

Prolonged Biologically Active Colonic Tissue Levels of Curcumin Achieved After Oral Administration—A Clinical Pilot Study Including Assessment of Patient Acceptability

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Abstract

Curcumin, the main constituent of turmeric, is suspected to possess cancer chemopreventive properties. Pharmacokinetic and pharmacodynamic parameters have been reported, but few data exist describing whether methodologies are suitably robust for curcuminoid detection in colonic biopsy specimens. Information on the acceptability of prolonged administration of daily curcumin is not available. This is of vital importance to implement chemoprevention strategies. This study aimed to quantify levels of curcuminoids in colorectal mucosa of patients undergoing colorectal endoscopy or surgical resection and to obtain information on the acceptability and compliance with daily curcumin. Curcumin C3 complex (2.35 g) was administered to patients once daily for 14 days before endoscopic biopsy or colonic resection. Safety and tolerance were monitored. Analysis of curcuminoids in plasma, urine, and colonic mucosa was conducted by ultraperformance liquid chromatography (UPLC)-UV with characterization by liquid chromatography/tandem mass spectrometry (LC/MS-MS). Twenty-four of 26 patients commencing curcumin completed the course. Six patients reported mild gastrointestinal adverse events. Curcuminoids were detectable in nine of 24 plasma samples, 24 of 24 urine samples, and in the colonic mucosa of all 23 biopsied participants. Mean tissue levels were 48.4 µg/g (127.8 nmol/g) of parent curcuminoids. The major conjugate, curcumin glucuronide, was detectable in 29 of 35 biopsies. High levels of topical curcumin persisted in the mucosa for up to 40 hours postadministration. Sixteen participants (67%) stated that they would take curcumin long-term should it be of proven benefit. In summary, pharmacologically active levels of curcumin were recovered from colonic mucosa. The regimen used here seems safe, and patients support its use in long-term trials. *Cancer Prev Res*; 6(2); 119–28. ©2012 AACR.

Introduction

Curcumin, the major constituent of the spice turmeric, has been the subject of increasing interest as having the potential to prevent and treat colorectal cancer (1) and may be of benefit in cardiovascular (2) and Alzheimer's disease (3). It has shown chemopreventive activity in a variety of *in vitro* cell-based systems and *in vivo* preclinical models (4–6). In the *Apc^{Min}* mouse model of inherited colorectal cancer (7), dietary curcumin reduced adenoma formation by 39%

to 64% (8, 9), decreased expression of COX-2 and endogenous DNA damage in adenoma tissue (10) and ameliorated levels of inflammatory markers interleukin-1b and chemokine ligand 2 within intestinal polyps (11). In clinical studies, curcumin has been administered safely at doses of up to 12 g daily over 3 months (12). Curcumin and related species (collectively known as curcuminoids) have been characterized and quantified in plasma as well as colorectal and liver tissues from patients with cancer receiving 1.8 g of daily curcumin (1, 13–15), whereas analogous studies have been conducted in healthy volunteers taking 12 g daily (16). The lower dose produced colonic tissue concentrations of curcumin of an order of magnitude associated with pharmacologic effects, both in cells *in vitro* and in rodents *in vivo* (17). However, it is unclear from these investigations how much of the curcumin recovered from the gut mucosa reflects material loosely adherent to the mucosal surface, as compared with agent absorbed into the tissue, which may elicit pharmacologic activity.

The ultimate goal of translational research using curcumin as a chemopreventive agent for colorectal cancer is to establish an optimal dose, schedule, and formulation for its

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administration to individuals at high risk of developing this disease. Unfortunately, information relating dose, schedules, and formulations to achieving relevant target tissue levels and clinical efficacy is currently incomplete. Thus, the aim of the study described here was to quantify levels of curcumin in normal colorectal mucosa in patients undergoing colorectal endoscopy or cancer resection. These individuals received the curcumin formulation "Curcumin C3 complex" (Sabinsa), which contains small amounts (combined total ~20%) of desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC) in addition to curcumin. The hypothesis tested was that most of the curcuminoids recovered from the gut tissue were absorbed into the mucosa, and thus capable of eliciting direct pharmacologic effect. Curcuminoids were measured using an ultraperformance liquid chromatography (UPLC)-UV method developed in our laboratory (18) with the identity of UV-detected curcuminoids confirmed with tandem mass spectrometry (MS-MS). Tissue levels could be compared with those known to elicit benefit in preclinical models.

