

# Pharmacokinetics of Curcumin Conjugate Metabolites in Healthy Human Subjects

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## Abstract

**Background:** Curcumin is a polyphenol, found in the spice turmeric, that has promising anticancer properties, but previous studies suggest that absorption of curcumin may be limited.

**Methods:** This study examined the pharmacokinetics of a curcumin preparation in healthy human volunteers 0.25 to 72 h after a single oral dose. Curcumin was administered at doses of 10 g ( $n = 6$ ) and 12 g ( $n = 6$ ). Subjects were randomly allocated to dose level for a total of six subjects at each dose level. Serum samples were assayed for free curcumin, for its glucuronide, and for its sulfate conjugate. The data were fit to a one-compartment absorption and elimination model.

**Results:** Using a high-performance liquid chromatography assay with a limit of detection of 50 ng/mL, only

one subject had detectable free curcumin at any of the 14 time points assayed, but curcumin glucuronides and sulfates were detected in all subjects. Based on the pharmacokinetic model, the area under the curve for the 10 and 12 g doses was estimated (mean  $\pm$  SE) to be  $35.33 \pm 3.78$  and  $26.57 \pm 2.97$   $\mu\text{g/mL} \times \text{h}$ , respectively, whereas  $C_{\text{max}}$  was  $2.30 \pm 0.26$  and  $1.73 \pm 0.19$   $\mu\text{g/mL}$ . The  $T_{\text{max}}$  and  $t_{1/2}$  were estimated to be  $3.29 \pm 0.43$  and  $6.77 \pm 0.83$  h. The ratio of glucuronide to sulfate was 1.92:1. The curcumin conjugates were present as either glucuronide or sulfate, not mixed conjugates.

**Conclusion:** Curcumin is absorbed after oral dosing in humans and can be detected as glucuronide and sulfate conjugates in plasma. (Cancer Epidemiol Biomarkers Prev 2008;17(6):1411-7)

## Introduction

Wide arrays of phenolic substances, especially those present in dietary and medicinal plants, have been reported to possess substantial antioxidant, anti-inflammatory, anticarcinogenic, and antimutagenic effects (1-3). Curcumin is a yellow, naturally occurring polyphenolic phytochemical derived from turmeric, the powdered rhizome of the herb *Curcuma longa* Linn. The spice turmeric is used in curries as a coloring and flavoring agent in various parts of the world, especially in the Indian subcontinent, an area that has a low incidence of colorectal cancer (4).

Several animal model studies have shown that curcumin suppresses carcinogenesis in skin (5-7), stomach (8, 9), colon (10, 11), breast (12-14), and liver (15). Curcumin is reported to induce apoptosis in a wide variety of tumor cells, including B- and T-cell leukemias (16, 17), colon (18), and breast carcinoma (19, 20). Chemopreventive activities of curcumin are thought to involve up-regulation of carcinogen-detoxifying enzymes (21-23) and antioxidants (24, 25), suppression of cyclooxygenase-2 expression (26-30), and inhibition of

nuclear factor- $\kappa$ B release (30-32). Inhibition of nuclear factor- $\kappa$ B release by curcumin also leads to the down-regulation of various proinflammatory cytokines (e.g., tumor necrosis factor and interleukins) and inhibition of the mRNA expression of several proinflammatory enzymes (e.g., cyclooxygenase, lipoxygenases, metalloproteinases, and nitric oxide synthase; ref. 33).

In animal studies, curcumin undergoes rapid metabolic reduction and conjugation, resulting in poor systemic bioavailability after oral administration (34-36). For example, an oral dose of 0.1 g/kg administered to mice yielded a peak plasma concentration of free curcumin that was only 2.25  $\mu\text{g/mL}$  (35). In rats, curcumin completely disappeared from plasma within 1 h after a 40 mg/kg i.v. dose (34). When given orally at a 500 mg/kg dose, peak concentrations of 1.8 ng/mL of free curcumin were detected in plasma (34). The major metabolites of curcumin (Fig. 1) identified in rat plasma were curcumin glucuronide and curcumin sulfate based on enzymatic hydrolysis studies. Hexahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin glucuronide were also present in minor amounts (34).

Data on the pharmacokinetic properties and metabolism of curcumin in humans are very limited. In a human study conducted in 25 patients with precancerous lesions, free curcumin concentrations (mean  $\pm$  SD) in plasma after taking 4, 6, and 8 g of curcumin per day for 3 months were 0.19, 0.20, and 0.60  $\mu\text{g/mL}$ , respectively (37). None of the curcumin conjugates or metabolites of curcumin were reported in that study. A study of six

Received 10/12/07; revised 3/31/08; accepted 4/8/08.

