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Protocatechuic Acid Is the Major Human Metabolite of Cyanidin-Glucosides[1–3]

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Abstract

The metabolic fate of dietary anthocyanins (ACN) has not been fully clarified in humans. In all previous studies, the proportion of total ACN absorbed and excreted in urine was <1% intake. This study aimed to elucidate the human metabolism of cyanidin-glucosides (CyG) contained in blood orange juice (BOJ). One liter of BOJ, containing 71 mg CyG, was consumed by 6 healthy, fasting volunteers. Blood, urine, and fecal samples were collected at baseline and at different times up to 24 h after juice consumption. The content of native CyG, glucuronidated/methylated derivatives, and various phenolic acids was determined by HPLC/MS/MS. The serum maximal concentration of cyanidin-3-glucoside (Cy-3-glc) was 1.9 ± 0.6 nmol/L and that of protocatechuic acid (PCA) was 492 ± 62 nmol/L at 0.5 h and 2 h after juice consumption, respectively. The calculated total amounts in plasma corresponded for Cy-3-glc to 0.02% and for PCA to 44% of CyG ingested. CyG and glucuronidated/methylated metabolites, but not PCA, were detected in urine. ACN recovered in 24-h urine collections represented ~1.2% of the ingested dose. Both CyG (1.90 ± 0.04 nmol/g) and PCA (277 ± 0.2 nmol/g) were recovered in 24-h fecal samples. Data explained the metabolic fate of 74% of BOJ ACN. PCA was for the first time, to our knowledge, identified in humans as a CyG metabolite, accounting for almost 73% of ingested CyG. A high concentration of PCA may explain the short-term increased plasma antioxidant activity observed after intake of cyanidin-rich food and it can also contribute to the numerous health benefits attributed to dietary ACN consumption. J. Nutr. 137: 2043–2048, 2007.

Introduction

Anthocyanins (ACN)[7] are water-soluble pigments responsible for blue, purple, and red color of many plants. They have a high nutritional importance, as ACN daily intake has been estimated, in the United States, at much higher than that of other flavonoids such as quercetin, kampferol, myricetin, apigenin, and luteolin (180–250 mg/d vs. 23 mg/d, respectively) (1).

In vitro studies demonstrated several biological properties for ACN, including antioxidant, antiobesity, cardiovascular-protective, and antiinflammatory activities (for review, see 2,3).

In vivo studies investigating the bioavailability of these compounds in humans demonstrated that the maximum plasma concentration (C_{max}) of ACN was in the range of 0.1–100 nmol/L in the native form upon ingestion of doses of 0.7–10.9 mg/kg body weight. Several authors (4–7) have shown an almost complete clearance of the bloodstream within 6 h following the ACN-rich food consumption.

Glucuronidation and/or methylation are the major ACN metabolic pathways. The glucuronidated and methylated conjugates represented the only metabolites in 24-h excreted urine in many intervention studies (8–15), whereas the sulfate conjugates were retrieved in only 2 of them (15,16).

All literature reviews reported a proportion of total ACN absorbed and excreted in the urine (in the native form and as metabolites) far below 1% intake (4,7,8,17–19), which is lower than that found for other flavonoids (18). Many of these studies also showed that 69% of the ACN disappeared from the gastrointestinal tract within 4 h after food ingestion. Some in vitro and animal studies suggest that part of this disappearance can be due to the degradation of ACN aglycones leading to the formation of the corresponding phenolic acids and aldehydes (20–23).

