min; 4°C) immediately after collection. Samples were also collected in serum separation tubes and allowed to stand for 30 min prior to centrifugation (1300 × *g*; 10 min; 21°C). All plasma and serum samples were aliquoted and frozen at −80°C until analysis. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Reading Research Ethics Committee (ref: 06/37).

2.5 Flow-mediated dilation

FMD of the brachial artery was the primary endpoint measure of the study and was measured following standard guidelines as previously described [6]. A single researcher, who was blinded to the measurement details, analyzed all image files and peak diameter was defined as the largest diameter obtained after the occlusion was released.

2.6 Plasma analysis of (poly)phenolic metabolites

Plasma flavonoid and phenolic acid analysis was performed using SPE followed by LC-qTOF as previously described [6].

2.7 Biochemical analysis

Plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol, glucose, and triacylglycerides were assayed on an ILAB 600 chemistry analyzer (Instrumentation Laboratory, Warrington, UK), as described elsewhere [6].

2.8 Power calculation and statistical analysis

Power calculations were performed for the primary endpoint, change in FMD response. Power was based on the intraindividual variability of the operator that performed the FMD analysis (5% CV, SD = 0.3%). At 80% power and 0.05 significance, the number of subjects required to detect a difference of 0.3% in the response of matched pairs in a crossover study is 10. This number is consistent with other studies carried out with similar endpoints and study design [16, 17]. Two-way repeated measured ANOVA were fitted to analyze the data using the SAS version 9.1 software package (SAS Institute, Cary, NC, US) and GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, US). Post hoc analysis was carried out using the Bonferroni test. Significance was defined as $p < 0.05$. Pharmacokinetics parameters were calculated as follows: the maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were determined from the individual data obtained from each participant; the area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal method. Correlation analysis was performed using Pearson's correlation coefficient.

Table 1. Phytochemical content of the test products

	Blueberry drink	Blueberry bun (×3)
Freeze-dried blueberry (g)	34	34
Total polyphenols (mg)	692 ± 13	637 ± 28
Total anthocyanins (mg)	339 ± 6.1	196 ± 7.7*
Total procyanidins (mg)	111 ± 4.1	140 ± 7.4
Monomers (mg)	22 ± 1.1	29 ± 0.8
Dimers (mg)	26 ± 1.1	42 ± 1.4*
Trimers (mg)	15 ± 0.6	23 ± 1.6*
Tetramers (mg)	14 ± 0.6	17 ± 1.5
Pentamers (mg)	9 ± 0.5	11 ± 1.5
Hexamers (mg)	8 ± 0.4	8 ± 0.8
Heptamers (mg)	6.5 ± 0.4	5 ± 0.5
Octamers (mg)	5 ± 0.8	4 ± 0.5
Nonamers (mg)	4 ± 0.8	0*
Decamers (mg)	2 ± 0.6	0*
Total oligomers (mg)	89 ± 2.9	111 ± 6.6
Quercetin (mg)	24 ± 0.2	25 ± 0.9
Chlorogenic acid (mg)	179 ± 1	221 ± 10*
Caffeic acid (mg)	16 ± 0.3	17 ± 0.9
Ferulic acid (mg)	22 ± 1.0	38 ± 1.3

Results are expressed as mean ± SEM (n = 3).
*Significantly different from the blueberry drink, $p < 0.05$.

3 Results

3.1 Composition of blueberry products tested

The (poly)phenol content of the wild blueberries tested is indicated in Table 1. Each blueberry containing baked product contained a total of 11.3 g of freeze-dried blueberry, so the three blueberry-containing products (buns) administered contained a total of 34 g of freeze-dried blueberry, with a total content of 637 mg of total polyphenols (Table 1). Although processing did not significantly change the total (poly)phenolic amount, the baked blueberry bun contained significantly less anthocyanins (−42%), significantly higher amounts of flavanol dimers and trimers (+36 and +28%, respectively), and no flavanol nonamers or decamers in comparison with the blueberry drink, and a higher chlorogenic acid content (+23%) (Table 1), as previously reported