**Grant support:** AICR-06A035, National Cancer Institute grant CN-55124, and University of Michigan General Clinical Research Center grant M01-RR00024.

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doi:10.1158/1055-9965.EPI-07-2693

patients with advanced colorectal cancer dosed with 3.6 g of curcumin daily for up to 3 months yielded 4.3, 5.8, and 3.3 ng/mL mean plasma concentrations of curcumin, curcumin glucuronide, and curcumin sulfate, respectively, 1 h after administration (38).

In animal models, no toxicity has been reported to date. Similarly, in humans to date, few adverse events due to curcumin even at very high doses have been reported (7). Whether the low toxicity is only a function of lack of bioavailability is an open question. We have recently reported a trial escalating curcuminoid dose from 0.5 to 10 g in healthy humans that showed few adverse events, but bioavailability of curcumin was limited (39). The present clinical study assessed the complete pharmacokinetic profile of curcumin and its conjugate metabolites in healthy human subjects administered either a 10 g or a 12 g single dose, the highest doses given to humans. The aims of this study were to (a) determine if curcumin, in capsule formulation, is absorbed and biotransformed in humans and (b) assess the human pharmacokinetics of curcumin and its conjugate metabolites.

## Materials and Methods

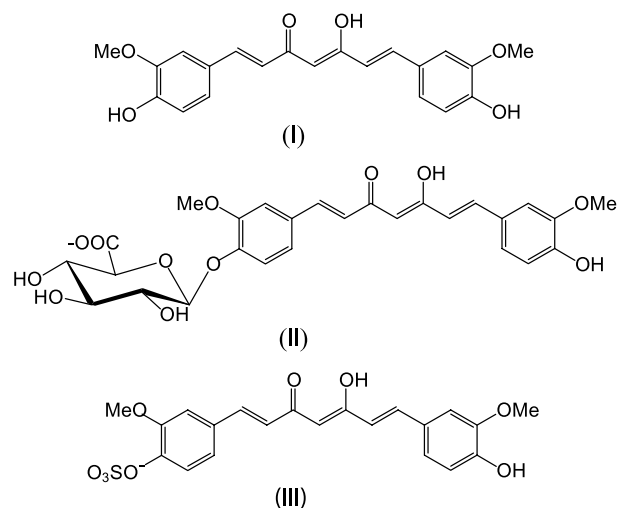
Curcuminoids were provided in a capsule form as a powder extract obtained from Alleppey finger turmeric (C<sup>3</sup> Complex, Sabinsa Corp.) in a standardized, Good Manufacturing Practice formulation. The mixture contained curcumin (75%), demethoxycurcumin (23%), and bisdemethoxycurcumin [2%; verified by high-performance liquid chromatography (HPLC) assay in our laboratory].  $\beta$ -17-Estradiol acetate and the enzymes  $\beta$ -glucuronidase (type IX-A from *Escherichia coli*) and sulfatase (type H-1 from *Helix pomatia*) were purchased from Sigma-Aldrich, Inc. Sodium phosphate and sodium acetate (American Chemical Society certified) were purchased from Fisher Scientific. HPLC-grade ethyl acetate and methanol were purchased from Burdick and Jackson (Honeywell International, Inc.).

**Clinical Trial Design.** Twelve healthy human subjects solicited by advertisement or word of mouth, 18 y of age or older, with normal organ function were recruited. Subjects included five males and seven females (all Caucasian, ages 18-65 y, body mass index range of 20-29). Subjects were not taking any medications chronically. They were educated on food sources of curcumin and asked to avoid all foods containing high concentrations of curcumin within the 14 d before drug administration. Subjects completed a food checklist to verify that they were not consuming any curcumin-rich foods before dosing. A standardized, high-fat breakfast with 750 calories and 35 g of total fat (42% fat) designed to slow gastric emptying time was fed to all subjects to allow maximum absorption of curcumin, which is highly lipophilic. Then, curcumin was administered at a dose of 10 g ( $n = 6$ ) or 12 g ( $n = 6$ ). Subjects were randomly allocated to dose level for a total of six subjects at each dose level. Curcumin was formulated as Sabinsa C<sup>3</sup> complex in 250 mg capsules (containing ~187 mg curcumin, 58 mg demethoxycurcumin, and 5 mg bisdemethoxycurcumin) and administered with 16 to 32 oz of water over a maximum of 30 min. Blood samples (10 mL) were collected in heparinized tubes before

