

# Xanthenes in Mangosteen Juice Are Absorbed and Partially Conjugated by Healthy Adults<sup>1-4</sup>

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## Abstract

The proposed health-promoting effects of the pericarp from mangosteen fruit have been attributed to a family of polyphenols referred to as xanthenes. The purpose of this study was to determine the bioavailability of xanthenes from 100% mangosteen juice in healthy adult participants ( $n = 10$ ). Pericarp particles accounted for 1% of the mass and 99% of the xanthone concentration in the juice. The juice provided  $5.3 \pm 0.1$  mmol/L total xanthenes with  $\alpha$ -mangostin, garcinones (C, D, and E),  $\gamma$ -mangostin, gartanins, and other identified xanthenes accounting for 58, 2, 6, 4, and 5%, respectively. Participants ingested 60 mL mangosteen juice with a high-fat breakfast. Free and conjugated (glucuronidated/sulfated) xanthenes were detected in serum and urine. There was marked variation in the AUC ( $762\text{--}4030$  nmol/L  $\times$  h), maximum concentration ( $113 \pm 107$  nmol/L), and time to maximum concentration ( $3.7 \pm 2.4$  h) for  $\alpha$ -mangostin in sera during the 24-h collection. Similarly, xanthenes in 24-h urine ranged from 0.9 to 11.1  $\mu$ mol and accounted for  $2.0 \pm 0.3\%$  (range 0.3–3.4%) of the ingested dose. There were no significant differences between female and male participants in mean pharmacokinetic values of  $\alpha$ -mangostin in serum and urinary xanthenes. Only  $15.4 \pm 0.7\%$  of total xanthenes in pericarp particles in the juice partitioned into mixed micelles during in vitro digestion. These results show that xanthenes in mangosteen juice are absorbed when ingested along with a high-fat meal, although release of xanthenes from pericarp particles during digestion may be limited. J. Nutr. 142: 675–680, 2012.

## Introduction

Mangosteen (*Garcinia magostana* Lin.) is a tree indigenous to Southeast Asia and Indonesia and is also grown in Hawaii and Puerto Rico (1). Mangosteen fruit has a purple exterior with an edible soft white pulp characterized by a sweet, slightly acidic flavor and surrounded by a brown pericarp or hull. The pericarp of the mangosteen has been used in traditional Thai medicine to treat inflammation, infections, wounds, and diarrhea (2). Since its introduction into the United States, juices and food products containing mangosteen fruit have become a top-selling botanical supplement, with sales of beverages alone exceeding \$200 million in 2008 (3). This commercial success has been largely the result of aggressive marketing of the putative health claims based on in vitro

observations and anecdotal reports. In vitro activities include anti-infectious (4–6), antioxidative (7,8), anti-inflammatory (9–11), antiproliferative and proapoptotic (12,13), and antimetastatic (14,15) properties. Such activities have been most strongly associated with a unique family of compounds referred to as xanthenes that are located in the pericarp of mangosteen (3,16). Xanthenes in mangosteen fruit have a distinct C<sub>6</sub>-C<sub>1</sub>-C<sub>6</sub> tricyclic ring system and often contain multiple hydroxy and isoprene groups. Mangostins and garcinones are the most abundant prenylated xanthenes in the mangosteen pericarp, along with many other xanthenes present in lower concentrations. Possible health-promoting activities of xanthenes are also supported by several animal studies. For example, dietary administration of  $\alpha$ -mangostin (0.05% by weight) significantly decreased development of aberrant crypt foci in 1,2-dimethylhydrazine-treated rats (17). Also, oral administration of 48  $\mu$ mol  $\alpha$ -mangostin/kg attenuated carrageenan-induced paw edema in mice (18).

The proposed health benefits of mangosteen rely on the assumption that the ingested compounds or their bioactive metabolites are delivered to target tissues. The bioavailability of mangosteen xanthenes has received very limited attention. Kondo et al. (19) recently reported the presence of  $\alpha$ -mangostin in plasma after subjects ingested 59 mL of a blended juice product containing mangosteen. No information was provided on other xanthenes in

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<sup>3</sup> Supplemental Tables 1 and 2 and Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

<sup>4</sup> This study was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT01425047.

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the juice or xanthone metabolites in plasma. Li et al. (20) reported that rats absorbed only 0.4% of  $\alpha$ -mangostin administered by gavage. Our primary objective in the present study was to determine the bioavailability of 7 of the more abundant xanthones present in mangosteen in healthy men and women who consumed a single dose of 100% mangosteen juice with a typical Western-style breakfast. The amounts of xanthones ingested, free and conjugated xanthones in urine, and  $\alpha$ -mangostin in serum were investigated. Several of the other xanthones were detected in serum. Finally, to determine the level of bioaccessibility of the target compounds, mangosteen juice was subjected to simulated gastric and small intestinal digestion, and the amount of xanthones released from pericarp particles was measured.

## Participants and Methods

**Chemicals and reagents.** All chemicals were purchased from Sigma Chemical Co. unless otherwise noted. HPLC-grade reagents were obtained from Fisher Scientific.  $\alpha$ - and  $\gamma$ -Mangostins, garcinones D and E, and 8-deoxygartanin were purified ( $\geq 98\%$  as assessed by NMR) as described elsewhere (21,22).