Curcumin was chosen as having the potential to combine many of the suggested mechanisms of aspirin and nonsteroidal anti-inflammatory agents used in chemoprevention (19) but to have the potential for better patient compliance as it seems to lack serious toxicity. Little is known about possible barriers to patient uptake and compliance in the context of chemoprevention, but this is a crucial issue when developing agents for clinical evaluation. Therefore, an additional component of this trial was to assess in a preliminary fashion the attitudes of this patient population toward curcumin and the experience of its use through the use of a questionnaire.

Materials and Methods

Investigational medicinal product

Standardized turmeric extract "Curcumin C3 complex," was provided by Sabinsa Corporation and encapsulated into gelatine capsules (Nova Laboratories) under conditions of Good Manufacturing Practice. Each capsule contained 470 mg of Curcumin C3 complex consisting of 80% curcumin and 20% DMC/BDMC.

Patients

This pilot study (EUDRACT-2007-001971-13) was sponsored by the University Hospitals of Leicester (UHL) Trust (Leicester, United Kingdom) and conducted at UHL and St Mark's Hospitals (SMH; Harrow, United Kingdom). The study was approved by the Northern and Yorkshire Research Ethics Committee (United Kingdom) and conducted in accordance with the Declaration of Helsinki and guidelines on Good Clinical Practices. Twenty-eight patients (20 awaiting lower gastrointestinal endoscopy and 8 scheduled for colorectal resection of primary disease) meeting the following criteria were recruited: positive fecal occult blood as part of colorectal screening program, awaiting diagnostic or surveillance endoscopy or diagnosis of colorectal cancer; age 18 years or older; provide informed

consent and comply with the protocol; reliable contraception if premenopausal female; no history in the past year of gastroduodenal ulcer; no significant medical or psychiatric problems; no use of investigational agents within the last 3 months; and no prior pelvic radiation. Nonsteroidal anti-inflammatory drug (NSAID) use was recorded and 3 patients completing the study took these regularly. Two participants who routinely took 75 mg oral daily aspirin continued to take their prescription during the trial. A third patient stopped taking aspirin for 2 weeks in anticipation of major surgery.

Study design

This was a pilot study administering 5×470 mg capsules (total 2.35 g curcuminoids) of daily oral Curcumin C3 complex for 14 days. This dose was chosen on the basis of the following 3 considerations: (i) previous trials at this center and elsewhere showed the safety and tolerability of this dose level when administered daily for up to 4 months. The dose was similar to that used in a study involving 4 patients with colorectal cancer, in whom 1.8 g curcumin resulted in a measurable pharmacodynamic change, that is, a reduction of malondialdehyde-DNA adduct levels in colorectal neoplastic tissue, compared with preadministration levels in biopsy samples (14). (ii) In a preclinical study in the *Apc^{Min+}* mouse model of gastrointestinal carcinogenesis (9), the minimal (dietary) dose which decreased adenoma number was 0.2% in the diet, translating into approximately 1.8 g per 80 kg human per day, when extrapolated on the basis of body surface area. (iii) The daily capsule number was compatible with patient acceptability. Capsules were taken once daily with food at the same time each day, with the final dose taken the day before the procedure. Plasma samples were obtained before curcumin ingestion and on the day of the procedure. A 24-hour urine collection was obtained between the last 2 doses. Tissue was collected from endoscopy or surgery scheduled for the day after the final dose of curcumin. The summary of enrollment procedures and participant movement through the study is shown in the CONSORT chart in Fig. 1. Participant compliance and the development of adverse events (AE) were monitored using a daily diary, which was supported by nonvalidated pre- and posttrial questionnaires that reported the thoughts and experiences of participants on curcumin. Adverse events were recorded according to Common Terminology Criteria Adverse Event reporting system version 3 (CTC-AE V3; ref. 20). The primary objective was to determine levels of curcumin and its metabolites achieved in normal colorectal tissue of patients following a 14-day course. Secondary objectives were to assess the practicality, acceptability, and safety of individuals taking curcumin.