dosing and at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 24, 36, 48, and 72 h after the last capsule was taken. All protocol procedures were done in the University of Michigan Medical Center General Clinical Research Center. The plasma fraction was separated from blood immediately and kept at  $-20^{\circ}\text{C}$  until assayed. Adverse events were graded based on National Cancer Institute Common Toxicity Criteria version 2.0 at 4, 24, 48, and 72 h after curcumin administration. The study protocol and the comprehensive written informed consent used in this study protocol were approved by the Institutional Review Boards of the University of Michigan Medical School before the start of the study.

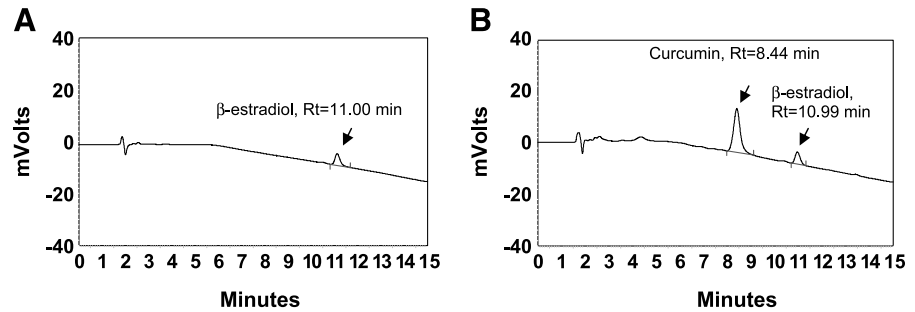
**Extraction of Curcumin from Plasma.** The extraction procedure was based on the previously validated method of Heath et al. (40). An aliquot of plasma (200  $\mu\text{L}$ ) was mixed with water (80  $\mu\text{L}$ ) and  $\beta$ -17-estradiol acetate (internal standard, 20  $\mu\text{L}$ ) and vortex mixed for 30 s. The solution was then extracted thrice with 1 mL of ethyl acetate/methanol (95:5, v/v) as an extracting reagent and vortex mixed for 3 min. The samples were centrifuged at 3,000 rpm for 15 min at  $4^{\circ}\text{C}$ . The upper organic layer was collected. The organic phases from the three extractions were pooled and evaporated under a stream of argon at room temperature. All the extraction procedures were done under dim light to prevent the degradation of curcumin. The extracts were then reconstituted in methanol (100  $\mu\text{L}$ ) before HPLC analysis.

**Enzymatic Hydrolysis of Curcumin Conjugates.** Samples were also assayed for curcumin and its conjugates after incubating the plasma samples with the enzymes  $\beta$ -glucuronidase and sulfatase using the method of Asai and Miyazawa (36). For these assays, plasma samples (200  $\mu\text{L}$ ) were mixed with water (80  $\mu\text{L}$ ) and internal standard (20  $\mu\text{L}$ ) and vortexed for 30 s. The samples were then mixed with  $\beta$ -glucuronidase (50  $\mu\text{L}$ , 446 units) in 0.1 mol/L phosphate buffer (pH 6.8) and sulfatase (45  $\mu\text{L}$ , 52 units) in 0.1 mol/L sodium acetate buffer (pH 5.0) and incubated at  $37^{\circ}\text{C}$  for 3.5 h. The



**Figure 1.** Structures of curcumin (I), curcumin glucuronide (II), and curcumin sulfate (III).

**Figure 2.** HPLC chromatograms of extracted plasma 1 h after a 12 g dose of curcumin. The plasma was analyzed without incubation (A) and after incubation with  $\beta$ -glucuronidase (440 units) and aryl sulfatase (50 units) for 3.5 h (B).



incubation time was determined by a test run, where samples were incubated with enzymes for 1, 2, 3, 4, 5, 6, and 15 h, showing that the optimal incubation time was 3.5 h. The samples were extracted thrice with 1 mL of ethyl acetate/methanol (95:5, v/v) using the extraction procedure mentioned above. The extracts were then reconstituted in methanol (100  $\mu$ L) before HPLC analysis. To determine the amount of glucuronide and sulfate conjugates in plasma, samples were incubated separately with  $\beta$ -glucuronidase and sulfatase enzymes before extraction.