**Mangosteen juice.** Four commercial sources of "mangosteen juice" were initially purchased in local natural health stores to determine xanthone concentration. Three of these products that were marketed as mangosteen juice were actually blends containing other fruit juices. Total xanthone concentration in the products ranged from  $0.48 \pm 0.03$  to  $5.32 \pm 0.19$  mmol/L. The highest concentration of xanthones was present in the 100% mangosteen juice product used in this study. The juice product was a colloidal suspension containing both liquid and pericarp particles (pulp). The profile of xanthones in mangosteen juice is presented in Table 1. The dry weight of pericarp in juice was  $10.6 \pm 0.2$  g/L, with particle sizes ranging from  $>74$  to  $<149$   $\mu\text{m}$ . Juice was centrifuged ( $10,000 \times g$ , 35 min at  $4^\circ\text{C}$ ) to separate the liquid and pericarp particles to determine the xanthone concentration of each fraction and to assess extent of bioaccessibility using in vitro digestion (see below).

**Participants.** Informed consent was obtained from all participants, and the study procedures were approved by the OSU<sup>11</sup> Biomedical Sciences Committee of the Institutional Review Board, Office of Responsible Research Practices. Nonsmoking, healthy adult participants (5 males and 5 females) were recruited by word of mouth among OSU students and staff. Study eligibility required participants to be non-smoking healthy adults with a BMI  $\leq 30$  kg/m<sup>2</sup> and normal renal function. Exclusion criteria included pregnancy, acute and chronic diseases, a history of gastrointestinal tract pathology, and either weight loss of  $\geq 10\%$  or use of dietary supplements, herbals, or antibiotics during the 6-mo period preceding entry into the study.

**Study design and sampling.** Participants were required to refrain from consuming mangosteen fruit, mangosteen juice, and other mangosteen-containing foods and beverages for 1 wk before the study and to refrain from drinking wine the day before and day of the study. After an overnight fast ( $\geq 10$  h), participants were admitted to the OSU CRC (0600–0700) for collection of baseline blood (0 h). Because the suggested serving of mangosteen juice products for proposed health-promoting activity on labels is 28–84 mL/d, participants received 60 mL of well-mixed mangosteen juice along with a typical fast-food (i.e., high-fat) breakfast consisting of English muffin (64 g), sausage (50 g), egg (65 g), and cheese (21 g) along with 6.8 g canola oil and 6.8 g soybean oil. Coffee (without creamer or fat-containing milk), tea, water, nonfat milk, or carbonated beverages were available. The total energy of the meal was 485 kcal, with protein, carbohydrate, and fat accounting for 23, 23, and

54%, respectively. The ratio of SFA : MUFA:PUFA was 2.1:1.6:1.0 (total fat = 28.1 g). This meal also contained 1.54 g dietary fiber. Subjects ingested the meal within 10 min and remained in the CRC for collection of blood at 1, 2, 3, 4, 6, and 8 h. After the 4-h collection of blood, participants consumed lunch consisting of white bread (50 g), butter (5 g), cheese (28.4 g), salad dressing (14.7 g), turkey breast (90 g), chicken noodle soup (126 g), crackers (6 g), pudding, (99 g) and the same selection of beverages as for the breakfast. Free access to water and coffee was provided throughout the day; a single participant drank a single cup of coffee after meals until release from the CRC. An optional snack of pretzels (28.4 g) and low-fat yogurt (245 g) was provided at 1500. Upon release from the CRC, participants were advised that there were no dietary restrictions for dinner other than to refrain from products containing mangosteen fruit, mangosteen-containing fruit juices/drinks, and wine for the remainder of the day. All subjects returned to the CRC the next morning for 24-h blood collection. Participants completed a dietary record of all ingested food and beverages, and analysis indicated complete compliance by all study participants.

A pretest sample of urine (baseline) was collected upon arrival at the CRC. Urine was also collected during the stay at the CRC (0–8 h) and continuously from 8 to 24 h by participants after release from the CRC. The total volume of urine for the 0–8-h and 8–24-h collections was measured, and an aliquot was stored at  $-20^\circ\text{C}$  for analysis of xanthones within 2 wk.

**Estimated bioaccessibility of xanthones in mangosteen juice during in vitro digestion.** Intact mangosteen juice (2 mL), the liquid fraction of the juice (2 mL), and pericarp particles from 2 mL intact mangosteen juice were separately mixed with 2.0 g nonfat yogurt containing 0–3% soybean oil to assess bioaccessibility. Samples were subjected to simulated gastric and small intestinal digestion as described in detail elsewhere (23). The recovery of xanthones after simulated digestion was  $>70\%$ , except for 8-deoxygartanin, which was 61%. The quantity of xanthones partitioning in the aqueous fraction of chyme after completion of simulated small intestinal digestion is referred to as being bioaccessible, i.e., having the potential to be absorbed.

