

## Comparative Absorption of a Standardized Curcuminoid Mixture and Its Lecithin Formulation

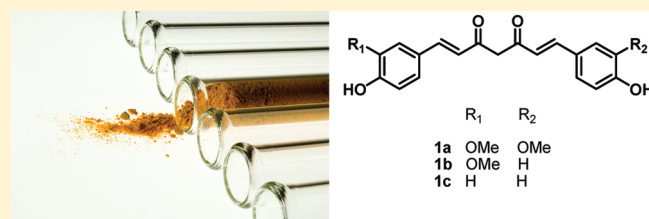
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**ABSTRACT:** The relative absorption of a standardized curcuminoid mixture and its corresponding lecithin formulation (Meriva) was investigated in a randomized, double-blind, cross-over human study. Clinically validated dosages were used for both products, and plasma levels of all three major curcuminoids [curcumin (**1a**), demethoxycurcumin (**1b**), and bisdemethoxycurcumin (**1c**)] were evaluated. Total curcuminoid absorption was about 29-fold higher for Meriva than for its corresponding unformulated curcuminoid mixture, but only phase-2 metabolites could be detected, and plasma concentrations were still significantly lower than those required for the inhibition of most anti-inflammatory targets of curcumin. Remarkably, phospholipid formulation increased the absorption of demethoxylated curcuminoids much more than that of curcumin (**1a**), with significant differences in plasma curcuminoid profile between Meriva and its corresponding unformulated curcuminoid mixture. Thus, the major plasma curcuminoid after administration of Meriva was not curcumin (**1a**), but demethoxycurcumin (**1b**), a more potent analogue in many *in vitro* anti-inflammatory assays. The improved absorption, and possibly also a better plasma curcuminoid profile, might underlie the clinical efficacy of Meriva at doses significantly lower than unformulated curcuminoid mixtures.



Over the past few decades, preclinical and clinical evidence has been accumulating that the pathological mechanisms involved in chronic diseases are multifactorial and that these conditions are therefore better addressed with a multitargeted rather than a monotargeted therapy.<sup>1–3</sup> The promiscuous targeting of multiple cellular end-points has therefore become a therapeutic virtue,<sup>4</sup> and agents that can modulate multiple cellular targets, once dismissed as unselective ligands, are now considered attractive research leads.<sup>4</sup> In this context, no compound better than curcumin (**1a**) exemplifies the biomedical relevance of promiscuous agents.<sup>5</sup> With over 100 molecular targets identified and almost 3000 preclinical investigations,<sup>6</sup> this compound is, undoubtedly, one of the best investigated natural products to date. Curcumin features a unique blend of Michael acceptor, metal chelating, and antioxidant properties that has so far substantially eluded all attempts of dissection.<sup>7</sup> The promiscuous binding properties of curcumin make it difficult to explore the chemical space around the pharmacophore of the natural product, since analogues would have to be assayed against multiple rather than single molecular end-points. Therefore, a large share of the medicinal chemistry research on curcumin has focused on improving the dismally low oral absorption of the natural product by suitable formulation.<sup>8</sup> Curcumin has a high hydrolytic instability at physiological pH<sup>9</sup> and an inherently low intestinal absorption.<sup>8</sup> It is therefore very

poorly bioavailable, with only conjugates being generally detectable in plasma even after dosages as high as 12 g/day.<sup>8,10</sup>

Two major strategies have been pursued to improve the bioavailability of curcumin.<sup>8</sup> The first is a combination with adjuvants capable of increasing the absorption of curcumin, like piperine, quercetin, or turmeric oil.<sup>9</sup> The curcumin–piperine formulation is the best documented and has been shown to almost double the human bioavailability of curcumin.<sup>11</sup> However, it has been argued that this improvement comes at the expense of an increased potential of interaction with mainstream drugs,<sup>12</sup> since piperine is an inhibitor of phase-1 and phase-2 xenobiotic metabolizing enzymes,<sup>13</sup> and curcumin has also been shown to inhibit various classes of cytochromes (including CPYP3A4) as well as P-glycoprotein activity.<sup>12,14</sup> The second strategy has been the inclusion of curcumin in a lipophilic matrix (liposomes, Phytosomes, and lipid micro- and nanoparticles) or encapsulation with micellar surfactants or casein.<sup>8</sup> Many of these formulations have led to dramatic improvement in the absorption of curcumin in animal experiments,<sup>8</sup> but little human data exist.