**Quantitation of Curcumin in Plasma.** Reverse-phase HPLC was used to quantify curcumin in plasma. The separations were done on a  $\mu$ Bondapak, C18 column (250  $\times$  4.6 mm inner diameter; 10  $\mu$ m particle size; Waters) at room temperature. The mobile phase consisted of 0.1% acetic acid/65% methanol/35% water (v/v/v; A) and 100% methanol (B). The extracted sample was eluted on a gradient mobile phase starting 100% of A at zero time to 100% B in 15 min in a linear gradient and then to 100% A in 2 min at a flow rate of 2 mL/min. The injection volume for all samples was 20  $\mu$ L and detection was done at 420 nm.

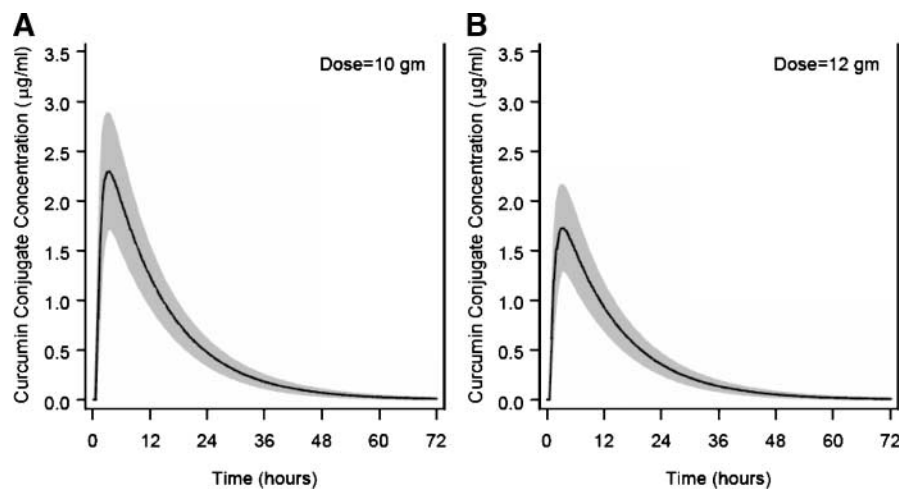
Standard curves were constructed using plasma added to curcumin. Plasma samples with no detectable curcumin (200  $\mu$ L) were added to varying amount of a standard solution of curcumin to yield final curcumin concentrations of 0, 0.025, 0.05, 0.075, 0.10, 0.125, 0.25, 0.5, 1.25, 2.5, 5, and 10  $\mu$ g/mL, respectively. Spiked samples were extracted as described above. Calibration curves were obtained by plotting the area ratios of curcumin to

internal standard,  $\beta$ -17-estradiol, against concentrations of curcumin in spiked plasma. Each sample was analyzed in duplicate.

**Analytic Assessment/Quality Control.** Curcumin and internal standards were well resolved by HPLC. Retention time of curcumin was 8.4 and internal standard was 11.0 min, respectively (Fig. 2). A linear relationship between curcumin plasma concentration and peak area response was found in the concentration ranges 0.075 to 10  $\mu$ g/mL. Interday coefficients of determination ( $R^2$ ) from 0.9979 to 0.9999 were observed over 4 d with curcumin-spiked plasma. The lower limit of quantitation of curcumin in plasma based on this method was 0.075  $\mu$ g/mL. The extraction efficiency of curcumin at 1.25 and 2.5  $\mu$ g/mL levels was 98.29% and 98.49%, respectively. The corresponding coefficients of variation were 1.8% and 1.2%, respectively. At 0.025  $\mu$ g/mL, the internal standard  $\beta$ -17-estradiol acetate showed 95.01% extraction efficiency with a coefficient of variation of 4%. The percentage recovery of curcumin at 1  $\mu$ g/mL was 95.14% with a coefficient of variation of 2.7%. The lower limit of detection was 0.050  $\mu$ g/mL.

We assayed the samples in random order, mixing samples from subjects receiving 10 and 12 g doses in assay batches (Fig. 3). We matched the subject identification number on the plasma tube to the dose administered, raw HPLC chromatogram data sets, and to the pharmacokinetic study case report form to verify that samples were not misidentified in the assay procedure and correlate dose administered to each chromatogram after analysis was complete.

**Figure 3.** Modeled time concentration plots of curcumin conjugates detected in plasma from 12 human subjects treated with a single 10 g (A) or 12 g (B) dose with confidence interval depicted in gray.