**Extraction and analysis of xanthones.** Aliquots of serum and urine were incubated with or without a glucuronidase/sulfatase enzyme mixture from *Helix pomatia* (S9626; Sigma Chemical Co.) for 2 h at  $37^\circ\text{C}$  to hydrolyze xanthone conjugates, followed by solid-phase extraction using C<sub>18</sub> cartridges (24). Mangosteen juice, the separated liquid and pericarp ("pulp") fractions of juice, and the chyme and aqueous fraction after in vitro digestion were extracted into ethyl acetate for analysis. The HPLC system and chromatographic separation of xanthones have been described elsewhere (21). Garcinones D and E,  $\alpha$ - and  $\gamma$ -mangostins, and 8-deoxygartanin were identified by comparison of time to elution and spectrum with standards ( $>98\%$  purity as determined by <sup>1</sup>H- and <sup>13</sup>C-NMR) (4). Concentrations were determined by comparison with known quantities of pure compounds that were prepared gravimetrically. The 5-point calibration curves were linear with a correlation coefficient  $>0.998$ , and the lower limit of detection of xanthones was 12.2 nmol/L (signal to noise ratio = 3). Because standards for garcinone C, gartanin, 6-dihydroxy-methoxy-dimethyl-pyranone, and tovo-phillin B were not available, each of these compounds was identified by comparison of time to elution and UV spectrum with values in the literature (21,22). Concentrations were estimated as equivalents of garcinone D for garcinone C, 8-deoxygartanin for gartanin, and  $\alpha$ -mangostin for 6-dihydroxy-methoxy-dimethyl-pyranone and tovo-phillin B.

Because the method described above lacked sufficient sensitivity to detect xanthones other than  $\alpha$ -mangostin in serum, several samples containing the highest concentration of  $\alpha$ -mangostin were selected for analysis by HPLC-MS to determine if other xanthones were present. A more rapid HPLC method was developed with the Synergi analytical column (4  $\mu\text{m}$ ,  $4.6 \times 150$  mm; Phenomenex). The mobile phase did not include acid because the signal improved several-fold using water and acetonitrile. The gradient was as follows: 40% to 95% acetonitrile over 17 min; 95–100% acetonitrile over 5 min; and 3 min reequilibration with 40% acetonitrile. An Alliance 2695 HPLC system was utilized with a flow rate of 1.3 mL/min and a column temperature at  $35^\circ\text{C}$ . Xanthone identities and elution order were confirmed by comparison with the longer HPLC method (described above) using pure standards. The HPLC eluate

<sup>11</sup> Abbreviations used: C<sub>max</sub>, maximum serum concentration; CRC, Clinical Research Center; OSU, Ohio State University; T<sub>max</sub>, time to the maximum serum concentration.

**TABLE 1** Xanthenes in mangosteen juice product ingested with breakfast<sup>1</sup>

Xanthenes	Juice $\mu\text{mol/L}$	Percentage of total xanthenes identified %	Distribution in juice	
			Liquid portion $\mu\text{mol/L}$	Pericarp
Garcinone C	291 $\pm$ 11.2	5.5	5.5 $\pm$ 0.2	263 $\pm$ 15.3
Garcinone D <sup>2</sup>	520 $\pm$ 10.9	10.2	5.8 $\pm$ 0.3	465 $\pm$ 23.5
Garcinone E <sup>2</sup>	239 $\pm$ 18.5	5.1	1.7 $\pm$ 0.2	214 $\pm$ 6.9
$\alpha$ -Mangostin <sup>2</sup>	3190 $\pm$ 123	59.9	12.2 $\pm$ 0.5	3100 $\pm$ 195
$\beta$ -Mangostin	121 $\pm$ 9.3	2.3	0.9 $\pm$ 0.1	111 $\pm$ 3.3
$\gamma$ -Mangostin <sup>2</sup>	356 $\pm$ 4.3	6.5	1.9 $\pm$ 0.2	326 $\pm$ 4.5
8-Deoxygartanin <sup>2</sup>	176 $\pm$ 4.5	3.1	2.4 $\pm$ 0.0	155 $\pm$ 5.0
Gartanin	157 $\pm$ 6.9	2.8	2.7 $\pm$ 0.2	149 $\pm$ 3.4
Tovophillin B	50 $\pm$ 2.9	1.1	ND	48 $\pm$ 0.7
1,6-Dihydroxy-methoxy-dimethyl-pyranone	193 $\pm$ 7.4	3.6	ND	176 $\pm$ 10.9
Total	5290 $\pm$ 166	100	33.0 $\pm$ 0.5	5000 $\pm$ 200

<sup>1</sup> Values are means  $\pm$  SD;  $n = 5$  independent replicates.

<sup>2</sup> Standards (>98% pure) available in our laboratory. Concentrations of garcinone C and gartanin were estimated as equivalents of garcinone D and 8-deoxygartanin, respectively, and 6-dihydroxy-methoxy-dimethyl-pyranone and tovophillin B were estimated as equivalents of  $\alpha$ -mangostin as described in Participants and Methods.

was interfaced with a quadrupole/time-of-flight hybrid mass spectrometer (QToF Premier; Micromass) via an electrospray probe operated in negative ion mode. The mass spectrometer was calibrated with sodium formate, and accuracy was maintained by lockspray using leucine enkephalin as a reference. Before introduction, the eluate was split so that approximately one-tenth of the flow was delivered to the MS source. MS parameters included the following: 450°C desolvation temperature (nitrogen), 35 V cone, 2.8 kV capillary, 8 eV collision energy, and argon Collision-induced dissociation with argon ( $3 \times 10^{-3}$  mBar).

**Miscellaneous analyses.** Urinary creatinine was analyzed by HPLC according to Achari et al. (25). Serum cholesterol was analyzed by enzymatic colorimetric method using the CHOL2 COBAS assay kit according to the manufacturer's instructions (Roche Diagnostics).