Table 2. Nutritional content of the test products

	Blueberry drink	Blueberry bun (×3)	Control bun (×3)
Energy (kcal/kJ)	44/185	464/1943	484/2027
Carbohydrates (g)	10.2	89.0	91.5
Sugars (g)	7.14	36.2	39.2
Fat (g)	0.5	8.0	8.7
Saturated fat (g)	0.0	3.5	3.9
Protein (g)	0.3	14.6	15.6
Sodium (g)	0.0	0.40	0.42

Values are the amount present per dose (i.e. in three of each bun).

Table 3. Baseline clinical characteristics of the study population, expressed as mean ± SEM ($n = 10$)

Baseline characteristics	
Age (years)	27 ± 1
BMI (kg/m^2)	25 ± 0.8
Total cholesterol (mmol/L)	4.3 ± 0.2
HDL (mmol/L)	1.2 ± 0.1
TAG (mmol/L)[a)]	0.8 ± 0.2
Glucose (mmol/L)	4.6 ± 0.1
SBP (mmHg)[b)]	124 ± 2.6
DBP (mmHg)[c)]	74 ± 2.5
Heart rate (beats/min)	66 ± 1.6
Brachial artery diameter (mm)	3.9 ± 0.1
FMD (%)	7.0 ± 0.1

a) TAG, triacylglycerides.
b) SBP, systolic blood pressure.
c) DBP, diastolic blood pressure.

[14]. The nutritional content of the test products is indicated in Table 2. The baked blueberry and control bun were matched for calories and macronutrients.

3.2 Baseline characteristics of subjects and tolerance of intervention

The baseline characteristics of subjects were all within the normal range for healthy individuals (Table 3). The interventions were well tolerated by all subjects who completed the study and intolerances or adverse events were not reported. The baseline FMD and blood pressure were within the normal range for healthy individuals.

3.3 Processed blueberry induced similar improvements in FMD as unprocessed blueberry

A significant biphasic increase in FMD was observed at 1, 2, and 6 h after consumption of the blueberry bun when compared with control bun ($p < 0.05$, Fig. 1). The maximum improvement of FMD occurred at 2 h postconsumption (2.6 ± 0.4 ΔFMD%). The effects observed on FMD were very similar to the ones after consumption of a freeze-dried blueberry drink, except for the fact that the maximum effect on FMD occurred at 2 h instead of 1 h (Fig. 1) consistent with slower gastric passage of solid foods as compared to liquids [18]. No significant changes in FMD between the blueberry intervention and control were observed at baseline and 4 h after consumption, and no significant changes in FMD were observed at any time after the consumption of the control bun. Blood pressure and heart rate were unaffected by blueberry ingestion (data not shown).

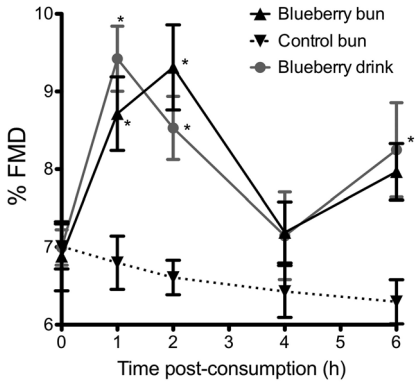

Figure 1. Time-course of FMD after consumption of three blueberry baked products (blueberry bun) containing 637 mg of total (poly)phenols, three baked control products (control bun), or a blueberry drink containing 692 mg of total (poly)phenols in healthy men ($n = 10$). Data are mean values ± SEM. $^*p < 0.05$ significantly different with respect to processed control at the specified time point.