**Statistical Methods and Pharmacokinetic Analysis.** Curcumin and curcumin conjugates were assessed in samples collected up to 72 h after dosing. The conjugate concentration of subject  $i$  at time  $t$ ,  $c_{it}$ , was modeled as a single-compartment pharmacokinetic process, with first-order absorption and elimination:

$$c_{it} = \frac{d_i k_{a_i}}{v_i (k_{a_i} - k_{e_i})} (e^{-k_{e_i}(t-\lambda)} - e^{-k_{a_i}(t-\lambda)}) + e_{it}$$

where  $d_i$  is the dose of subject  $i$ ,  $k_{a_i}$  and  $k_{e_i}$  are the absorption and elimination variables of the  $i^{\text{th}}$  subject,  $v_i$  is the lumped bioavailability/volume variable,  $\lambda$  is the lag time for absorption, and  $e_{it}$  is an additive normal error term with variance  $[\sigma E(c_{it})]^2$ , where  $E$  is the expectation operator. To consider intersubject variation,  $k_{e_i}$  and  $v_i$  were modeled as exponential functions of independent, normally distributed random variables:

$$k_{a_i} = e^{b_{1i}}; b_{1i} \sim N(\beta_1, \sigma_1^2) \\ v_i = e^{b_{2i}}; b_{2i} \sim N(\beta_2, \sigma_2^2)$$

The population means of  $k_{e_i}$ ,  $v_i$ , and  $k_{a_i}$ , respectively, were estimated by  $e^{\beta_1}$ ,  $e^{\beta_2}$ , and  $e^{\beta_3}$ , and the SEs of those population means were calculated by means of the delta method. These primary model variables were estimated using SAS PROC NLINMIXED (SAS Institute), from which estimates of area under the curve (AUC),  $T_{\max}$ ,  $C_{\max}$ , and  $t_{1/2}$  were derived; their SEs and 95% confidence intervals, which describe the precision of the estimates, were calculated by means of the delta method (41). Goodness of fit was evaluated by means of plots of observed versus predicted responses and plots of residuals versus time. Prediction intervals, which describe the distribution of values in the population, were estimated by parametric bootstrap (42). It was observed from the raw data and model variables that the concentration profiles did not linearly increase with dose. The model above was rerun, with  $d_i = 10$  or  $d_i = 10 + \delta$ , for subjects in the 10 and 12 g arms, respectively.

## Results

**Subjects and Adverse Events.** Twelve healthy Caucasian subjects are shown in Table 1 (five males and seven

**Table 1. Description of study subjects**

Dose (g)	Sex	Age (y)	BMI
10	Female	26	22.2
10	Female	40	21.3
10	Female	33	28.8
10	Female	65	27.4
10	Male	18	21.4
10	Male	20	20.8
Mean (range)	4 females 2 males	33.6 (18-65)	24.38 (20.8-28.8)
12	Female	30	23.0
12	Female	19	25.6
12	Female	19	26.8
12	Male	55	21.1
12	Male	51	20.4
12	Male	37	29.4
Mean (range)	3 males 3 females	35.16 (19-55)	23.65 (20.4-29.4)

Abbreviation: BMI, body mass index.

**Table 2. Curcumin conjugate pharmacokinetic variables estimated from the raw data**

	Dose = 10 g (n = 6)		Dose = 12 g (n = 6)	
	Mean $\pm$ SD	(Min, max)	Mean $\pm$ SD	(Min, max)
AUC	39.8 $\pm$ 7.6	(30.8, 47.8)	26.8 $\pm$ 10.9	(8.2, 40.3)
$C_{\max}$	3.2 $\pm$ 0.56	(2.4, 3.8)	2.1 $\pm$ 0.73	(1.3, 3.2)
$t_{1/2}$	10.3 $\pm$ 4.4	(6.0, 18.8)	8.8 $\pm$ 2.7	(5.2, 14.2)
$T_{\max}$	4.33 $\pm$ 3.2	(2, 10)	3.67 $\pm$ 2.0	(2, 6)

NOTE: AUC was determined by the trapezoid rule, and  $t_{1/2}$  by means of linear interpolation. Units for AUC,  $C_{\max}$ ,  $t_{1/2}$  and  $T_{\max}$  are  $\mu\text{g}\cdot\text{h}/\text{mL}$ ,  $\mu\text{g}/\text{mL}$ , b and h, respectively.

females; mean age, 35 years; range, 18-65 years; body mass index, 20-29; recruited from July 2003 to September 2003). Adverse events reported were all grade 1 by National Cancer Institute Common Toxicity Criteria (version 2.0). No adverse events greater than grade 1 were reported. The major adverse events included one subject with headache and one with sore arms hours after dosing, likely unrelated to treatment, and two subjects with yellow, loose stool the day after dosing, most likely due to the lack of curcumin absorption in the gastrointestinal tract. One subject consumed curcumin-containing foods four times weekly but had no detectable curcumin or conjugates at baseline. Interestingly, this subject had the lowest AUC for curcumin glucuronide and curcumin sulfate compared with all other subjects.