**Analysis of serum  $\alpha$ -mangostin data.** Postprandial absorption of  $\alpha$ -mangostin in serum was expressed as baseline-corrected AUC during the 24-h period after ingesting breakfast with mangosteen juice by trapezoidal approximation using Igor Pro 4.0 software (Wave Metrics). The  $T_{\text{max}}$  and  $C_{\text{max}}$  also were determined. The amount of xanthenes excreted into urine was estimated by the product of concentration of total xanthenes (free + conjugated) in urine and total urine volume.

**Statistical analysis.** A Welch unpaired  $t$  test (GraphPad Software) was used to assess whether there were significant differences between general characteristics, pharmacokinetic parameters for  $\alpha$ -mangostin in serum, and urinary xanthenes for female and male participants. Welch paired  $t$ -test was used to compare within-group changes over time for xanthenes in urine. The impact of the amount of fat on the partitioning of individual xanthenes into the aqueous fraction of chyme during simulated digestion of mangosteen juice was analyzed by 1-factor ANOVA followed by Tukey's honestly significant difference test (SPSS). Differences at  $P < 0.05$  were considered significant. Values shown in the text are means  $\pm$  SD.

## Results

All 10 participants completed the study, and none reported gastrointestinal distress after consuming the test sample of 60 mL mangosteen juice. BMI was within the healthy range for all participants. Serum cholesterol and urinary creatinine were normal in healthy adults (26). These variables and age did not differ between female and male participants (Supplemental Table 1).

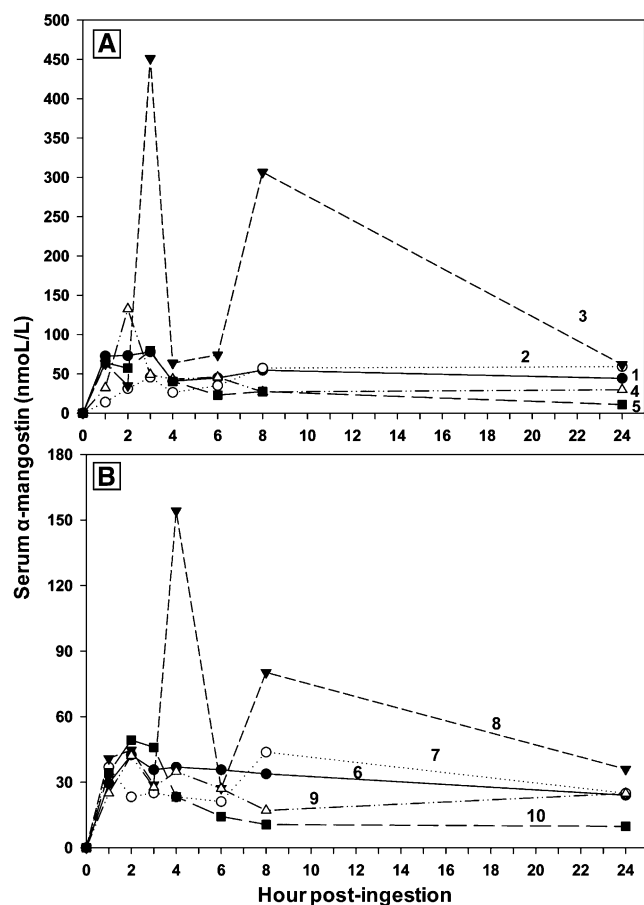
**Xanthenes in serum.**  $\alpha$ -Mangostin was the only xanthone detected in serum by HPLC-Diode-Array Detector, indicating that

the concentration of other xanthenes was <12.2 nmol/L. The AUC,  $C_{\text{max}}$ , and  $T_{\text{max}}$  of serum  $\alpha$ -mangostin were similar in female and male participants (Table 2). The serum concentration of  $\alpha$ -mangostin increased rapidly after ingestion of the mangosteen juice with the breakfast meal (Fig. 1). One participant (no. 3) had a markedly greater AUC (4030 nmol/L  $\times$  h) and  $C_{\text{max}}$  (450 nmol/L) compared with all other participants. After a steep decline, the concentration of  $\alpha$ -mangostin in the serum of participants 1, 3, 7, and 8 increased again at 6–8 h, although the maxima of the second peaks were less than those of the respective initial peaks. These data suggested one or more of the following possibilities: the xanthone-free meal at lunch stimulated absorption of the xanthenes retained in enterocytes or enhanced lymphatic flow into circulation, xanthenes were reabsorbed by enterohepatic circulation, or xanthenes also may be absorbed in the large intestine. Serum  $\alpha$ -mangostin did not differ significantly between 8 h ( $71 \pm 87$  nmol/L) and 24 h ( $33 \pm 18$  nmol/L). The  $C_{\text{max}}$  of total  $\alpha$ -mangostin ranged from 42 to 450 nmol/L, and the  $T_{\text{max}}$  for total  $\alpha$ -mangostin was 2–4 h in 8 of the participants, and 8 h for the 2 remaining participants (Table 2).