In particular, protocatechuic acid (PCA) and *p*-hydroxybenzoic acid were identified in rat plasma and tissues after administration of a high dose of cyanidin-3-glucoside (Cy-3-glc) and pelargonidin,

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[3] Supplemental Figures 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
[7] Abbreviations used: ACN, anthocyanin; BOJ, blood orange juice; C_{max}, maximal concentration; Cy, cyanidin aglycone CyG, cyanidin-glucoside; Cy-3-glc, cyanidin-3-glucoside; Cy-gluc, cyanidin-glucuronide; Cy-mal-glc, cyanidin-3-(6″-malonyl) glucoside; Cy-met-gluc, cyanidin-methyl-glucuronide; PCA, protocatechuic acid; PelG, pelargonidin glycosides; Pel-gluc, pelargonidin-glucuronide; t_{max}, time of peak concentration.
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respectively (20,23). However, these phenolic acids have never been found in the plasma of humans following ACN consumption (6,8).

Blood oranges are typically produced in Sicily and Florida and blood orange juice (BOJ) is among the most important dietary source of cyanidin-glucosides (CyG). To our knowledge, only 2 studies, 1 in rats and 1 in humans, dealt with the bioavailability of red orange ACN (24,25).

The aim of this study was to clarify the human metabolic fate of dietary CyG by investigating blood, urine, and fecal samples of healthy volunteers who consumed 1 L of BOJ. The study was focused on CyG in the native forms and on their glucuronidated and methylated metabolites as well as on PCA.

Materials and Methods

BOJ. A commercial pasteurized BOJ produced by Oranfrizer (Scordia) was used as the ACN source. Analysis was performed by HPLC with diode array detection using 2 LC-10ADvp pumps (Shimadzu), a control system SCL-10Avp, and a SPDM10Avp diode array detection. The analytical conditions previously described by Fiore and co-workers (26) were applied and juice total CyG concentration was calculated as 71 mg/L, with Cy-3-glc and cyanidin-3-(6″-malonyl) glucoside (Cy-mal-glc) concentrations at 43 mg/L and 28 mg/L, respectively.

Subjects. Six healthy volunteers (3 male and 3 female) 20–24 y of age, weighing between 58 and 81 kg and with BMI between 21 and 31 were recruited for this study. Exclusion criteria were gastrointestinal disorders, metabolic diseases, habitual consumption of nonsteroidal antiinflammatory drugs, and use of antibiotic or hormone therapy as well as particular dietary regimes during the 3 mo before the experiment. All the selected subjects were informed about the study design and they gave informed written consent before starting the experiment.

The procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Study design. During the 2 d before the experiment, subjects were instructed to avoid the consumption of major dietary sources of polyphenol compounds such as fruits, fruit juices, vegetables, coffee, whole grains, and related products. We also asked participants to avoid the most commonly consumed ACN-rich foods such as red grapes and red wine, eggplant, red onion, red cabbage, and plums. Allowed foods were milk, tuna, white bread, chicken, pasta, and rice.

On the morning of the experimental day, after a 12-h fasting, subjects were asked to consume 1 L of BOJ in ~15 min. Blood, urine, and fecal samples were collected before the experiment (t0) and at different time points during the experiment. We collected blood samples at 0.5 (t0.5), 1 (t1), 2 (t2), 4 (t4), and 6 (t6) h; urine samples at 2 (t2), 4 (t4), 6 (t6), 12 (t12), and 24 (t24) h; and fecal samples at 24 (t24) h following BOJ ingestion. A biologist at the doctor's office of the Clinical Centre San Ciro (Portici) performed the blood drawings.

Sample treatment. Blood samples were allowed to clot at room temperature for 1 h and then centrifuged at 2800 × *g*; 15 min at 4°C to obtain serum. A 10-mL aliquot of each urine sample was added to 0.005% BHT (v:v) to limit oxidation processes. Fecal extracts were obtained by diluting each fecal sample with 10 mmol/L PBS, 1:10 (wt:v), centrifuging at 2800 × *g*; 15 min at 5°C, and collecting the supernatants. Serum, urine, and fecal extracts were prepared as described above and stored at −80°C until analysis. Urinary creatinine was measured in each sample by an automated system based on the buffered Jaffe reaction and analyzed by the COBAS Integra (Roche).