Commercially available natural curcumin is a mixture of three curcuminoids: curcumin (**1a**, ca. 70–75%), demethoxycurcumin (**1b**, ca. 15–20%), and bisdemethoxycurcumin (**1c**, ca. 5–10%). Most published literature on curcumin does not make a clear

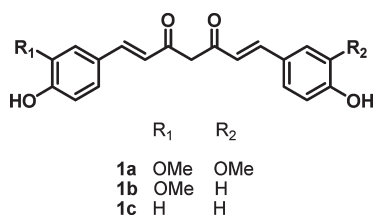
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Table 1. Curcuminoid Content of the Study Capsules

curcuminoid	Meriva (mg per capsule)	reference (mg per capsule)	high dose (mg per dose)	low dose (mg per dose)	reference (mg per dose)
curcumin (1a)	33	259	297	165	1295
demethoxycurcumin (1b)	8	79	68	38	396
bisdemethoxycurcumin (1c)	1	22	11	6	108
total curcuminoids	42	360	376	209	1799

distinction between pure **1a** (monomolecular curcumin, generally obtained by synthesis) and the mixture of the three curcuminoids obtained by extraction from turmeric. In this study, two formulations of a mixture of natural curcuminoids, a noncomplexed powder and a phospholipid formulation (Meriva), were compared. Both materials had a similar ratio of all three natural curcuminoids (see below).<sup>15</sup> Curcumin is sparingly soluble in both water and lipophilic organic solvents, but has polar groups that make it a good candidate for phospholipid complexation via hydrogen bonding and dipole interactions with the polar head groups of phosphatidylcholine.<sup>16</sup> As a result, phospholipid particles bearing curcumin at their surface can be formed,<sup>17</sup> and curcumin has indeed been shown to strongly bind to phospholipid micelles, positioning the water-labile  $\beta$ -diketone moiety into the lipid bilayer and shielding it from hydrolytic retro-Claisen fragmentation, the major mechanism of degradation in water.<sup>16</sup> Based on this previous research, a novel curcumin formulation was developed under the brand name Meriva by combining a standardized mixture of natural curcuminoids and lecithin in a 1:2 ratio, with 2 parts of microcrystalline cellulose being also added to improve the physical state.<sup>18</sup> A comparative analysis of the hydrolytic stability of Meriva and noncomplexed curcumin showed a dramatic increase in stability in the pH range of the small intestine (pH 7–8), the major site of absorption for polyphenolic compounds (Giori, A.; Franceschi, F.; Appendino, G. Unpublished data). Apart from hydrolytic stabilization, phospholipid formulation could also increase the absorption of curcumin in a direct way, since phospholipids can be rapidly taken up into biomembranes by pinocytosis, shuttling their guest to cells in this process.<sup>17</sup> These results were verified by an animal study, which showed a >20-fold increase in absorption of curcumin from Meriva as compared to unformulated natural curcumin.<sup>19</sup> As an extension of these studies, we have now compared the relative human absorption of a standardized, natural curcuminoid mixture with two doses of the corresponding phospholipid formulation Meriva.



## RESULTS AND DISCUSSION

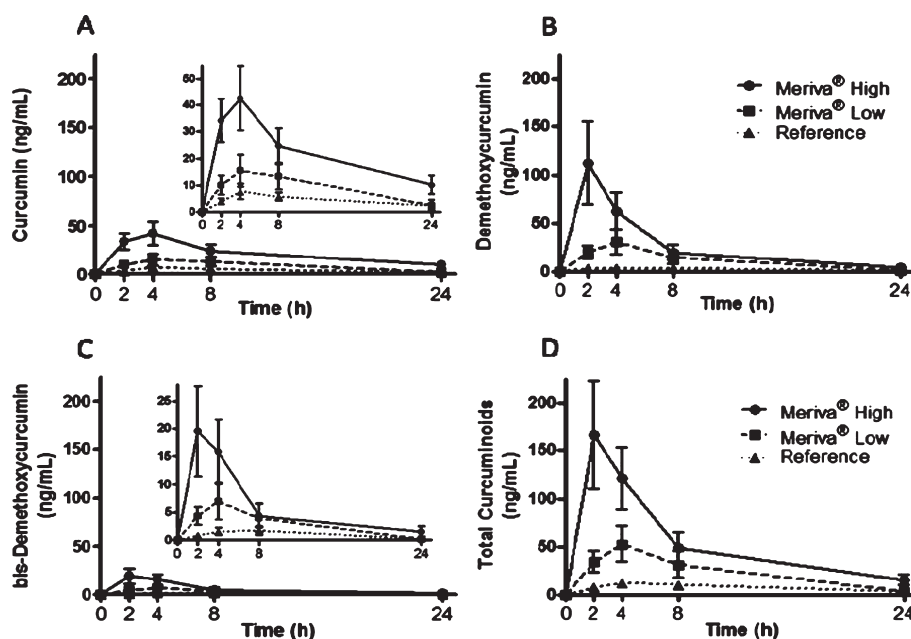
A randomized, double-blind, crossover study was carried out in nine volunteers, measuring the plasma concentrations of three curcuminoids [curcumin (**1a**), demethoxycurcumin (**1b**), and bisdemethoxycurcumin (**1c**)] after supplementation with two dosages of Meriva and one dosage of the same batch of