**TABLE 2** Pharmacokinetic parameters of free and conjugated  $\alpha$ -mangostin in serum collected from 10 healthy participants<sup>1</sup>

Participant	AUC $\text{nmol/L} \times \text{h}$	$C_{\text{max}}$ $\text{nmol/L}$	$T_{\text{max}}$ $\text{h}$
Females			
1	1605	78	3
2	962	57	8
3	4029	450	3
4	1484	132	2
5	1249	79	3
Mean $\pm$ SD	1870 $\pm$ 1230	159 $\pm$ 165	3.8 $\pm$ 2.4
Males			
6	1004	42	2
7	771	44	8
8	1798	154	4
9	816	42	2
10	762	49	2
Mean $\pm$ SD	1030 $\pm$ 440	66 $\pm$ 49	3.6 $\pm$ 2.6

<sup>1</sup>  $C_{\text{max}}$ , maximum serum concentration;  $T_{\text{max}}$ , time to the maximum serum concentration.



**FIGURE 1** Profile of  $\alpha$ -mangostin concentration in serum of female (A) and male (B) participants during a 24-h period after ingestion of 60 mL of mangosteen juice with a breakfast meal. Numbers above the lines identify individual participants listed in Tables 2 and 3.

The mean ratio of free to conjugated  $\alpha$ -mangostin in serum was  $\sim 2.7:1.0$  ( $73.0 \pm 3.7\%$  vs.  $27.0 \pm 3.7\%$ ) from 1–8 h and  $2.8:1.0$  ( $73.5 \pm 23.4\%$  vs.  $26.5 \pm 23.4\%$ ) at 24 h. However, there was considerable interindividual variation in the extent to which serum  $\alpha$ -mangostin was conjugated during the collection period. The ranges of conjugated  $\alpha$ -mangostin in serum among participants were 9–48%, 6–49%, 4–35%, 3–70%, 4–42%, <1–79%, and <1–73% at 1, 2, 3, 4, 6, 8, and 24 h, respectively. Serum from 3 participants collected at  $C_{max}$  was analyzed by HPLC-MS to provide additional sensitivity to identify xanthenes other than  $\alpha$ -mangostin. Preliminary HPLC-accurate mass data indicated the presence of garcinone D,  $\alpha$ -mangostin, 8-deoxygartanin, and gartanin in serum (Supplemental Fig. 1).  $\gamma$ -Mangostin, garcinone E, and tophillin B also were detected in serum from 1 of the 3 participants (no. 3). Double peaks were observed for garcinone D (peaks 1a and 1b) and 8-deoxygartanin (peaks 2a and 2b) (Supplemental Fig. 1) in serum from all participants. These likely are cyclized isomers of the parent compounds because they exhibited the same accurate mass and similar retention behavior. Standards, as well as pericarp extracts, also displayed these minor forms.

**Xanthenes in urine.** Xanthenes were not detected in the urine of participants at baseline. Both free and conjugated xanthenes were present in urine of all participants after ingestion of the mangosteen juice (Table 3). The relative quantities of the different xanthenes in urine were similar to those in mangosteen juice with the exception of the absence of garcinone C (data not

shown). The amount of total xanthenes in urine during the 24-h collection period represented  $2.0 \pm 0.3\%$  of the ingested dose and did not differ between males and females. The rate of xanthone excretion during the 0–8-h collection period ( $342 \pm 49$  nmol/h) was greater than between the 8- and 24-h collection period ( $205 \pm 20$  nmol/h) ( $P < 0.01$ ).

There was marked interindividual variability in the relative amounts of free and conjugated species of each xanthone excreted during the 24-h collection period (Table 3). For example, the percentages of total garcinone D and  $\alpha$ -mangostin in urine that were conjugated ranged from 2 to 80% and from 9 to 54%, respectively (data not shown). The extent of conjugation of the various xanthenes in urine from a participant was similar and independent of gender (data not shown).

**Bioaccessibility of xanthenes from mangosteen juice during in vitro digestion.** Partitioning of xanthenes into the aqueous or bioaccessible fraction of chyme generated during simulated digestion of mangosteen juice increased in proportion to the amount of soybean oil added to the mixture of mangosteen juice and yogurt ( $P < 0.001$ ) (Supplemental Fig. 2). In the absence of oil, 8–15% of the xanthenes were present in the aqueous fraction of chyme. This increased to 27–33% during digestion of the juice and yogurt mixture containing 3% soybean oil. There were <1% of xanthenes partitioned in the aqueous fraction of chyme when the bile extract was omitted during digestion of the juice containing 3% soybean oil (data not shown), suggesting that the bioaccessibility of xanthenes was partially dependent on their incorporation into mixed micelles. Partitioning of xanthenes from the separated liquid and pericarp fractions of mangosteen juice into the bioaccessible fraction of chyme during simulated digestion was also compared. The quantity of xanthenes released from the pericarp into the aqueous fraction of chyme greatly exceeded that from the liquid fraction because the pericarp contained 99% of the xanthenes in the juice (Supplemental Table 2). In contrast, the efficiency of partitioning of xanthenes in the aqueous fraction of juice (liquid part) in the aqueous fraction of chyme ( $84.7 \pm 1.4\%$ ) greatly exceeded that during the digestion of pericarp ( $15.4 \pm 0.7\%$ ) ( $P < 0.001$ ).