Sample collection

Plasma samples were obtained between 6.5 and 35.5 hours after curcumin dose (median 24.5 hours). Plasma and urine were obtained from 24 participants. Four biopsy samples were taken from healthy mucosa at endoscopy

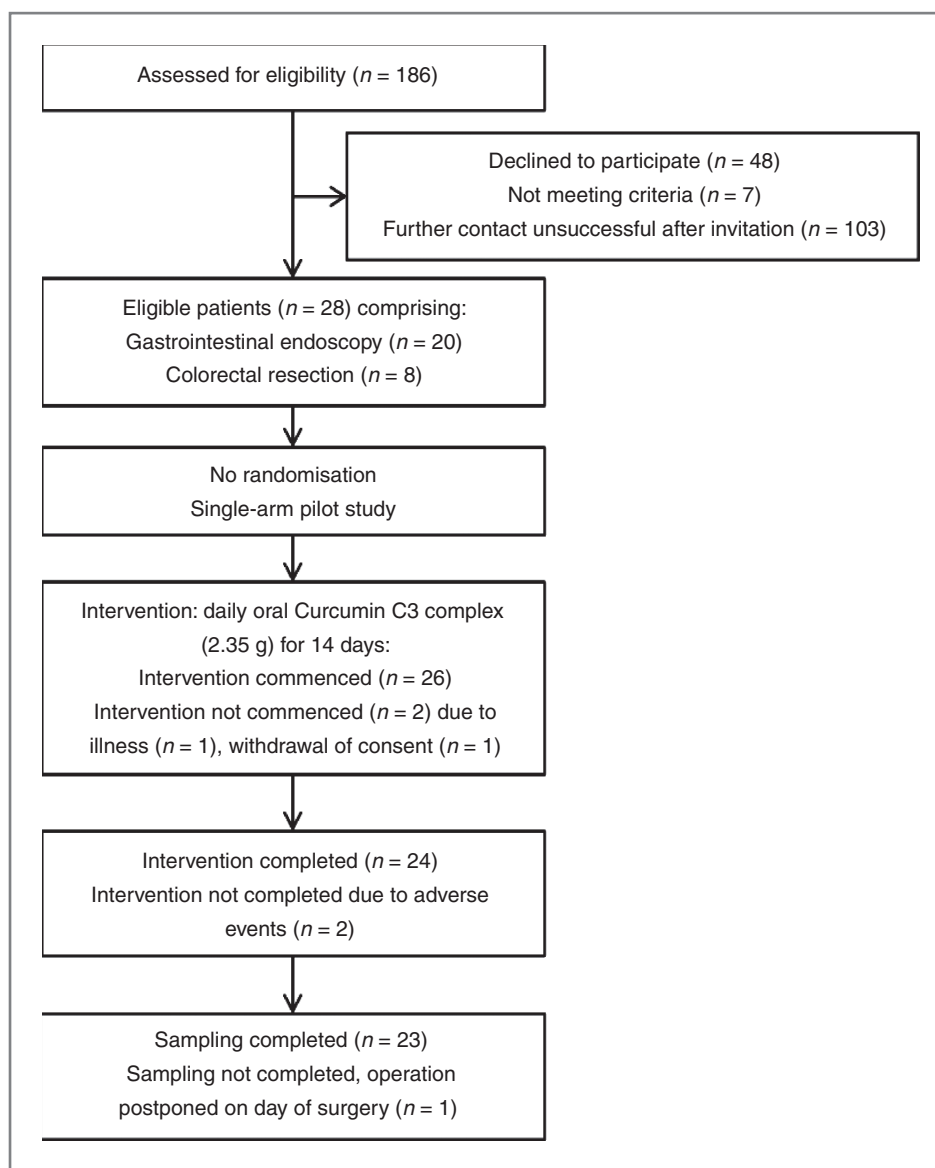


Figure 1. CONSORT chart for this study.

using cold forceps. For patients with colorectal cancer, tissue that was surplus to diagnostic requirements was sampled. Complete bowel preparation with large volume osmotic laxative was used before colonoscopic examination and a phosphate enema was used before sigmoidoscopy. Preoperative bowel preparation for patients undergoing left-sided resection consisted of a 72 hours low-residue diet followed by a phosphate enema. Colonic mucosal biopsies were obtained from 23 participants (sampling time postdose: 14–39.5 hours, median 26.5 hours). All samples were stored at -80°C until analysis.

Materials and reagents

Acetonitrile, ammonium acetate, methanol, acetone, and organophosphoric acid were purchased from Fisher Scientific. Glacial acetic acid, dimethylsulfoxide, potassium chloride (KCl), and quercetin were from Sigma and S9 human liver micro-

somes with uridine 5'-diphospho-glucuronic acid (UDPGA) cofactor solutions A and B were from Becton Dickinson.