curcuminoid mixture used for the formulation with lecithin. The dosages were inspired by previous clinical studies for inflammatory conditions, where active dosages of around 1–2 g/day of nonformulated curcuminoid mixtures<sup>20</sup> and around 200–300 mg of curcuminoids as Meriva were used,<sup>1</sup> while the choice of the first time point was done in accordance to previous data that showed  $t_{max}$  values for curcumin around 4 h.<sup>10</sup> For this study, subjects consumed, in random order and on separate study days, five (low-dose) or nine (high-dose) capsules of Meriva, corresponding to 209 and 376 mg total curcuminoids, or, alternatively, five capsules of the corresponding nonformulated curcuminoid mixture (reference) containing 1799 mg of total curcuminoids. The composition of the two types of capsules is listed in Table 1.

Free curcumin (**1a**) could not be detected in any plasma samples, in accordance with previous studies that have mostly failed to detect unconjugated curcumin in human plasma even after the administration of megadoses of curcumin.<sup>8</sup> Consequently, all plasma samples were treated with *Helix pomatia* glucuronidase/sulfatase before HPLC-MS/MS analysis. The peak plasma total curcuminoid concentration ( $c_{max}$ ) reached with the high dosage of Meriva was  $206.9 \pm 164.7$  ng/mL, and the corresponding time of the peak plasma curcuminoid concentration ( $t_{max}$ ) was reached at  $2.7 \pm 1$  h after the administration. For the low dosage,  $c_{max}$  was  $68.9 \pm 50.8$  ng/mL and  $t_{max}$  was  $3.3 \pm 1$  h. For the reference curcuminoid mixture, these values were  $14.4 \pm 12.5$  ng/mL and  $6.9 \pm 6.7$  h. Within the context of curcumin human absorption, the >200 ng/mL concentration of conjugated curcuminoids is still lower than the low micromolar concentration of free curcumin required for in vitro direct activity against its various targets.<sup>7</sup>

Pharmacokinetic data for each administration were graphed, and statistical analysis was performed using GraphPad Prism Version 5.04 software (GraphPad Software, San Diego, CA). The average of each group's plasma analyte concentrations were plotted against time (Figure 1). The area under the curve (AUC), the  $c_{max}$  and the  $t_{max}$  were calculated for each curcuminoid and for the total curcuminoid mixture and are presented in Table 2. The AUC was calculated using the trapezoidal method from time 0 to 24 h, and the values were normalized to curcuminoid intake by dividing the observed AUC by the corresponding curcuminoid dosage of each administration. The resulting normalized AUCs, expressed in ng/mL(plasma)  $\times$  h/mg ingested, were divided by the AUC of the reference to calculate the relative absorption values (Table 2) (for statistical details, see the Experimental Section).

From these data, the average absorption of curcumin (**1a**) was calculated to be ca. 18-fold higher from Meriva than from the corresponding unformulated mixture of curcuminoids. Moreover, the overall curcuminoid absorption was about 29-fold higher for the Phytosome formulation compared to the unformulated reference, since the plasma concentrations of demethoxycurcumin (**1b**) and bisdemethoxycurcumin (**1c**) from intake of



**Figure 1.** Pharmacokinetic data for curcumin (**1a**), demethoxycurcumin (**1b**), bisdemethoxycurcumin (**1c**), and total curcuminoids for each dosage. Concentrations are expressed in ng/mL and refer to enzymatically hydrolyzed plasma samples. Circles (●) represent high dosages of Meriva; squares (■) represent low-dose Meriva; and triangles (▲) represent the reference material. Insets (A and C) show an expanded view of the original data. The data shown are baseline subtracted means  $\pm$  SEM.