3.4 Processing produced significant changes in blueberry (poly)phenol metabolites

In line with the increases in FMD, after consumption of the blueberry products significant increases in some of the plasma (poly)phenol metabolites were observed at 1, 2, and 6 h postconsumption. At 1–2 h postconsumption, increases in the plasma levels of four metabolites (ferulic, isoferulic, vanillic, and *m*-hydroxyphenylacetic acids) after the processed blueberry bun intake, and increases in the levels of six metabolites after the blueberry drink (ferulic, isoferulic, benzoic, vanillic, salicylic and caffeic acids) were observed (Fig. 2). At 6 h postconsumption, four metabolites (ferulic, isoferulic, hydroxyhippuric, and dihydro-*m*-coumaric acids) increased significantly after the processed blueberry bun consumption, whereas increases in eight metabolites (hippuric, hydroxyhippuric, homovanillic, dihydro-*m*-coumaric, salicylic, dihydroferulic, and gentisic acids) after blueberry drink were observed (Figs. 2 and 3).

When the AUC of the individual plasma phenolic metabolites concentration over time after blueberry bun consumption was compared with the AUC of phenolic metabolites after blueberry drink intake, significant differences were observed in eight phenolic metabolites, with

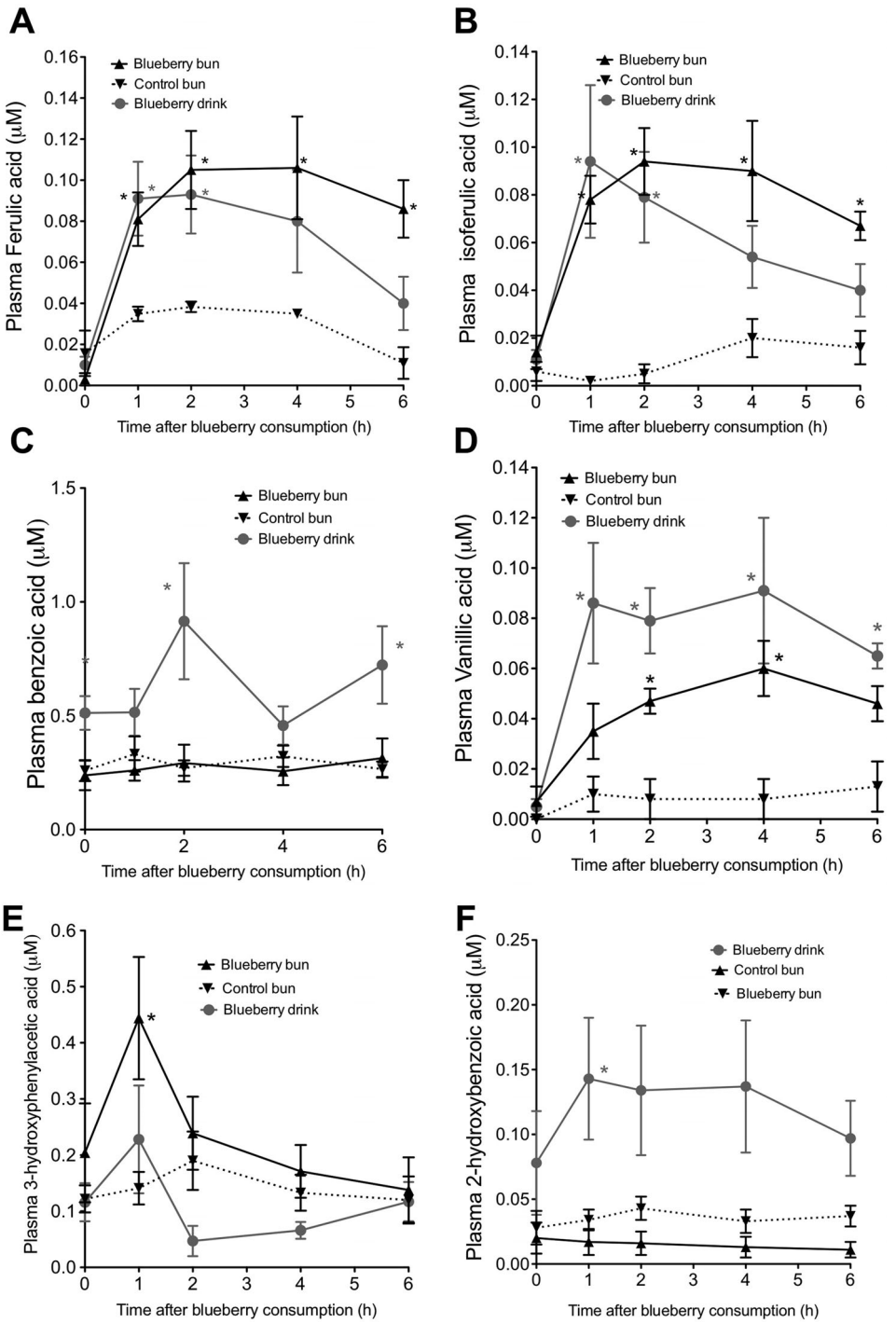