**TABLE 3** Excretion (24 h) of xanthenes into urine accounts for  $\sim 2\%$  of the dose of mangosteen juice ingested by female and male participants

Participants	Urinary xanthenes			Apparent absorption %
	nmol/24 h	Free:conjugated	nmol/mg Creatinine	
<b>Females</b>				
1	8380	40:60	18.5	2.6
2	5344	49:51	10.5	1.6
3	11,151	38:62	8.3	3.4
4	4932	52:48	4.4	10.5
5	898	65:35	2.9	0.3
Mean $\pm$ SD	$6140 \pm 3860$	$49 \pm 10.8:51 \pm 10.8$	$8.9 \pm 6.2$	$1.9 \pm 1.2$
<b>Males</b>				
6	8798	43:57	21.5	2.1
7	9949	73:27	8.3	3.0
8	4946	78:22	8.0	1.5
9	4602	42:58	4.1	1.4
10	1071	55:45	2.4	0.3
Mean $\pm$ SD	$5870 \pm 3560$	$58 \pm 16.7:42 \pm 15.7$	$8.9 \pm 7.5$	$1.7 \pm 1.0$

## Discussion

This is the first study to our knowledge to examine the bioavailability of multiple xanthenes in human participants after consumption of mangosteen juice as part of a meal. Both free and glucuronidated/sulfated conjugates of the most abundant xanthenes in mangosteen juice were present in serum and urine. The relative amounts of the individual xanthenes in urine were similar to those in the juice, suggesting that the apparent absorption of these compounds was minimally affected by their structural differences. Absorption from this juice product was estimated to be 2% of the ingested dose as assessed by the quantity of total xanthenes in urine collected for 24 h. The concentration of  $\alpha$ -mangostin in serum and the total quantity of xanthenes in urine varied  $\sim$ 10-fold among participants. Results from simulated gastric and small intestinal digestion of mangosteen juice suggested that the apparently low bioavailability was primarily due to inefficient release of xanthenes from pericarp particles. Transformation of ingested xanthenes to bioactive metabolites by gut bacteria and human cells (27), secretion of xanthenes into bile, and retention in tissues beyond the 24-h collection period may underestimate the extent of absorption based solely on 24-h urinary content.

Kondo et al. (19) previously reported the presence of  $\alpha$ -mangostin in serum from human participants who ingested 59 mL of a blended juice containing mangosteen, aloe vera, green tea, and multivitamins before breakfast. "Mangostin" (not defined) concentration was 3.8 mmol/L and the  $C_{\max}$  for  $\alpha$ -mangostin in plasma was  $7.6 \pm 3.7$  nmol/L. The  $T_{\max}$  was 1 h, and the xanthone was detected for  $\leq 6$  h postingestion. The quantities of mangostins ( $\alpha$ ,  $\beta$ , and  $\gamma$ -mangostin) and total xanthenes in mangosteen juice in the present study were 3.7 and 5.3 mmol/L, respectively (Table 1). Apparent absorption was greater in our study with the  $C_{\max}$  for  $\alpha$ -mangostin increased by 5-fold. Differences that accounted for the greater absorption of  $\alpha$ -mangostin in our study include determination of both free and conjugated  $\alpha$ -mangostin and co-consumption of the juice with a breakfast meal containing a high amount of fat. We previously reported that  $\alpha$ -mangostin was partially conjugated by Caco-2 human intestinal cells and that free and conjugated compounds effluxed across the basolateral membrane (28). A prandial-like state in Caco-2 cell cultures, i.e., presence of oleate: taurocholate micelles in the apical compartment, stimulated trans-epithelial flux of free, but not conjugated,  $\alpha$ -mangostin, suggesting that the free compound was incorporated into chylomicrons and secreted. For this reason, mangosteen juice and a high-fat breakfast were consumed together in the present study to increase partitioning of xanthenes into mixed micelles for delivery to enterocytes and subsequent secretion into lymph. The  $T_{\max}$  of 3.7 h in the present study compared with the  $T_{\max}$  of 1 h reported by Kondo et al. (19) is likely due to slower gastric emptying when the juice was consumed with the high-fat meal. Finally, it is possible that compounds in tea and aloe vera present in the juice blend ingested by participants in the previous study (19) may have attenuated the bioaccessibility and absorption of xanthenes.

Li et al. (20) recently reported the first pharmacokinetic investigation of  $\alpha$ -mangostin. Rats were administered pure  $\alpha$ -mangostin solubilized in 2% ethanol and Tween 80 for intravenous and oral delivery, respectively. The pharmacokinetic profile of intravenously administered  $\alpha$ -mangostin included a rapid tissue distribution phase and a slow elimination phase. However, the oral bioavailability of  $\alpha$ -mangostin from a 20-mg dose without a meal was extremely low ( $\sim$ 0.4%). We have found both free and phase 2 metabolites of xanthenes in tissues of mice chronically fed an AIN-93G diet containing 5% fat and 0.1% (wt:wt)  $\alpha$ -mangostin, confirming the absorption and metabolism of dietary xanthenes (29).

Whole juice and its aqueous and pericarp components were separately subjected to simulated gastric and small intestinal digestion to provide insight on the apparent poor bioavailability of xanthenes in the participants. Only 15% of the xanthenes in this juice product partitioned in the bioaccessible fraction of chyme. Because 99% of the xanthenes were in pericarp, the release of xanthenes from undigested material appears to be the limiting factor for xanthone bioaccessibility from the juice. Bioavailability of dietary compounds is affected by numerous factors including size of ingested food particles (e.g., 30) because increased surface area increases the access of digestive enzymes. Materials that are not digested in the upper gut pass to the large intestine where fermentation of the matrix may release compounds that can be transformed to bioactive compounds by the microbiota. The fate of pericarp xanthenes in the large intestine and innovative strategies to enhance release of xanthenes from the pericarp during preparation of juice products merit investigation.