**Table 2.** Area Under the Curve (AUC),  $c_{max}$ ,  $t_{max}$  and Relative Absorption for Each Treatment of Curcuminoids<sup>a</sup>

curcuminoid	formulation	AUC (ng/mL)	$c_{max}$ (ng/mL)	$t_{max}$ (h)	relative absorption <sup>b</sup>
curcumin ( <b>1a</b> )	Meriva high	538.0 $\pm$ 130.7	50.3 $\pm$ 12.7	3.8 $\pm$ 0.6	19.2 <sup>c</sup>
	Meriva low	272.6 $\pm$ 68.52	24.2 $\pm$ 5.9	4.2 $\pm$ 0.8	17.5 <sup>c</sup>
	reference	122.5 $\pm$ 29.3	9.0 $\pm$ 2.8	6.9 $\pm$ 2.2	1
demethoxycurcumin ( <b>1b</b> )	Meriva high	655.0 $\pm$ 195.7	134.6 $\pm$ 40.6	2.4 $\pm$ 0.3	68.3 <sup>d</sup>
	Meriva low	297.4 $\pm$ 107.3	39.1 $\pm$ 11.4	3.1 $\pm$ 0.4	55.5 <sup>d</sup>
	reference	55.8 $\pm$ 15.5	4.2 $\pm$ 1.1	4.4 $\pm$ 1.0	1
bisdemethoxycurcumin ( <b>1c</b> )	Meriva high	142.2 $\pm$ 58.2	24.9 $\pm$ 8.1	2.2 $\pm$ 0.4	56.8 <sup>e</sup>
	Meriva low	70.1 $\pm$ 34.3	8.8 $\pm$ 3.1	2.4 $\pm$ 0.6	51.3 <sup>e</sup>
	reference	24.6 $\pm$ 10.3	2.1 $\pm$ 0.8	3.4 $\pm$ 1.2	1
total curcuminoids	Meriva high	1336.0 $\pm$ 357.1	206.9 $\pm$ 54.9	2.7 $\pm$ 0.3	31.5 <sup>f</sup>
	Meriva low	640.2 $\pm$ 197.7	68.9 $\pm$ 16.9	3.3 $\pm$ 0.3	27.2 <sup>f</sup>
	reference	202.8 $\pm$ 53.8	14.4 $\pm$ 4.2	6.9 $\pm$ 2.2	1

<sup>a</sup> Actual results not baseline subtracted, and errors are SEM. <sup>b</sup> AUC normalized. <sup>c</sup> Average: 18.3. <sup>d</sup> Average: 61.9. <sup>e</sup> Average: 54.1. <sup>f</sup> Average: 29.4.

Meriva were 50- to 60-fold higher than from the corresponding unformulated curcuminoid mixture. Remarkably, the major plasma curcuminoid was demethoxycurcumin (**1b**) and not curcumin (**1a**) with both dosages of Meriva investigated. The marked differences in the plasma curcuminoid profile could not be accounted for by the nature of the starting materials, since Meriva capsules and the noncomplexed curcumin capsules contained very similar curcuminoid profiles. Thus, the ratio **1a/1b/1c** was 1/0.25/0.04 in the capsules (Table 1), but 1/0.50/0.20 in the plasma samples from the reference curcuminoid mixture, and 1/1.20/0.26 in the plasma from the administration of Meriva. These data show that demethoxylated forms of curcumin (**1b** and **1c**) have a better intrinsic absorption than curcumin (**1a**) and that formulation with phospholipids increases these differences in bioavailability.<sup>22</sup> Interestingly,

turmeric is often used in cuisine associated with lecithin-rich ingredients like eggs or vegetable oils, and these observations might well hold also for the dietary intake of curcuminoids.

The reasons for this unexpected increase in the plasma concentrations of the demethoxylated curcuminoids **1b** and **1c** over curcumin (**1a**) are unknown. It is, in principle, possible that a reductive microbial metabolism of curcumin (**1a**) might be involved, in a process not unlike the one that generates enterolactone and enterodiols from flax lignans.<sup>23</sup> The hydrolytic stabilization of curcumin at intestinal pH might, in fact, translate into a significant curcumin load for the gut microflora, known to be able to reductively demethoxylate dietary phenolics.<sup>24</sup> The possibility that **1b** and **1c** are generated from curcumin (**1a**) by liver metabolism seems unlikely, since dietary phenolics are generally oxidatively *O*-demethylated rather than reductively