**Detection of Curcumin and Conjugate Metabolites in Plasma Samples.** Plasma samples of all subjects were analyzed with and without incubation with deconjugating enzymes using a slightly acidic pH because curcumin is not stable at pH 7.4. Figure 2 shows HPLC chromatograms of plasma with and without hydrolysis of conjugates. Free curcumin was detected in the plasma of only one subject without incubation, 30 min after ingestion of a 10 g dose. No free curcumin was detected in any other plasma samples from any other subject. Subsequent results refer exclusively to curcumin conjugates.

$C_{\max}$ ,  $t_{1/2}$ , and AUC of total curcumin conjugates calculated from the raw data are presented in Table 2. One subject on the 10 g dose level had a  $T_{\max}$  of 10 h, but no curcumin conjugates were detected in this subject before 2 h and after 48 h of drug intake. When the  $T_{\max}$  was within the range of 1 to 4 h, curcumin conjugates were completely eliminated from plasma at the 24-h time point. The concentrations (mean  $\pm$  SD) of curcumin glucuronide and curcumin sulfate at  $T_{\max}$  at the 10 g dose level were  $2.04 \pm 0.31 \mu\text{g}/\text{mL}$  (range, 1.57-2.39  $\mu\text{g}/\text{mL}$ ) and  $1.06 \pm 0.40 \mu\text{g}/\text{mL}$  (range, 0.44-1.62  $\mu\text{g}/\text{mL}$ ), respectively, and at the 12 g dose level were  $1.40 \pm 0.74 \mu\text{g}/\text{mL}$  (range, 0.61-2.56  $\mu\text{g}/\text{mL}$ ) and  $0.87 \pm 0.44 \mu\text{g}/\text{mL}$  (range, 0.41-1.65  $\mu\text{g}/\text{mL}$ ), respectively (Table 3).

The kinetic variables of the total curcumin conjugates from the biexponential model are shown in Table 4. A typical subject has an absorption rate constant ( $k_a$ ) of  $1.05 \pm 0.26 \text{ h}^{-1}$  (mean  $\pm$  SE), elimination rate constant ( $k$ ) of  $0.08 \pm 0.005 \text{ h}^{-1}$  (mean  $\pm$  SE), and half-life ( $t_{1/2}$ ) of  $6.77 \pm 0.83 \text{ h}$  (mean  $\pm$  SE). Based on the pharmacokinetic model, AUC (mean  $\pm$  SE) for the 10 and 12 g doses was  $35.33 \pm 3.78$  and  $26.57 \pm 2.97 \mu\text{g}/\text{mL} \times \text{h}$ , respectively. The  $C_{\max}$  (mean  $\pm$  SE) for the 10 and 12 doses was  $2.30 \pm 0.26$  and  $1.73 \pm 0.19 \mu\text{g}/\text{mL}$ , respectively. Note



**Table 3. The fraction of curcumin glucuronide and curcumin sulfate in plasma at  $C_{max}$  after administration of 10 and 12 g curcumin based on enzymatic hydrolysis done in duplicate**

Dose (g)	Curcumin glucuronide ( $\mu\text{g}/\text{mL}$ )	Curcumin sulfate ( $\mu\text{g}/\text{mL}$ )	Total curcumin	
			Separate assay ( $\mu\text{g}/\text{mL}$ )	Mixed assay ( $\mu\text{g}/\text{mL}$ )
10	$2.04 \pm 0.31^*$	$1.06 \pm 0.40$	$3.10 \pm 0.60$	$3.09 \pm 0.53$
12	$1.40 \pm 0.74$	$0.87 \pm 0.44$	$2.27 \pm 1.17$	$2.03 \pm 0.74$

NOTE: Total curcumin in plasma was assayed after incubating plasma with enzymes  $\beta$ -glucuronidase and sulfatase separately (separate assay) and combined (mixed assay).