Figure 2. Plasma levels of (A) ferulic acid [3-(3′-methoxy-4′-hydroxyphenyl)-prop-2-enoic acid]; (B) isoferulic acid [3-(3′-hydroxy-4′-methoxyphenyl)prop-2-enoic acid]; (C) benzoic acid; (D) vanillic acid [3-methoxy-4-hydroxybenzoic acid]; (E) *m*-hydroxyphenylacetic acid [3′-hydroxyphenylacetic acid]; (F) salicylic acid [2-hydroxybenzoic acid) following blueberry bun, control bun, or blueberry drink consumption ($n = 10$). Values presented are means ± SEM. $*p < 0.05$ significantly different with respect to processed control at the specified time point.

m-hydroxyphenylacetic, ferulic, isoferulic, and hydroxyhippuric acids being higher after the blueberry bun than after the blueberry drink consumption and hippuric, benzoic, salicylic, and sinapic acids being lower (Table 4). Significant changes were also observed between blueberry bun and blueberry drink interventions in the maximum concentration in plasma (C_{max}), with lower C_{max} of hippuric and benzoic acids but higher C_{max} of *m*-hydroxyphenylacetic acid after consumption of the blueberry bun.

In a multivariate regression analysis including all plasma metabolites increasing over the 1–2 h time frame (Fig. 3) and coinciding with the first peak of FMD response, vanillic acid ($r = 0.64$, $p = 0.007$) and ferulic acid ($r = 0.67$, $p = 0.004$) predicted the magnitude of FMD increase after the blueberry