*C-demethoxylated* by liver enzymes,<sup>25</sup> and phase-2 metabolism to conjugates is generally the primary metabolic pathway for dietary phenolics.<sup>25</sup> The unique plasma curcuminoid profile might play a role in the clinical efficacy of Meriva at dosages much lower than those of unformulated curcumin, since demethoxycurcumin is more potent than curcumin in many molecular assays of anti-inflammatory activity.<sup>26</sup> The presence of demethoxylated curcuminoids in most “curcumin” samples has, surprisingly, been largely overlooked, and the fragmentary state of our knowledge on the *in vivo* biological profile of these compounds makes it difficult to evaluate the clinical meaning of differences in the plasma curcuminoid profile. However, based on *in vitro* studies, a better anti-inflammatory curcuminoid profile seems possible for Meriva compared to unformulated curcuminoid mixtures. This would certainly be worthy of further evaluation, and our data add to the growing body of information suggesting that curcuminoids have different biological profiles, drawing attention to their different bioavailability and to the need to specify the composition of “curcumin” whenever this compound is used in both cellular and clinical studies.

Curcumin has been a sort of “forbidden fruit” for biomedical research,<sup>5</sup> since its poor oral bioavailability has substantially hampered clinical development, despite the very promising indications of the preclinical research.<sup>6</sup> We have demonstrated that formulation with phospholipids<sup>27</sup> improves the human absorption of curcumin, without, however, leading to pharmacologically active plasma concentrations and with only phase-2 metabolites being detectable.<sup>23</sup> While phase-2 metabolites might play a role *in vivo*, either as pro-drugs or as targeting agents,<sup>28</sup> the failure to reach pharmacologically active plasma curcuminoid concentrations even with clinically validated dosages of Meriva<sup>21</sup> raises the issue of how to evaluate effective dosages of multi-targeted agents whose action *in vivo* might be the result of the combinatorial binding to several protein targets and/or the epigenetic modulation of their expression.<sup>29</sup>

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Pure curcuminoid standards (>98% purity by HPLC) were used (Chromadex, Irvine, CA). A 95% soybean-based phospholipid–curcumin formulation (Meriva) was provided by Indena (Milan, Italy). Capsules of nonformulated 95% curcuminoid mixture prepared from the same batch used for the lecithin formulation were provided by USANA Health Science, Inc.  $\beta$ -Glucuronidase/sulfatase (EC 3.2.1.31) from *Helix pomatia* was purchased from Sigma (St. Louis, MO).<sup>30</sup> HPLC grade 2-propanol, formic acid, ethyl acetate, and other chemicals used in the buffer system were purchased from Pharmco-AAPER (Brookfield, CT). HPLC-MS/MS: Agilent HPLC system (1100 series, USA) and Agilent 6410 tandem mass spectrometer with (+) ESI, equipped with an Agilent C<sub>18</sub> analytical column (75 mm × 4.6 mm).

**Chromatographic Analysis of Curcuminoids.** The HPLC-MS/MS procedure used was adapted from Liu et al.<sup>31</sup> Concentrated stock solutions of curcumin (1a), demethoxycurcumin (1b), and bisdemethoxycurcumin (1c) were prepared by dissolving 5.0 mg of each compound in 200 mL of methanol to give 25  $\mu$ g/mL stock solutions. Three standard solutions were prepared by combining the stock solutions and diluting with methanol to yield final concentrations of 0.5, 25, and 50 ng/50  $\mu$ L. A calibration curve was prepared for each subject by spiking blank plasma from the baseline blood draw with 50  $\mu$ L of the appropriate working solution to yield concentrations of 5, 250, and 500 ng/mL. These calibration standards were run multiple times between samples, resulting in 18 “three-point” calibration curves that

**Table 3. Statistical Analysis [Friedman test results; number of treatment groups = 3 (low, high, reference) for all groups]**

group	Friedman statistic	p value
AUC curcumin (1a)	16.22	<0.0001
$c_{\max}$ curcumin (1a)	16.22	<0.0001
$t_{\max}$ curcumin (1a)	2.111	0.03285
AUC demethoxycurcumin (1b)	18	<0.0001
$c_{\max}$ demethoxycurcumin (1b)	18	<0.0001
AUC bisdemethoxycurcumin (1c)	12.97	0.0003
$c_{\max}$ bisdemethoxycurcumin (1c)	13.61	0.0002
AUC total curcuminoids	16.22	<0.0001
$t_{\max}$ total curcuminoids	18	<0.0001

also served as a system suitability check. Plasma spiking was used to create the calibration curve instead of changing the injection volume in order to compensate for matrix effects. The analysis was carried out using 2-propanol/0.03% formic acid (35:65, v/v) as mobile phase, with an injection volume of 10  $\mu$ L, a run time of 15 min, and a flow rate of 1.0 mL/min. Autosample carryover was determined by injecting the most concentrated calibration standard followed by a blank sample. No carryover was observed, as indicated by an inability to detect curcumin peaks in the blank sample. The sensitivity of the multiple reactions monitoring (MRM) was optimized by testing with an infusion of 100 ng/mL curcuminoid solution. The mass spectrometer was operated under MRM mode with a collision energy of 10 eV. The transitions monitored were  $m/z$  369.2  $\rightarrow$  285.2 for curcumin (1a), 339.2  $\rightarrow$  255.0 for demethoxycurcumin (1b), and 309.1  $\rightarrow$  225.0 for bisdemethoxycurcumin (1c). The limit of detection was calculated from the signal-to-noise ratio to be 0.5 ng/mL, an order of magnitude below the lowest standard concentration used.