Determination of ACN and phenolic acids. ACN and phenolic acids were extracted from blood, urine and fecal samples using the procedure previously described by Kay and co-workers (13). Briefly, a solid-phase extraction using C18 cartridges (Supelclean ENVI-18, 6 mL, 500 mg; Sigma) after preconditioning with methanol (0.1% trifluoroacetic acid, pH 2.1, followed by oxalic acid added to water, pH 2.2) was performed. Blood serum (1.5 mL for ACN or 0.5 mL for phenolic acids determination, respectively), urine (1 mL), or fecal extracts (1 mL) were loaded onto the cartridge after acidifying with 6 mmol/L HCl and diluting with 10 mmol/L oxalic acid. The slow draining of the samples was followed by washing with oxalic acid acidified water. Both ACN and phenolic acids were eluted with 6 mL methanol (0.1% trifluoroacetic acid) and evaporated under a N_2 flow at room temperature. The residue was redissolved in 50 μL methanol (0.1% trifluoroacetic acid) before HPLC/MS/MS analysis.

HPLC/MS/MS analysis. The HPLC/MS/MS analyses were performed on API 3000 triple quadrupole mass spectrometer (Applied Biosystem Sciex), with a Turboionspray interface, coupled with an HPLC binary micropump (Perkin Elmer mod. series 200). All the analyses were performed using drying gas (N_2) at 400°C.

Chromatographic separations of ACN were performed on a Luna 3μ C18 100 A (50 × 2.00 mm) (Phenomenex) column using the following: mobile phases, water, 0.5% trifluoroacetic acid (solvent A), and methanol (solvent B). The following gradient elution was applied: 0–10 min 80% solvent A and 20% solvent B; 10–12 min 100% solvent B; and 12–14 min 80% solvent A and 20% solvent B.

For phenolic acids, a Prodigy C18 particle size 5 μ (250 mm × 460 mm) column (Phenomenex) and the following mobile phases were used: water, 0.1% formic acid (solvent A), and methanol (solvent B). The following gradient elution was used: 0–10 min 95% solvent A and 5% solvent B; 10–12 min 55% solvent A and 45% solvent B; 12–15 min 45% solvent A and 55% solvent B; 15–22 min 100% solvent B; and 22–24 min 95% solvent A and 5% solvent B.

The MS/MS detection was performed by acquiring data in positive ion mode for ACN and in negative ion mode for phenolic acids. The quantification was carried out in multiple reaction monitoring in both cases. Ions corresponding to $[M+H]^+$ for ACN and to deprotonated molecules $[M+H]^-$ for phenolic acids, each by specific molecular weights and fragments (**Table 1**), were monitored. The ions produced in MS/MS were obtained through fragmentation by a specific collision energy of a selected ion precursor, applying a voltage of 4500 V.

The quantification of ACN was obtained using a calibration curve obtained with pure Cy-3-glc (Exrasynthèse). For PCA and other phenolic compounds, a specific calibration curve obtained with the pure compounds purchased from Sigma was used. The minimal detection limits for ACN and phenolic acids were 11.1 μmol/L and 65.3 μmol/L, respectively.

Statistical analysis. We recorded the plasma and urine C_{max} and the time of peak concentration (t_{max}) as observed. The area under the

TABLE 1 HPLC/MS/MS identification of ACN and phenolic acids[1]

ACN	[M+H]+	MS/MS fragments
		m/z
Cy-3-glc	449	287
Cy-mal-glc	535	373, 287
Cy	287	213, 241
Cy-gluc	463	287
Cy-met-gluc	477	301
Cy-met-glc	463	301
Pel-gluc	447	271
Phenolic acids	**[M+H]−**	
Ferulic acid	193	134, 178
Caffeic acid	179	135
Vanillic acid	166.8	123
Hyppuric acid	178	134
PCA	153	109

[1] The analytical conditions specifically applied for ACN or phenolic acid determination in biological samples are reported in "Materials and Methods."

curves (AUC) for serum concentration time (0–6 h) and urine concentration time (0–24 h) was estimated using the linear trapezoidal rule. All data were expressed as means ± SEM. The significance of differences between the baseline (0 h) and the subsequent time points was assessed by ANOVA for repeated measures and the Dunnett's 2-tailed t test, assuming the baseline values as reference category. $P < 0.05$ was considered significant.