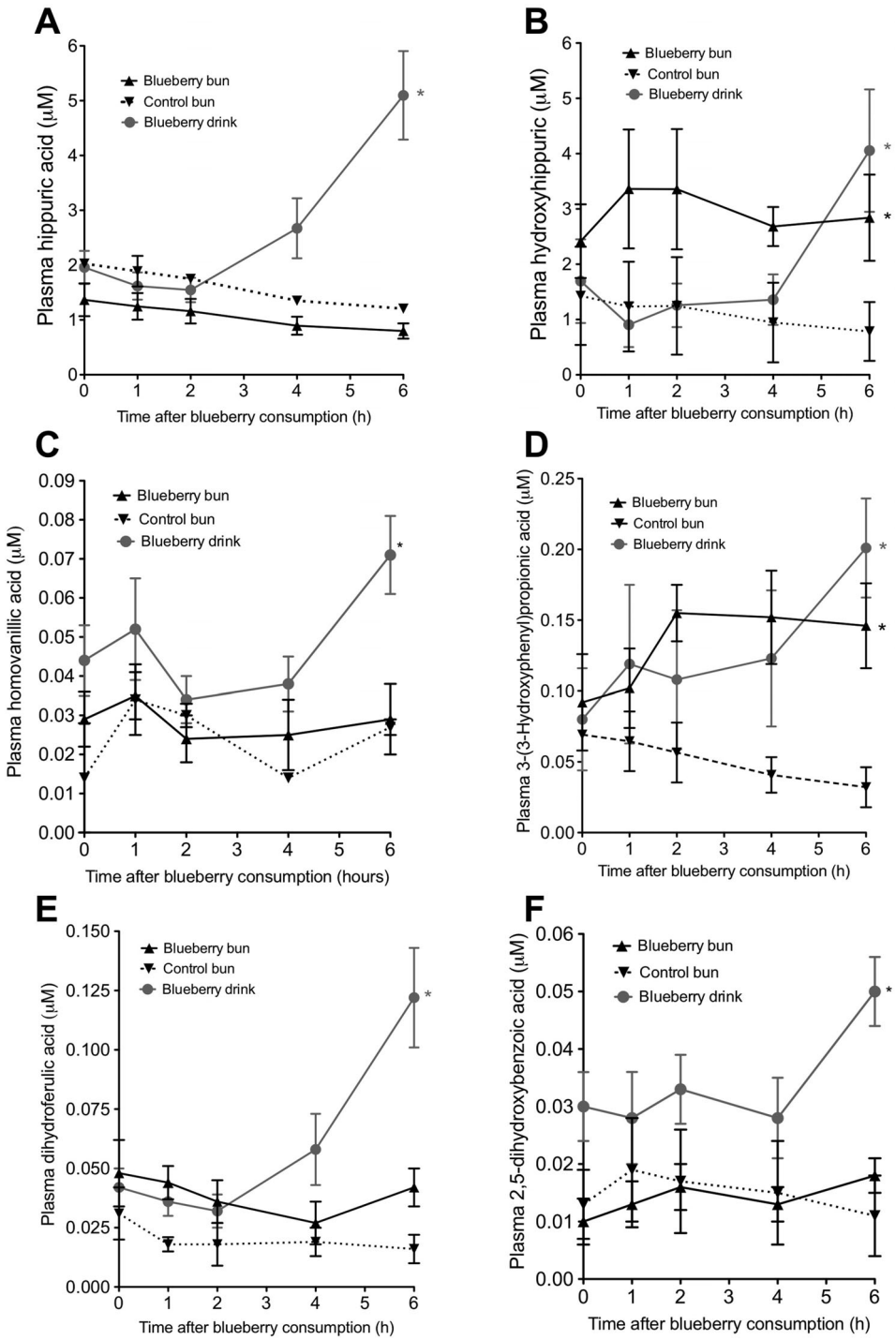

Figure 3. Plasma levels of (A) hippuric acid [2-benzamidoacetic acid]; (B) hydroxyhippuric acid [3-hydroxybenzoylamino)-acetic acid]; (C) homovanillic acid [3′-methoxy-4′-hydroxyphenylacetic acid]; (D) dihydro-*m*-coumaric acid [3-(3′(hydroxyl) phenyl)propionic acid]; (E) dihydroferulic acid [3-(3′-methoxy-4′-hydroxy phenyl)propionic acid]; (F) gentisic acid [2,5-dihydroxybenzoic acid], following blueberry bun, control bun, or blueberry drink consumption ($n = 10$). Values presented are means ± SEM. $^*p < 0.05$ significantly different with respect to processed control at the specified time point.

buns, whereas after the blueberry drink benzoic acid ($r = 0.49$, $p = 0.004$) and vanillic acid ($r = 0.60$, $p = 0.0136$) correlated with the FMD response, suggesting that these metabolites may be, at least in part, involved in mediating the observed increases in endothelium-dependent vascular function between 1–2 h. Similar multivariate regression analysis conducted using values obtained between 4 and 6 h (the second increase in FMD) with those metabolites observed to increase over this time frame (Fig. 4), indicated that hippuric acid ($r = 0.56$, $p = 0.014$), hydroxyhippuric acid ($r = 0.55$, $p = 0.017$), and homovanillic acid ($r = 0.83$, $p = 0.04$) all predicted the magnitude of FMD increase after the unprocessed blueberry. However, no significant correlations were found between the plasma metabolites and