**Product Administration.** The three dosages were administered with 8 fluid oz. of water in two-piece hard-shell capsules to eight healthy male subjects and one female subject, age 35  $\pm$  10 years. On each study day, subjects were required to complete an overnight fast and donate a baseline blood sample of both serum and plasma. Next, they received one oral dosage of one of the three randomized treatments. Subjects then ate a standardized breakfast consisting of one plain bagel (99 g) with cream cheese (25 g) followed by venous blood draws at 2, 4, 8, and 24 h postadministration. After the 4 h blood draw, the volunteers were fed a standardized lunch consisting of one plain bagel (99 g) with cream cheese (25 g). Subjects were released after the 8 h blood draw and were allowed to resume their normal dietary intake but restricting foods known to contain curcumin. After fasting overnight, volunteers returned the next morning for the 24 h blood draw. After a seven-day washout period, each subject returned to the clinical site to receive the next randomly assigned treatment. Each laboratory visit was identical to the first and repeated until all three treatments were administered.

**Sample Preparation.** A 0.2 mL aliquot of plasma was transferred to a clean microcentrifuge tube and next treated with 100  $\mu$ L of a solution containing 1000 U of  $\beta$ -glucuronidase in 0.1 M phosphate buffer (pH 6.86) and 50  $\mu$ L of methanol. The resulting mixture was then thoroughly vortexed and incubated at 37 °C for 1 h to hydrolyze the phase-2 conjugates of curcuminoids. After incubation, curcuminoids were extracted with 1 mL of ethyl acetate, and the mixture was vortexed for 1 min, followed by sonication in a water bath for 15 min. After centrifugation at 15000g for 6 min, the upper organic layer was transferred to a 2 mL microcentrifuge tube and evaporated to dryness at 30 °C under negative pressure in a centrifugal concentrator. This process was repeated for a total of two extractions. The dried extract was reconstituted in 100  $\mu$ L of methanol, and 10  $\mu$ L was injected into the HPLC-MS/MS.

**Analysis of Capsules Used in the Study.** The curcuminoid concentration of both the reference and the Meriva capsule was determined by breaking open each capsule and dissolving 5 mg of the powder obtained in 250 mL of methanol. The solution was shaken for 20 min, and 1  $\mu$ L injected into the HPLC-MS/MS, using the conditions described above.

**Statistical Analysis.** For comparison of treatment effects, one-way repeated measures analysis of variance by ranks (Friedman test) with a Dunn's multiple comparison post hoc test was used. Results were considered significant if the *p* value was  $\leq 0.05$  relative to the reference material (see Table 3 for details). Statistical analysis was performed using GraphPad Prism Version 5.04 software (GraphPad Software, San Diego, CA).