Results

Determination of ACN and phenolic acids in serum samples. Human serum samples at baseline (t0) contained neither ACN, in native or glucuronidated/methylated form, nor any of the monitored phenolic acids. Among all ACN and phenolic acids monitored, only Cy-3-glc and PCA were found within the 6 h following BOJ ingestion.

The blood concentration time curve of Cy-3-glc determined by MS/MS (**Fig. 1**) showed for this compound a C_{max} of 1.9 ± 0.6 nmol/L and t_{max} of 0.5 h. The concentration decreased to 1.1 ± 0.2 nmol/L 2 h after ingestion and reached a plateau during the next 4 h. The AUC value was 7.0 ± 1.2 nmol·h·L^{-1}.

PCA was quantified in the serum samples of all subjects after BOJ consumption by MS/MS using the specific transition mass-to-charge ratio (m/z) 153 → m/z 109. PCA C_{max} was 492 ± 62 nmol/L at t_{max} 2 h; 6 h after BOJ consumption, PCA concentration returned close to baseline (Fig. 1). The blood PCA AUC value was 11.0 ± 2.3 μmol·h·L^{-1}.

Determination of ACN and phenolic acids in urine samples. At baseline, all urine samples did not contain PCA or any form of ACN. The urine samples collected after BOJ consumption analyzed by HPLC/MS/MS clearly showed the peaks of the native ACN and of their metabolites (**Fig. 2**). Samples up to 24 h after BOJ consumption contained both parent ACN and glucuronidated and methylated compounds, whereas PCA was never found (**Fig. 3**).

The t_{max} of urinary excretion of both CyG in the native forms was 2 h and returned to baseline 24 h after BOJ consumption.

Large inter-individual differences in urinary excretion of these compounds were found: Cy-3-glc was retrieved in postjuice consumption urines of all subjects, whereas Cy-mal-glc was found in urines of only 4/6 subjects at a lower mean concentration than the nonacylated compound (0.07 ± 0.01 nmol/mmol creatinine vs. 0.77 ± 0.35 nmol/mmol creatinine, respectively). The AUC values were 306 ± 54 nmol·h·mmol creatinine^{-1} for urinary Cy-3-glc and 23.6 ± 4.2 nmol·h·mmol creatinine^{-1} for Cy-mal-glc.

Among ACN metabolites, cyanidin in glucuronidated and/or methylated form as well as glucuronidated pelargonidin (specific transition m/z 447 → m/z 271) were found. Cyanidin-glucuronide (Cy-gluc) and pelargonidin-glucuronide (Pel-gluc) had a t_{max} between 6 and 12 h after juice consumption, whereas glucuronidated and methylated cyanidin (Cy-met-gluc) had a t_{max} of 12 h (Fig. 3). Although pelargonidin glucosides were present only in trace amounts in the BOJ, urine AUC of Pel-gluc (1128 ± 430 nmol·h·mmol creatinine^{-1}) and Cy-gluc (1042 ± 259 nmol·h·mmol creatinine^{-1}) was comparable. Cy-met-glu was found in the urine of 5/6 subjects after juice consumption and its total concentration in 24-h urine was 457 ± 131 nmol·h·mmol creatinine^{-1}.