Table 4. Pharmacokinetics of the major plasma (poly)phenol metabolites after consumption of a blueberry drink containing 692 mg of total (poly)phenols or three blueberry buns containing 637 mg of total (poly)phenols in healthy young men

	C_{max}		T_{max}		AUC (0–6 h)	
	BB drink	Bun	BB drink	Bun	BB drink	Bun
Benzoic acid	1211	433*	3.5	3.3	2778	1644*
Baffeic acid (3-(3′,4′-dihydroxyphenyl)pro-2enoic acid)	49	44	2.1	2.8	216	202
Dihydroferulic acid (3-(3′-methoxy-4′-hydroxyphenyl)propionic acid)	106	57	5.6	3.0	279	219
Dihydro-*m*-coumaric acid (3-(3′-hydroxyphenyl)propionic acid)	202	217	4.9	3.1	622	830
Ferulic acid (3-(3′-methoxy-4′-hydroxyphenyl)-prop-2-enoic acid)	113	122	2.0	3.1	354	538*
Gentisic acid (2,5-dihydroxybenzoic acid)	57	21	3.5	3.6	201	85
Hippuric acid (2-benzamidoacetic acid)	4686	1768*	5.7	3.4	12478	7062*
Homovanillic acid (3′-methoxy-4′-hydroxyphenylacetic acid)	71	46	5.0	4.6	222	165
Hydroxyhippuric acid (3-hydroxybenzoylamino)acetic acid)	3041	2727	6.0	4.0	7468	11282*
Isoferulic acid (3-(3′-hydroxy-4′-methoxyphenyl)prop-2-enoic acid)	109	112	2.8	4.0	280	428*
Kaempferol (3,5,7-trihydroxy-2(4-hydroxyphenyl)chromen-4-one)	8.0	11	3.1	3.6	38	45
m-Hydroxyphenylacetic acid (3′-hydroxyphenylacetic acid)	258	456*	2.2	1.5	505	1284*
Phenylacetic acid (2-phenylacetic acid)	44	80	1.5	5.3	244	155
p-Hydroxybenzoic acid (4-hydroxybenzoic acid)	23	42	3.6	3.5	83	94
p-Hydroxyphenylacetic acid (4′-hydroxyphenylacetic acid)	262	297	2.8	1.9	1146	1400
p-Hydroxysalicylic acid (2.4-dihydroxybenzoic acid)	22	144	3	3.8	75	38
Protocatechuic acid (3,4-dihydroxybenzoic acid)	142	148	2.3	3.3	564	529
Pyrocatechuic acid (2,3-dihydroxybenzoic acid)	27	26	3.3	4.8	72	110
Salicylic acid (2-hydroxybenzoic acid)	167	51	2.1	2.9	624	215*
Sinapic acid (3-(4′-hydroxy-3′,5′-dimethoxyphenyl)prop-2-enoic acid)	35	29	1.6	2.6	204	79*
Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid)	299	153	1.8	4.0	608	666
Vanillic acid (3-methoxy-4-hydroxybenzoic acid)	113	65	1.9	3.6	384	274
Total					29 445	27 344

Results are presented as averages ($n = 9$).
*Significantly different from the blueberry drink (BB) drink, $p < 0.05$.

the FMD increases 6 h after consumption of the processed blueberry product.