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## REFERENCES

- Keith, C. T.; Borisy, A. A.; Stockwell, B. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 71–78.
- Sams-Dodd, F. *Drug Discovery Today* **2005**, *10*, 139–147.
- Morphy, R.; Kay, C.; Rankovic, Z. *Drug Discovery Today* **2004**, *9*, 641–651.
- Mencher, S. K.; Wang, L. G. *BMC Clin. Pharmacol.* **2005**, *5*, 3.
- Goel, A.; Jhurani, S.; Aggarwal, B. B. *Mol. Nutr. Food Res.* **2008**, *52*, 1010–1030.
- (a) Epstein, J.; Sanderson, I. R.; Macdonald, T. T. *Br. J. Nutr.* **2010**, *103*, 1545–1557. (b) Aggarwal, B. B.; Sung, B. *Trends Pharmacol. Sci.* **2009**, *30*, 85–94.
- Anand, P.; Thomas, S. G.; Kunnumakkara, A. B.; Sundaram, C.; Harikumar, K. B.; Sung, B.; Tharakan, S. T.; Priyadarsini, I. K.; Rajasekharan, K. N.; Aggarwal, B. B. *Biochem. Pharmacol.* **2008**, *76*, 1590–1661.
- (a) Bisht, S.; Maitra, A. *Curr. Drug Discovery Technol.* **2009**, 192–199. (b) Anand, P.; Kunnumakkara, A. B.; Newman, R. A.; Aggarwal, B. B. *Mol. Pharmaceutics* **2007**, *4*, 807–818. (c) Lao, C. D.; Ruffin, M. T.; Normolle, D.; Heath, D. D.; Murray, S. I.; Bailey, J. M.; Boggs, M. E.; Crowell, J.; Rock, C. L.; Brenner, D. E. *BMC Complement. Altern. Med.* **2006**, *6*, 10.
- Wang, Y. J.; Pan, M.-H.; Cheng, A.-L.; Lin, L.-I.; Ho, Y.-S.; Hsieh, C.-Y.; Lin, J.-K. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867–1876.
- Vareed, S. K.; Kakarala, M.; Ruffin, M. T.; Crowell, J. A.; Normolle, D. P.; Djuric, Z.; Brenner, D. E. *Cancer Epidemiol. Biomarkers Prev.* **2008**, *17*, 1411–1417. In this study, only one of the six participants who received a 12 g/day dosage of curcuminoid mixture had plasma levels of free curcumin that reached the detection threshold (50 ng/mL). In all the other volunteers and in the six participants who received a dosage of 10 g curcumin/day, only conjugates were detected. In a recent study, none of the five cancer patients who received a daily dosage of 8 g curcumin had detectable plasma concentrations of unconjugated curcumin. Kanai, M.; Yoshimura, K.; Asada, M.; Imaizumi, A.; Suzuki, C.; Matsumoto, S.; Nishimura, T.; Mori, Y.; Masui, T.; Kawaguchi, Y.; Yanagihara, K.; Yazumi, S.; Chiba, T.; Aggarwal, B. B. *Cancer Chemother. Pharmacol.* **2010** in press.
- See: <http://www.bioperine.com/curcumin.html>.
- (a) Burgos-Morón, E.; Calderón-Montaño, J. M.; Robles, A.; López-Lázaro, M. *Int. J. Cancer* **2010**, *126*, 1771–1775. (b) Mancuso, C.; Barone, E. *Trends Pharmacol. Sci.* **2009**, *30*, 333–334.
- (a) Bhardwaj, R. K.; Glaeser, H.; Becquemont, L.; Klotz, U.; Gupta, S. K.; Fromm, M. F. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 645–650. (b) Srinivasan, K. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 735–748.
- (a) Volak, L. P.; Chirmai, S.; Cashman, J. R.; Court, M. H. *Drug Metab. Dispos.* **2008**, *36*, 1594–1605. No significant effect of curcumin on the expression of P450 or PgP was, however, reported. (b) Graber-Maier, A.; Berger-Büter, K.; Aeschlimann, J.; Bittle, C.; Kreuter, M.; Drewe, J.; Gutmann, H. *Planta Med.* **2010** in press. (c) Price, R. J.; Scott, M. P.; Giddings, A. M.; Walters, D. G.; Stierum, R. H.; Meredith, C.; Lake, B. G. *Xenobiotica* **2008**, *38*, 574–586.
- For sake of clarity, throughout the text curcumin refers to monomolecular **1a**, while the mixture of the three curcuminoids will be referred to as natural curcumin or curcuminoids.
- Jeffrey, B.; Fritz, M.; Brender, J. F.; Smith, P. E. S.; Lee, D.-K.; Ramamoorthy, A. *J. Am. Chem. Soc.* **2010**, *131*, 4490–4498.
- Semalty, A.; Semalty, M.; Rawat, M. S.; Franceschi, F. *Fitoterapia* **2010**, *81*, 306–314.
- Giori A.; Franceschi F. *Eur. Pat. Appl.* EP1837030, 2006.
- Marczylo, T. H.; Vershoyle, R. D.; Cooke, D. N.; Morazzoni, P.; Steward, W. P.; Gescher, A. J. *Cancer Chemother. Pharmacol.* **2007**, *60*, 171–177.
- Jurenka, J. S. *Altern. Med. Rev.* **2009**, *14*, 141–153. A direct comparison between a clinical dosage of curcumin as Meriva and the same amount of uncomplexed curcumin was not possible because uncomplexed curcumin does not produce consistently detectable plasma concentrations in humans at dosages lower than 2 g.<sup>8</sup> On the other hand, comparison of Meriva with very high dosages of uncomplexed curcumin would be biased by saturation effects and by the nonlinearity of its absorption,<sup>8</sup> affording unrealistic values of improved absorption. The 1.8 g reference dosage is a compromise between detectability and clinical activity of uncomplexed curcumin. Satooskar, R. R.; Shah, S. J.; Shenoy, S. G. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1986**, *24*, 651–654. Kuptniratsaikul, V.; Thanakhumtorn, S.; Chinswangwatanakul, P.; Wattanamongkornsil, L.; Thamlikitkul, V. *J. Compl. Altern. Med.* **2009**, *15*, 891–897. It should be pointed out that the published clinical trials on curcumin are small, often nonrandomized, and essentially open-label and that some of them gave negative results, even with higher dosages (4 g/day) of curcumin than those we have used as reference (see ref 12).
- (a) Belcaro, G.; Cesarone, M. R.; Dugall, M.; Pellegrini, L.; Ledda, A.; Grossi, M. G.; Togni, S.; Appendino, G. *Panminerva Med.* **2010**, *52* (Suppl. 1), 55S–62S. (b) Allegri, P.; Mastromarino, A.; Neri, P. *Clin. Ophthalmol.* **2010**, *4*, 41–46. (c) Belcaro, G.; Cesarone, M. R.; Dugall, M.; Pellegrini, L.; Ledda, A.; Grossi, M. G.; Togni, S.; Appendino, G. *Altern. Med. Rev.* **2010**, *15*, 337–344.
- The rate of absorption of curcuminoids increased with the dosage, and the first time point (2 h) for demethoxycurcumin (**1b**) and bisdemethoxycurcumin (**1c**) at the high dosage of Meriva was also the peak concentration. It is, therefore, possible that the AUC of these compounds might, actually, be underestimated at this dosage, with the effect of lecithin formulation being overall higher than reported. We are grateful to one reviewer for bringing this issue to our attention. The effect of dosage on the rate of absorption is, undoubtedly, intriguing and well worth further investigation.
- In the case of flax lignans, it is, however, the *para*-oxygenated function that is reductively removed by gut microorganisms. Lampe, J. W. *J. Nutr.* **2003**, *133* (Suppl. 3), 956S–964S.
- (a) Aura, A. M. *Phytochem. Rev.* **2008**, *7*, 407–429. (b) Possemiers, S.; Bolca, S.; Verstraete, W.; Heyerick, A. *Fitoterapia* **2011**, *82*, 45–58.
- Crozier, A.; Jaganath, I. B.; Clifford, M. N. *Nat. Prod. Rep.* **2009**, *26*, 1001–1043.
- (a) Zhang, L.; Wu, C.; Zhao, S.; Yuan, D.; Lian, G.; Wang, X.; Wang, L.; Yang, J. *Int. Immunopharmacol.* **2010**, *10*, 331–338. (b) Luthra, P. M.; Kumar, R.; Prakash, A. *Biochem. Biophys. Res. Commun.* **2009**, *384*, 420–425. (c) Ahmed, T.; Gilani, A. H. *Pharmacol., Biochem. Behav.* **2009**, *91*, 554–559. (d) Yodkeere, S.; Chaiwangyen, W.; Garbisa, S.;