\*Mean  $\pm$  SD of six subjects.

that the modeled  $C_{max}$  and AUC are, like the direct estimates from the raw concentrations in Table 2, greater for the 10 g than for the 12 g subjects. This difference was not due to a single outlier.

Table 4 also presents estimates of the variation in AUC,  $C_{max}$ ,  $T_{max}$ , and  $t_{1/2}$  in the subjects. After a 10 g dose, 95% of the population would be expected to show  $C_{max}$  between 0.71 and 7.04  $\mu\text{g}/\text{mL}$ , with half-life between 3.19 and 14.46 h.

The subjects in the 10 g dose group were similar in body mass index or mean age compared with the 12 g dose group (Table 1). Pharmacokinetic variables were analyzed by sex, age, and body mass index but were not found to differ significantly based on these variables; however, this study was not powered to detect such differences.

The amount of glucuronide conjugate assessed at each participant's observed  $T_{max}$  ranged between 20% and 67% higher than the concentration of sulfate conjugate (Table 3). Total curcumin released after incubating plasma with  $\beta$ -glucuronidase and sulfatase enzymes separately was almost equal to the curcumin released after incubating plasma with  $\beta$ -glucuronidase and sulfatase enzymes together (Table 3). These results indicate that most of the curcumin conjugates are either glucuronides or sulfates and not mixed conjugates.

## Discussion

Prior clinical reports at lower doses have suggested that orally administered curcumin is poorly bioavailable. Gram doses were required to detect curcumin in blood, using 90% to 99% pure curcumin preparations, and the

highest dose tested, 8 g, resulted in  $<1 \mu\text{g}/\text{mL}$  curcumin (37, 38, 43). A dose-escalation study detected free curcumin concentrations of 0.03 to 0.06  $\mu\text{g}/\text{mL}$  1 to 4 h after a 10 or 12 g oral dose of curcuminoids in only one of three subjects at each dose level (39). In the present single-dose pharmacokinetic study, we also failed to detect free curcumin in all but one subject using the Sabinsa curcuminoid ( $C^3$ ) mixture that was 75% curcumin. Repetitive daily administration of curcumin may cause accumulation of curcumin at the gastrointestinal mucosal interface, resulting in more free curcumin delivered to systemic sites after multiple dosing, compared with a single dose (37, 38, 43). Alternatively, at the extremely high doses we used, curcumin transport may be saturated at the gut epithelium, whereas conjugation is not. Curcumin absorption may not be limited. Rather, it may induce its own biotransformation (both conjugation and CYP reduction enzymes) in the gut epithelium or in the liver. In this scenario, one should detect proportionately higher conjugate concentrations at higher doses unless the excess conjugates are eliminated via the gut.

Data to date do not support the concept that curcumin serves as a prodrug (34, 44, 45). For example, data suggest that tetrahydrocurcumin, a major reduction metabolite, is a less potent inhibitor of nuclear factor- $\kappa$ B, cyclooxygenase-1, 5-lipoxygenase, and proliferation at equivalent concentrations as curcumin (45, 46). The potential biological effect of the high concentrations and long systemic exposure to conjugates remains unclear. Nevertheless, our findings that the majority of curcumin is biotransformed before measurement at the systemic (peripheral venous) site are consistent with the very low

**Table 4. Pharmacokinetic variables derived from pharmacokinetic model of curcumin conjugates**

	Mean $\pm$ SE	95% confidence interval	95% prediction interval
AUC ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ), 10 g dose	$35.33 \pm 3.78$	26.90-43.75	10.62-117.01
AUC ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ), 12 g dose	$26.57 \pm 2.97$	19.96-33.18	7.86-89.46
$C_{max}$ ( $\mu\text{g}/\text{mL}$ ), 10 g dose	$2.30 \pm 0.26$	1.72-2.88	0.71-7.04
$C_{max}$ ( $\mu\text{g}/\text{mL}$ ), 12 g dose	$1.73 \pm 0.19$	1.30-2.16	0.52-5.41
$T_{max}$ (h)	$3.29 \pm 0.43$	2.34-4.24	1.56-7.37
$t_{1/2}$ (h)	$6.77 \pm 0.83$	4.82-8.71	3.19-14.46
$k_a$ ( $\text{h}^{-1}$ )	$1.05 \pm 0.26$	0.47-1.64	NA
$k_e$ ( $\text{h}^{-1}$ )	$0.08 \pm 0.0050$	0.069-0.091	NA
$v$	$3.54 \pm 0.41$	2.63-4.46	NA
Lag (h)	$0.68 \pm 0.016$	0.64-0.71	NA
Dose (g)	$-2.48 \pm 0.98$	-4.66 to -0.30	NA

NOTE: The mean  $\pm$  SE of the estimate, 95% confidence intervals for the variable estimates, and 95% prediction intervals for the population are presented. Abbreviations:  $C_{max}$ , peak concentration;  $T_{max}$ , time of peak concentration;  $t_{1/2}$ , half-life;  $k_a$ , absorption constant;  $k_e$ , excretion constant;  $v$ , bioavailability/volume; Lag, absorption lag; NA, not available.

concentrations of free curcumin detected by both Cheng et al. (37) and Sharma et al. (38).