Determination of ACN and phenolic acids in fecal samples. HPLC/MS/MS analyses of fecal samples showed that native CyG and PCA were in the 24-h samples of all subjects but 1 (**Supplemental Figs. 1, 2**), which showed PCA and not CyG. None of these compounds was found in the fecal samples at baseline and none of the glucuronidated/methylated ACN metabolites were found at baseline and 24-h samples. Mean concentrations ($n = 5$) of Cy-3-glc and Cy-mal-glc were 1.45 ± 0.07 nmol/g feces and 0.45 ± 0.01 nmol/g feces, respectively. PCA was retrieved at a mean concentration ($n = 6$) of 277 ± 0.2 nmol/g feces.

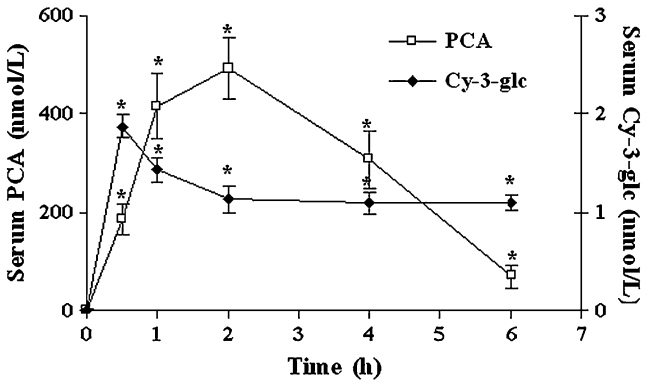

FIGURE 1 Serum concentration time curves (0–6 h) of Cy-3-glc (left axis) and PCA (right axis) of healthy subjects after BOJ consumption. Values are means ± SEM, $n = 6$. *Different from baseline, $P < 0.05$.

Discussion

The results obtained in this study were summarized and the amount of each compound retrieved in biological samples was reported together with the percentage relative to ingested CyG (**Table 2**). About 1.2% of ingested CyG were recovered in urine; Cy-gluc accounted for 83% of this (of which 20% were also methylated) and native CyG accounted for the remaining 17%. This figure is consistent with the previous in vivo studies dealing with the metabolism of ACN and reporting that glucuronidated and methylated compounds were the most abundant ACN metabolites (8–10,14–16,27).

BOJ was used as the dietary source of ACN, because with Cy-3-glc and Cy-mal-glc accounting for >82% of BOJ total ACN, the metabolic fate of CyG could be investigated without relevant interference of other ACN (26,28–30).

The mean dose of CyG ingested with 1 L of BOJ was 1 mg/kg body weight (2 μmol/kg body weight), which is far below the mean dose used in human studies (ACN dose ranged from 12 mg/kg body weight to 1.9 g/kg body weight) (18). In only 2 previous human studies (4,5), volunteers consumed foods containing a dose of total ACN comparable to that used in this study (2.7 mg/kg body weight and 3.6 mg/kg body weight). The first study (4) obtained data very similar to those here reported. The authors administered red fruit extract and found native CyG in plasma starting from 30 min after consumption and a plasma (0–6 h) cyanidin total amount of ~0.04% of the ingested dose (vs. 0.02% in this study). They too did not retrieve glucuronidated and methylated compounds or cyanidin aglycone (Cy) in plasma.

In the study performed by Matsumoto and co-workers (5), a black currant concentrate providing several ACN was used and the results were quite different to those here reported, as a high recovery in plasma and a low recovery in urine was shown. In fact, the recovery in plasma (0–4 h) was 0.2% of the ingested CyG