Limtrakul, P. *J. Nutr. Biochem.* **2009**, *20*, 87–95. (e) Zhang, L. J.; Wu, C. F.; Meng, X. L.; Yuan, D.; Cai, X. D.; Wang, Q. L.; Yang, J. Y. *Neurosci. Lett.* **2008**, *447*, 48–53.

(27) Interestingly, turmeric is mainly consumed in fatty dishes (Duke, J. A. *CRC Handbook of Medicinal Spices*; CRC Press: Boca Raton, FL, 2003; pp 137–144), potentially increasing the bioavailability of “dietary” curcumin.

(28) (a) Roycik, M. D.; Fang, X.; Sang, Q. X. *Curr. Pharm. Des.* **2009**, *15*, 1295–1308. (b) Nakayama, S. K. *Methods Enzymol.* **2005**, *400*, 263–272.

(29) Fu, S.; Kurzrock, R. *Cancer* **2010**, *116*, 4670–4676.

(30) *Helix pomatia*  $\beta$ -glucuronidase has also sulfatase activity and is routinely used for the complete hydrolysis of phase-2 conjugates of phenolic compounds. Voelkel, W.; Kiranoglu, M.; Fromme, H. *Environ. Res.* **2011**, *111*, 143–148.

(31) Liu, A.; Lou, H.; Zhao, L.; Fan, P. *J. Pharm. Biomed. Anal.* **2006**, *40*, 720–727.