Curcuminoid sulfates were chemically synthesized in-house (21) using sulfur trioxide–dimethylformaldehyde (DMF) complex (Sigma). Curcumin C3 complex (200 mg) was dissolved in pyridine (1.25 mL) and DMF (3.75 mL) then sulfur trioxide–DMF complex (166 mg) was added to the solution, which was incubated for 4 hours at 37°C . Sodium bicarbonate (116 mg) was then added and TLC was carried out for crude separation of new compounds supported by MS-MS. The reaction mixture was filtered and dried *in vacuo*. The crude reaction mixture was purified by flash column chromatography using silica (silica 60) and ethanol/dichloromethane (1:9 \rightarrow 4:6 \rightarrow 7:3) as mobile phase. Pooled fractions were dried, redissolved in methanol, and filtered before precise analysis by UPLC-UV and liquid chromatography/tandem mass spectrometry (LC/

MS-MS) by the method described later. The dominant curcumin monosulfates eluted after the parent compounds, at approximately 12 minutes (Fig. 2A). The retention time of curcumin parent compounds ranged from 10.2 to 10.7 minutes.

Curcumin glucuronides were biosynthesized *in vitro* following the previous method (18) with the following modifications: Curcumin C3 complex (2 μ L of 2.5 mmol/L stock = 1.84 μ g) was incubated for 30 minutes at 37°C with S9 human liver microsome preparation (12.5 μ L of 20 mg/mL; BD Biosciences) and UDPGA cofactor solutions A (20 μ L) and B (50 μ L) made up to 250 μ L with water. The reaction was stopped by the addition of ice-cold acetone (250 μ L), and the mixture was incubated at -20°C for 15 minutes. Protein was removed by centrifugation (6,000 \times g, 5 minutes) and the supernatant analyzed as described later. The dominant curcumin monoglucuronides eluted before the parent compounds, at approximately 7.5 minutes (Fig. 2A).

Sample preparation and analysis

Extractions were conducted using methods previously validated in our laboratory (18) and adjusted to cater for

minute mucosal biopsies and 1 mL plasma and urine samples. In brief, 1 mL of plasma or urine was diluted with 1 mL water and acidified with *o*-phosphoric acid (0.5% final concentration). The acidified samples (2 mL) were loaded onto 2 1 cm³ Oasis HLB cartridges (Waters), washed with acidified methanol [methanol:water:glacial acetic acid (25:25:1)] preconditioned with 1 mL water and then 1 mL methanol and eluted with acidified methanol (2% glacial acetic acid). The eluant was evaporated to dryness at 45°C under nitrogen, and the combined residue resuspended in 40 μ L of methanol:glacial acetic acid (95:5). A final centrifugation was conducted (16,000 \times g, 10 minutes) and the volume injected on to the column was 20 μ L. The parent curcuminoids and curcuminoid glucuronides could be recovered by this method, with extraction efficiencies of more than 60% and 25% respectively, curcumin sulfates were recovered at only trace levels (<1%) in the eluant obtained from HLB cartridges.

To quantify the levels of curcumin adherent to the mucosa, homogenization was conducted both with and without first extensively washing the biopsies. The assay was