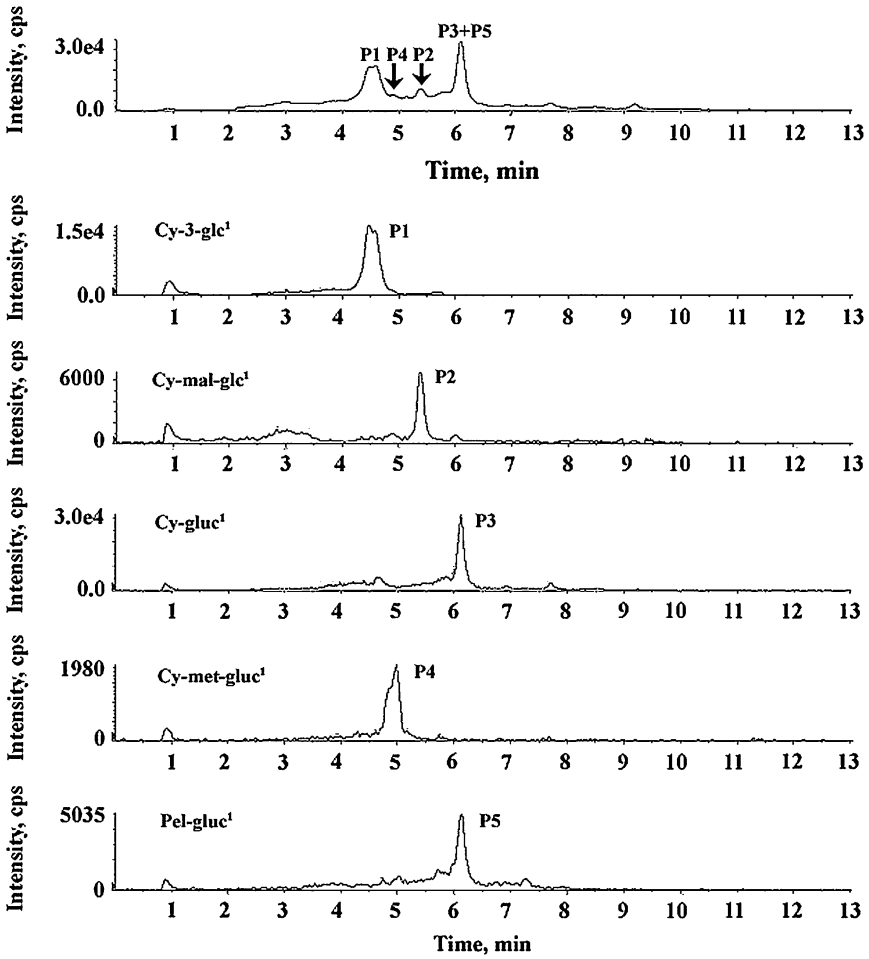

FIGURE 2 HPLC/MS/MS profile of a representative urine sample after BOJ consumption. 1, Obtained extracting the specific transitions of the native CyG and glucuronidated/methylated metabolites, as reported in Table 1.

(vs. 0.02% in this study) and that found in urine was 0.06% of dose (vs. 1.2% in this study).

An interesting feature in our study is the recovery of a high amount of Pel-gluc (42% of the total glucuronides recovered) in urine. Pelargonidin glycosides (PelG) were previously identified in BOJ (26). In accordance with Fiore and co-workers (26), who reported that pelargonidin-rhamnosil-glucoside and pelargonidin-glucoside are among the minor ACN components of BOJ, we found PelG in the BOJ at a concentration of 0.7 mg/L. On the other hand, Wu and co-workers (10), in a study aimed at comparing absorption and metabolism of PelG and CyG, found a total urinary recovery of the pelargonidin derivatives ~7 times higher than that of cyanidin (0.6% vs. 0.09%). These features may explain why in this study the Pel-gluc in 24-h urine collections is present at an amount comparable to that of Cy-gluc (1.17 μmol vs. 1.20 μmol, respectively).

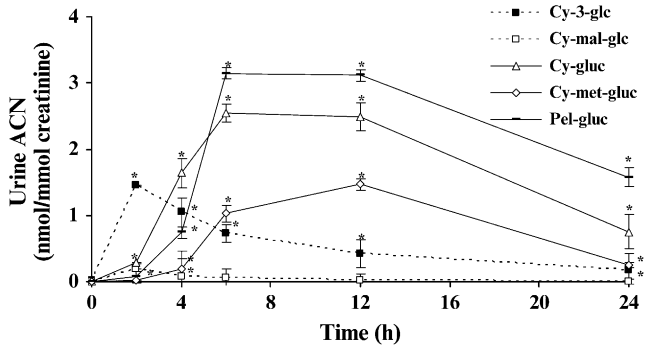

FIGURE 3 Urine concentration time curves (0–24 h) of native CyG and 3 main metabolites of healthy subjects after BOJ consumption. Values are means ± SEM, $n = 6$. *Different from baseline, $P < 0.05$.