4 Discussion

Our results showed significant improvements in FMD at 1, 2, and 6 h postconsumption of blueberry-containing baked buns (Fig. 1). The consumption of a blueberry drink produced a similar biphasic increase on FMD, in agreement with a previous study carried out with a nearly identical population [6]. However, despite kinetic differences, the magnitude of vascular effects exerted by blueberries was not changed by processing. The maximal FMD occur at 2 h postconsumption of the blueberry baked buns, and at 1 h postconsumption of the blueberry drink. This may be explained by a faster gastric passage and (poly)phenol release from the blueberry drink being a liquid as compared to the solid bun [18]. No significant differences in the plasma AUC of total (poly)phenol metabolites were found between treatments. Although our results suggest that (poly)phenols in the blueberry products represent the biologically active moiety in the blueberries administered, the mechanism of action is still unknown. Furthermore, it is unknown if an individual blueberry-derived polyphenol possesses distinct biological activities. In order to obtain first mechanistic insight, we analyzed temporal and quantitative associations between vascular functions and plasma levels and AUCs. The peak plasma levels of ferulic, isoferulic, m-hydroxyphenylacetic, and hydroxyhippuric acids were higher after the processed bun consumption (Fig. 2, Table 4). From these, only ferulic acid correlated with the FMD response at 1–2 h postconsumption. Plasma vanillic acid also correlated with the FMD response at 1–2 h postconsumption, with no correlations found between plasma metabolites and FMD response at 6 h postconsumption. For the blueberry drink, benzoic and vanillic acids correlated with the FMD response at 1–2 h, whereas hippuric, hydroxyhippuric, and homovanillic acids correlated with the effects at 6 h postconsumption. The levels of hippuric and homovanillic acids were significantly lower for the blueberry bun at 6 h, however hydroxyhippuric acid levels were similar between both treatments (Fig. 3). Which compounds are responsible for the observed effects on FMD at 6 h postconsumption of the blueberry buns is unknown. Indeed, and although evidence on the bioactivity of colonic metabolites in vitro is accumulating [2], it needs yet to be proven whether they possess any bioactivity in vivo. One potential interpretation of these results is that the individual compounds possess biological activities that are additive, and that the changes in the composition of (poly)phenols by processing does not change the net amount of biologically active compounds. Whether plasma phenolic metabolites and catabolites exert a synergistic effect on FMD and possess a

"class effect" or whether only certain metabolites are bioactive is presently unknown, and further work is needed in this area.

The differences in plasma blueberry (poly)phenols observed here may be due to the differences in the levels of chlorogenic acids and anthocyanins between the processed bun and blueberry drink. However, it is difficult to distinguish from which blueberry (poly)phenols the metabolites are derived from, as all those metabolites have been suggested to be formed after metabolic transformation, degradation, or colonic transformation by the gut microbiota of anthocyanins [19, 20], and chlorogenic acids [21–23] postabsorption, but might also be derived from procyanidins and quercetin. Indeed, many of the phenolic acids produced in the colon are rather generic and not only linked to a single parent compound [24]. Only a few studies have explored the effect of processing on (poly)phenol absorption and bioaccessibility. The bioavailability of chlorogenic acids was studied in cherry tomatoes [25] and artichokes [26], reporting significantly higher increases in plasma levels of hydroxycinnamic acid metabolites (chlorogenic, caffeic, ferulic, dihydrocaffeic, and dihydroferulic acids) after cooking. Mateo Anson et al. [27] studied the bioaccessibility of ferulic acid from wheat fractions and breads consumed by human subjects and observed that wheat ferulic acid had a low bioaccessibility (0.01%) due to its binding affinity to polysaccharides. However, the bioaccessibility was higher when free ferulic acid was added to flour, and also fermentation of wheat prior to baking broke the ferulic acid ester links to fiber, thus releasing ferulic acid and subsequently improving its bioavailability. This could explain the increase we observed in the plasma levels of ferulic and isoferulic acid after consumption of the processed product. We hypothesized that processing may break the ferulic acid esters linked to the fiber of the blueberry freeze-dried product and lead to an easier liberation of ferulic acid from the blueberry bun matrix in the stomach and the small intestine. Ferulic and isoferulic acids (free or esterified with saccharides in simple bound forms) would be more bioaccessible, and thus may be absorbed in the small intestine before reaching the colon, avoiding the degradation by microflora. A similar finding had been previously observed after consumption of ferulic acid in rats by Zhao et al. [28] and recently discussed by Erk et al. [29]. The increase in chlorogenic acid content in the processed bun may also be responsible for the increase in ferulic and isoferulic acids derivatives in plasma. On the other hand, in the unprocessed blueberry drink, ferulic acid interactions with the freeze-dried blueberry matrix might be stronger, explaining their lower absorption in the small intestine. A higher amount of ferulic acids and other blueberry (poly)phenols (mainly procyanidins and anthocyanins) will reach the colon and will be degraded by the gut microflora, leading to several phenolic acid metabolites that peak at 6 h in the blueberry drink intervention but not after the blueberry bun intake.