In this present study, a single high dose of curcumin resulted in rapid appearance of curcumin conjugates in human plasma with a lag of 0.68 h after dose with  $T_{max}$  of ~4 h as shown in Table 4. In the rat, curcumin glucuronide and curcumin sulfate were the predominant metabolites (35, 36). In a previously published human study, using 4 months of oral dosing of 3.6 g of curcumin daily, both glucuronide and sulfate conjugates were detected in plasma but at very low levels (3–6 ng/mL) similar to that of free curcumin (34). Curcumin conjugates in our study were present at levels that were ~1,000-fold higher (1–3 µg/mL) when extracting curcumin after hydrolysis of conjugates. The present study suggests that curcumin was rapidly biotransformed at anatomic sites, potentially gastric mucosa, liver, or both, before peripheral venous circulation.

Surprisingly, the  $C_{max}$  and AUC of curcumin conjugates in the 10 g arm were significantly higher than in the 12 g arm ( $P < 0.01$ ; Table 4). Our review did not find bias in the sample handling, processing, or analytic procedures.

This outcome could be explained by saturation of a transport mechanism, resulting in no incremental curcumin absorption at higher doses, induction of metabolism at higher doses, and other mechanisms discussed earlier. Because the subjects took large numbers of capsules (40 capsules for the 10 g dose, 48 capsules for the 12 g dose), variation in the time required to swallow all of these capsules might have affected the absorption. The potentially longer time required to swallow the 48 capsule dose may have resulted in a lower  $C_{max}$  and lower absorption compared with the 40 capsule dose.

In this trial, no serious adverse events were reported after curcumin dosing. All adverse events reported were grade 1. Although the small size of this trial precludes any formal safety end point analysis and statistical certainty of safety, the safety profile observed here is consistent with previous clinical and preclinical data (7, 37–39).

A high rate of curcumin conjugation, through glucuronidation and sulfation, may explain low concentrations of free curcumin when administered orally in previous studies (34, 37, 38). The potential activation of curcumin conjugates by intracellular glucuronidases or sulfatases should be explored further. Ireson et al. (34) reported that curcumin metabolites, specifically hexahydrocurcumin, tetrahydrocurcumin, and curcumin sulfate, had very low activity for inhibition of phorbol ester-induced prostaglandin  $E_2$  production in human colonic epithelial cells compared with the parent compound. If, however, conjugates are deconjugated to free curcumin, as occurs in inflammation (47, 48), one can speculate that the curcumin conjugate concentrations detected here are sufficient to modulate key curcumin-associated intracellular targets. *In vitro*, concentrations in the range of 3.6 to 36 µg/mL of curcumin were necessary to modulate cellular targets (e.g., cyclooxygenase-2) in human colonic cells (30, 34). The concentrations of curcumin conjugates observed in human plasma after consumption of 10 or 12 g single doses described here resulted in plasma concentrations sufficient to elicit pharmacologic activity *in vitro*, if deconjugated. Alternatively, curcumin con-

jugates have recently been shown to have interesting biological properties. Conjugation, therefore, may not necessarily be an inactivation pathway (49, 50). Preliminary data in rodents have shown modulation of carcinogenesis targets at systemic sites after curcumin administration (10, 34, 51).

The results of this study permit the following two conclusions to be drawn about oral curcumin in humans: (a) consumption of 10 or 12 g curcumin single doses generates mainly curcumin glucuronide and curcumin sulfate and (b) glucuronide conjugate predominates over sulfate conjugate in plasma with no evidence of a mixed conjugate being formed. These results indicate that curcumin is absorbed after oral administration and could be transported to sites other than the intestinal tract.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Acknowledgments

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We thank the volunteers who participated in this study and Bill C. Frame for his contributions to the pharmacokinetic analysis.

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*Cancer Epidemiol Biomarkers Prev* 2008;17:1411-1417.

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