The main finding of this work (Table 2) is that PCA is the major metabolite of CyG in humans, accounting for 44.4% of the ingested CyG in 6-h postconsumption bloodstream. A PCA mean concentration of 2.0 nmol/g was found in fecal samples collected the day after BOJ consumption, thus suggesting that the production of PCA in vivo by human colon microflora occurs. This result showed that PCA retrieved in feces accounted for 28.1% of the ingested CyG. Adding the amounts of PCA recovered in bloodstream to that found in feces, PCA can be considered the major human metabolite of Cy-3-glc and Cy-mal-glc, representing alone the metabolic fate of 72.5% of the ingested ACN. To the best of our knowledge, this is the first time PCA has been retrieved in human serum and feces after ingestion of an ACN-rich food.

The presence of PCA is not surprising, considering in vitro and animal data published in the last few years. Tsuda and co-workers (20) reported the presence of PCA in plasma of rats fed Cy-3-glc. They found that plasma concentration of PCA was 8 times higher than that of Cy-3-glc and, in accordance to our data, they did not find the Cy. Moreover, they found Cy and PCA in the intestine and demonstrated in vitro that PCA was formed 15 min after the addition of Cy to plasma. In a recent in vitro study focused on the pH-dependent stability of various ACN, it was reported that at pH 7.4, Cy almost completely disappeared

TABLE 2 Repartition of parental CyG and their metabolites in human biological fluids after BOJ consumption[1]

	Ingested	Blood[2]	Urine	Feces
		μmol (%)		
CyG	148 ± 0.01 (100)	0.03 ± 0.01 (0.02)	0.27 ± 0.02 (0.2)	0.28 ± 0.01 (0.2)
Cy-gluc			1.20 ± 0.11 (0.8)	
Cy-met-gluc			0.38 ± 0.07 (0.3)	
PCA		65.7 ± 0.10 (44)		41.6 ± 0.03 (28)
Total		65.7 ± 0.11 (44)	1.85 ± 0.20 (1.2)	41.9 ± 0.04 (28)

[1] Values are means ± SEM, $n = 6$, except CyG in feces, $n = 5$ (percentage of CyG).
[2] Amounts recovered in blood and in fecal samples were calculated assuming a mean volume of 6 L blood and a mean stool weight of 150 g (31–37).

after 60 min to form different dimerization products (via the quinoid anhydrobase), PCA, and an aldehyde (via the α-diketone intermediate). On the contrary, Cy-monodiglucosides and even more Cy-diglucosides were stable at neutral pH (38). A previous study (39) also showed that CyG can be converted into PCA in vitro.

The fundamental role of deglycosilation of ACN to form the corresponding phenolic acids and aldehydes has also been reported in studies focused on the role of gut microflora in the metabolism of these compounds (21,22,38). In fact, in some in vitro studies demonstrated that after incubating ACN with pig or human fecal microflora, the production of the corresponding phenolic acids and aldehydes was observed. The first step of bacterial biotransformation was in both cases the cleavage of sugar moiety leading to the formation of ACN aglycone. This compound could be further metabolized by microflora or naturally degraded due to pH conditions (21,22,38).

Finally, in the discussion of an article reporting data about a recent human study (13) on CyG bioavailability, the authors found that traces of PCA were detectable in plasma and urine, but they did not correlate the presence of PCA to the dietary CyG.