The profile of the individual xanthenes in urine was similar to that in mangosteen juice, suggesting that there may be limited impact of xanthone structure on absorption and excretion. In contrast, there were marked variations in the concentration of  $\alpha$ -mangostin in serum, the amounts of xanthenes in urine, and the ratio of free to conjugated metabolites of xanthenes in serum and urine among participants consuming the same quantity of mangosteen juice. Heterogeneity in serum concentrations of dietary phytochemicals is common, and the genetic and physiologic basis of such differences are only beginning to be understood. For example, polymorphisms in xenobiotic metabolizing enzymes and transporters are known to influence the absorption and biotransformation of drugs and phytochemical compounds (31,32).

The presence of free  $\alpha$ -mangostin and other xanthenes in serum is particularly interesting because concentrations of  $\leq 10$   $\mu$ mol/L exhibit antiinflammatory, antiproliferative, and proapoptotic effects in vitro (9–12). The question is whether concentrations of xanthenes in body fluids and tissues are sufficient to mediate the proposed health-promoting activities in humans.  $C_{\max}$  for  $\alpha$ -mangostin in this study in participants consuming 60 mL of 100% mangosteen juice were submicromolar. Chronic consumption of  $\leq 500$  mL mangosteen juice for 8 wk was not associated with reported side effects or significant changes in vital signs (33), suggesting that tissue concentrations of xanthenes may approach those shown to mediate phenotypic changes in cellular studies. It is interesting that reduced C-reactive protein and other antiinflammatory markers in serum have been recently reported for 2 randomized, double-blinded, placebo-controlled studies in healthy participants consuming a mangosteen juice blend daily for 4 and 8 wk (33,34). Because the blend used in these trials contained numerous components in addition to mangosteen juice, it is unclear if the observed antiinflammatory activity was mediated by xanthenes. Further investigation using pure mangosteen juice will facilitate evaluation of proposed health-promoting activities of xanthenes in vivo.

The results from this study clearly show that xanthenes are absorbed and can be transformed to phase 2 metabolites. The relative bioactivities of the ingested xanthenes and their metabolites in humans merit investigation. The above data provide a foundation for the design of future investigations to further elucidate the absorption, metabolism, and potential efficacy of dietary xanthenes.