Higher levels of hydroxyhippuric acid were found after the blueberry bun intake, whereas in the blueberry drink human subjects, we observed higher levels of benzoic and salicylic acids. A possible explanation is that processing facilitates or leads to the hydroxylation and glycination of the benzoic acid (or just glycination of the salicylic acid) present in the blueberry freeze-dried product, leading to the formation of hydroxyhippuric acid in the blueberry bun before or just after its ingestion. In the blueberry drink intervention, hydroxyhippuric acid absorption might occur only after its production by the colonic microflora metabolism of blueberry (poly)phenols.

Another important consideration is the effect of the food matrix on the liberation of the blueberry (poly)phenols. The blueberry buns had more calories than the blueberry drink, with higher carbohydrate, fat, and protein content (Table 2). It has been shown that consumption of these macronutrients together with (poly)phenols can affect plasma levels of (poly)phenol metabolites [30–34]. Thus, the simultaneous intake of other food components in the bun intervention (milk, sugar, flour) may be responsible for some of the differences observed in the absorption of blueberry (poly)phenols in our study. Renouf et al. [31] showed that addition of milk, sugar, and nondairy creamer to coffee did not alter the overall bioavailability of coffee phenolic acids, although sugar and nondairy creamer had a small effect on the C_{max} and T_{max} of ferulic and isoferulic acids in plasma. The ingestion of blackcurrant anthocyanins with cereal and milk did not significantly alter the total amount of anthocyanins absorbed, but the rate of absorption and subsequent decline was slower [34]. A delayed absorption of anthocyanins was also shown after strawberries ingested with cream, although maximum concentration in plasma was not altered [30]. Those studies investigated concomitant consumption of (poly)phenols with other foods, but no processing was performed on the test products, which may explain the differences with the present study.

Also important is the variability in the FMD response and the plasma concentrations of metabolites in the study participants. Although in the present study improvements in FMD after blueberry consumption was observed in all participants, significant differences were found in the magnitude of the changes, together with differences in the plasma levels of phenolic metabolites. This suggests that some individuals are more responsive than others to the consumption of dietary polyphenols. Potential sources of interindividual variation can be differences in age, gender, genetic polymorphisms, dietary background, or differences in the gut microbiota. More research is needed to further determine the role of each of these factors in the variability observed.

In conclusion, our data suggest that the effects on endothelial function observed after blueberry intake are not significantly affected by processing in the blueberry baked product tested, even if changes in the (poly)phenol content of blueberry during processing led to some differences in the plasma metabolite profiles postconsumption. However, caution should be expressed when extrapolating the present findings to commercially available (poly)phenol-containing processed products. The baked products tested here had a

much higher blueberry content (equivalent to 80 g of fresh blueberry per bun) than other commonly commercially available blueberry-containing baked products such as blueberry muffins (typically 15–20 g fresh blueberries per muffin), and the (poly)phenol content was well preserved during processing, which may be due to short baking time (12 min) at relatively lower temperature (180°C) and to the presence of yeast, which might have helped to preserve the polyphenols [14]. Thus, processing conditions need to be taken into account in order to preserve the biological activity of (poly)phenol rich foods.

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Potential conflict of interest statement: We declare that we received by way of a gift the wild blueberry freeze-dried powder from the Wild Blueberry Association of North America. TWG is a director of Eccentricities Ltd. The other authors have declared no conflict of interest.

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