On the basis of all these in vitro, ex vivo, and animal studies and considering the human data in this study, we summarized the absorption and metabolism of CyG in humans in a scheme (**Fig. 4**). This scheme takes into account the amount of compounds recovered in the different biological samples together with the time course of their detection.

The rapid t_{max} (0.5 h in serum and 2 h in urine) of the parent compounds in blood and urine seems to confirm the important role of gastric absorption previously reported by other investigators (25,40,41). After absorption from the stomach into the bloodstream, the native CyG are glucuronidated and excreted within a few hours.

PCA can be formed both in the small intestine or the bloodstream and these 2 possibilities have been highlighted in the scheme. Considering that PCA has a t_{max} of 2 h and that Cy aglycone was not detected in the bloodstream, we hypothesize that CyG undergo deglycosilation by small intestine β-glycosidases and that the aglycone is degraded into PCA directly in the intestinal lumen or after absorption in the blood.

The recovery of CyG and PCA in the 24-h feces indicated the in vivo production of PCA by intestinal microflora. A fraction of the fecal PCA and CyG may be absorbed from the colon. This slow and continuous release of antioxidant compounds into the bloodstream may have a great physiological relevance to sustain the concentration of blood antioxidants over 24 h. This phenomenon can explain the findings of Riso and co-workers (24), who reported that Cy-3-glc is found in fasting blood the day after a 21-d intervention with BOJ in humans.

The presence of Cy-met-gluc in urine and not in plasma was justified by kidney biotransformation (Fig. 4). Liver and kidney are the major sites of glucuronidation and methylation of ACN in vivo through the action of UDP-glucuronyl-transferase (4,20, 41,42). It is possible that glucuronidated and methylated compounds, formed in the kidney, are directly excreted in urine and are thus not detected in the bloodstream (4, this work).

The finding of a relevant concentration of PCA in human blood after BOJ ingestion has interesting physiological and nutritional implications. The considerable amount of PCA in the blood starting a few minutes after ingestion can explain the acute increase of plasma antioxidant activity found in humans after ingestion of foods containing ACN (43,44). Many authors noted that the increased antioxidant activity did not correlate with the small amount of ACN or their glucuronidated/methylated metabolites recovered in bloodstream, whereas it can be explained by the concentration of PCA found in this study also, considering its marked antioxidant activity (39). On the other hand, the absence of PCA in 24-h urine collections suggests that it can be used as an in vivo antioxidant, thus being potentially transformed and/or linked to other compounds (e.g. serum albumin).

In this study, we demonstrated the metabolic fate of ~74% of CyG ingested with BOJ in humans. For the first time, to our knowledge, PCA has been quantified in the bloodstream and feces of humans after ingestion of dietary CyG, accounting for almost 73% of the ingested dose. Thus, PCA can be considered the major human metabolite of Cy-3-glc and Cy-mal-glc and it may be responsible for several health benefits related to CyG-rich food consumption.

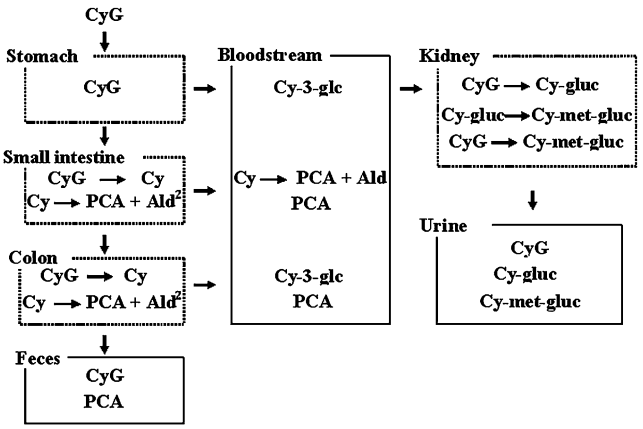

FIGURE 4 Proposed pathways for absorption and metabolism of CyG in humans as indicated by the results of this study. 2, Aldehyde.

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