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## Literature Cited

- Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M. *Garcinia mangostana* L: a phytochemical and pharmacological review. *Phytother Res.* 2009;23:1047–65.
- Chairungsrilerd N, Takeuchi K, Ohizumi Y, Nozoe S, Ohta T. Mangostanol, a prenyl xanthone from *Garcinia mangostana*. *Phytochemistry.* 1996;43:1099–102.
- Sloan EW. Getting ahead of the curve: phytochemicals. *Nutraceutical World.* 2010;13:16–7.
- Suksamrarn S, Suwannapoch N, Phakhodee W, Thanuhiranlert J, Ratananukul P, Chimnoi N, Suksamrarn A. Antimycobacterial activity of prenylated xanthenes from the fruits of *Garcinia mangostana*. *Chem Pharm Bull (Tokyo).* 2003;51:857–9.
- Vlietinck AJ, De Bruyne T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. *Planta Med.* 1998;64:97–109.
- Riscoe M, Kelly JX, Winter R. Xanthenes as anti-malarial agent: discovery, mode of action and optimization. *Curr Med Chem.* 2005;12:2539–49.
- Williams P, Ongsakul M, Proudfoot J, Croft K, Beilin L. Mangostin inhibits the oxidative modification of human low density lipoprotein. *Free Radic Res.* 1995;23:175–84.
- Ngawhirunpat T, Opanasopi P, Sukma M, Sittisombut C, Kat A, Adachi I. Antioxidant, free radical-scavenging activity and cytotoxicity of different solvent extracts and their phenolic constituents from the fruit hull of mangosteen (*Garcinia mangostana*). *Pharm Biol.* 2010;48:55–62.
- Nakatani K, Yamakuni T, Kondo N, Arakawa T, Oosawa K, Shimura S, Inoue H, Ohizumi Y.  $\gamma$ -Mangostin inhibits inhibitor- $\kappa$ B kinase activity and decreases lipopolysaccharide-induced cyclooxygenase-2 gene expression in C6 rat glioma cells. *Mol Pharmacol.* 2004;66:667–74.
- Romier-Crouzet B, Van De Walle J, During A, July A, Rousseau C, Henry O, Larondelle Y, Schneider YJ. Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. *Food Chem Toxicol.* 2009;47:1221–30.
- Bumrungpert A, Kalpravidh RW, Chuang C, Overman A, Martinez K, Kennedy A, McIntosh M. Xanthenes from mangosteen inhibit inflammation in human macrophages and in human adipocytes exposed to macrophage-conditioned media. *J Nutr.* 2010;140:842–7.
- Matsumoto K, Akao Y, Ohguchi K, Ito T, Tanaka T, Iinuma M, Nozawa Y. Xanthenes induce cell-cycle arrest and apoptosis in human colon cancer DLD-1 cells. *Bioorg Med Chem.* 2005;13:6064–9.
- Nakagawa Y, Iinuma M, Naoe T, Nozawa Y, Akao Y. Characterized mechanism of alpha-mangostin-induced cell death: caspase-independent apoptosis with release of endo-nuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer DLD-1 cells. *Bioorg Med Chem.* 2007;15:5620–8.
- Shih Y-W, Chien S-T, Chen P-S, Lee J-H, Wu S-H, Yin L-T.  $\alpha$ -Mangostin suppresses phorbol 12-myristate 13-acetate-induced MMP-2/MMP-6 expressions via  $\alpha$ v $\beta$ 3 integrin/FAK/ERK and NF- $\kappa$ B signaling pathway in human lung adenocarcinoma A549 cells. *Cell Biochem Biophys.* 2010;58:31–44.
- Hung S-H, Shen K-H, Wu C-H, Liu C-L, Shih Y-W.  $\alpha$ -Mangostin suppresses PC-3 carcinoma cell metastasis by inhibiting matrix metalloproteinase-2/9 and urokinase-plasminogen expression through the JNK-signaling pathway. *J Agric Food Chem.* 2009;57:1291–8.
- Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Anti-cancer effects of xanthenes from pericarp of mangosteen. *Int J Mol Sci.* 2008;9:355–70.
- Nabandith V, Suzuki M, Morika T, Kaneshiro T, Kinjo T, Matsumoto K, Iinuma M, Yoshinmi N. Inhibitory effects of crude alpha-mangostin, a xanthone derivative, on two different categories of coloreneoplastic lesions induced by 1,2-dimethylhydrazine in the rat. *Asian Pac J Cancer Prev.* 2004;5:433–8.
- Chen L-G, Yang L-L, Wang C-C. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. *Food Chem Toxicol.* 2008;46:688–93.
- Kondo M, Zhang L, Ji H, Kou Y, Ou B. Bioavailability and antioxidant activity of a xanthone-rich mangosteen (*Garcinia mangostana*) product in humans. *J Agric Food Chem.* 2009;57:8788–92.
- Li L, Brunner I, Han A, Hamburger M, Kinghorn AD, Frye R, Butterweck V. Pharmacokinetics of  $\alpha$ -mangostin in rats after intravenous and oral application. *Mol Nutr Food Res.* 2011;55:567–74.
- Chaivisuthangkura A, Malaikaew Y, Chaovanalikit A, Jaratrungratawee A, Panseeta P, Rataananukul P, Suksamrarn S. Prenylated xanthone composition of *Garcinia mangostana* (mangosteen) fruit hull. *Chromatographia.* 2009;69:315–8.
- Jung H-A, Su B-N, Keller WJ, Mehta RG, Kinghorn AD. Antioxidant xanthenes from the pericarp of *Garcinia mangostana* (mangosteen). *J Agric Food Chem.* 2006;54:2077–82.
- Chitchumroonchokchai C, Schwartz SJ, Failla ML. Assessment of lutein bioavailability from meals and supplements using simulated digestion and Caco-2 human intestinal cells. *J Nutr.* 2004;134:2280–6.
- Walsh KR, Haak SJ, Bohn T, Tian Q, Schwartz SJ, Failla ML. Isoflavonoid glucosides are deconjugated and absorbed in the small intestine of human subjects with ileostomies. *Am J Clin Nutr.* 2007;85:1050–6.
- Achhari R, Mayersohn M, Conrad KA. Creatinine in human plasma and urine. *J Chromatogr Sci.* 1983;21:278–81.
- Bishop ML, Fody EP, Schoeff L, editors. *Clinical chemistry.* 5 ed. New York: Lippincott Williams & Wilkins; 2005.
- Foti RS, Pearson JT, Rock DA, Wahlstrom JL, Wienkers LC. *In vitro* inhibition of multiple cytochrome P450 isoforms by xanthone derivatives from mangosteen extract. *Drug Metab Dispos.* 2009;37:1848–55.
- Bumrungpert A, Kalpravidh RW, Suksamrarn S, Chaivisuthangkura A, Chitchumroonchokchai C, Failla ML. Bioaccessibility, biotransformation and transport of  $\alpha$ -mangostin from *Garcinia mangostana* (Mangosteen) using simulated digestion and Caco-2 human intestinal cells. *Mol Nutr Food Res.* 2009;53:554–61.
- Chitchumroonchokchai C, Suksamrarn S, Thomas-Ahner JM, Clinton SK, Failla ML. Alpha-mangostin reduces HT-29 colon cancer cell proliferation *in vitro* and inhibits transplantable tumorigenesis *in vivo*. *FASEB J.* 2010; 24:928.10.
- Castenmiller JJM, West CE, Linssen JPH, van het Hof KH, Voragen AGJ. The food matrix of spinach is a limiting factor in determining the bioavailability of  $\beta$ -carotene and to a lesser extent of lutein in humans. *J Nutr.* 1999;129:349–55.
- Burchell B. Genetic variation of human UDP-glucuronosyltransferase: implications in disease and drug glucuronidation. *Am J Pharmacogenomics.* 2003;3:37–52.
- Nowell S, Falany CN. Pharmacogenetics of human cytosolic sulfo-transferases. *Oncogene.* 2006;25:1673–8.
- Tang Y-P, Li P-G, Kondo M, Ji H-P, Kou Y, Ou B. Effect of mangosteen dietary supplement on human immune function: A randomized, double-blind, placebo-controlled trial. *J Med Food.* 2009;12:755–63.
- Udani JK, Singh BB, Barrett ML, Singh VJ. Evaluation of mangosteen juice blend on biomarkers of inflammation in obese subjects: a pilot dose finding study. *Nutr J.* 2009;8:48.