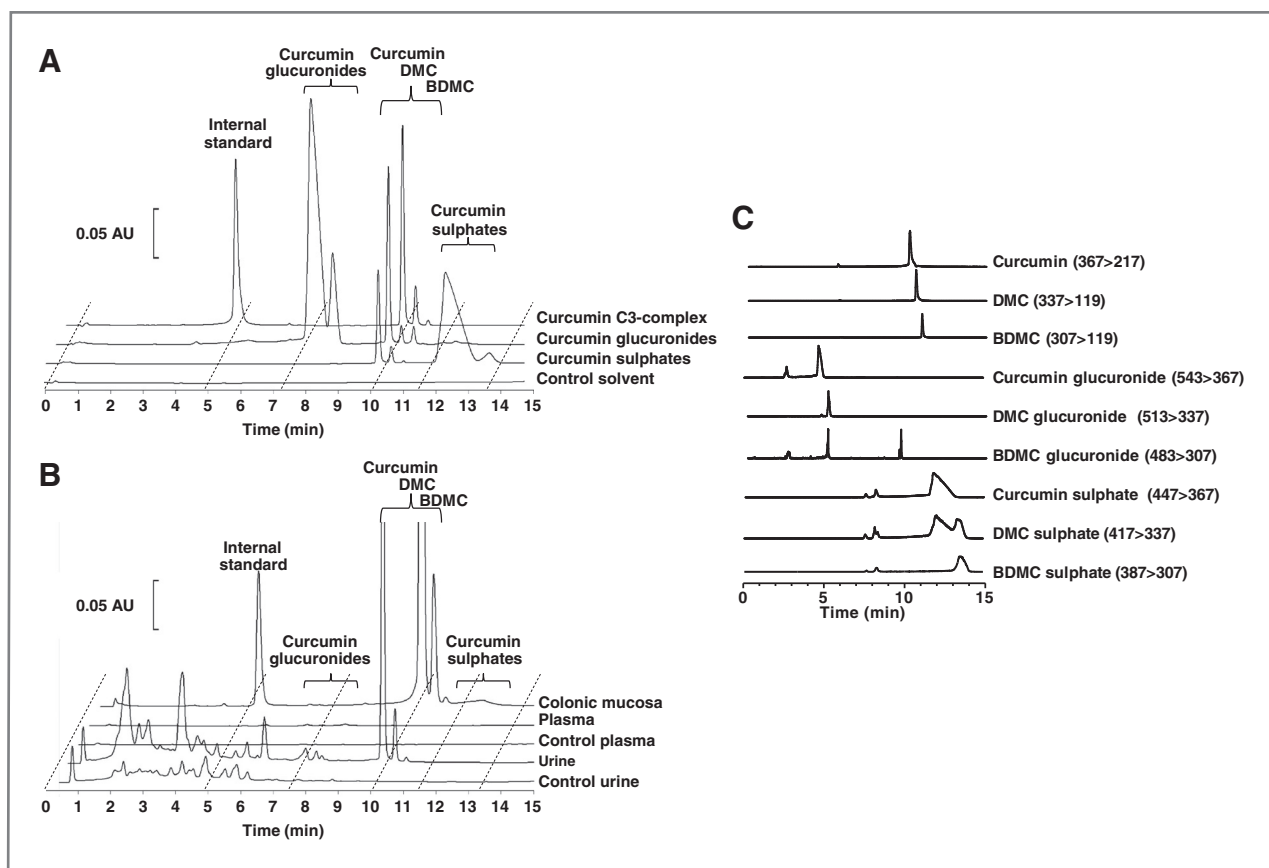


Figure 2. UPLC-UV chromatography of (A) standards of Curcumin C3 complex, biosynthetic curcumin glucuronides, chemically synthesized curcumin sulfates and control solvent, and (B) extracts of colonic mucosa, plasma, and urine from patients who had received oral Curcumin C3 complex (2.35 g daily for 14 days) and plasma and urine from individuals who had yet to receive the agent (control biomatrix). Plasma and tissue biomatrices were obtained approximately 24 hours after the final dose of curcumin. Urine was collected continuously for the 24 hours between the last 2 doses. C, LC/MS-MS of curcuminoids present in curcumin and metabolite standards obtained in SRM mode with the *m/z* transitions for each analyte in brackets.

validated with rodent and human colonic mucosa. Preliminary experiments using a small surplus of tissue showed that surface levels of curcumin reached a steady state after 7 separate washes, therefore, one of each set of biopsies was washed 7 times in 1.15% KCl. All samples were then homogenized in 1.15% KCl, and quercetin (150 µg/mL) added as an internal standard before extraction with acidified acetone (10% glacial acetic acid). Solid matter was removed by centrifugation (16,000 × *g*, 10 minutes), the supernatant evaporated to dryness at 45°C under nitrogen and the residue redissolved in 40 µL of methanol:glacial acetic acid (90:10). A final centrifugation was conducted (16,000 × *g*, 10 minutes). Washes obtained from the tissue samples were also processed by this extraction method.

Curcuminoids were separated and quantified using an Acquity UPLC system (Waters) with a BEH RP18 (2.1 × 100 mm, 1.7 µm) column (Waters) at 35°C, as described previously (18). In brief, the mobile phase A consisted of 5% aqueous acetonitrile adjusted to pH 3.5 with acetic acid, whereas mobile phase B was 100% acetonitrile. The elution gradient at 0 minutes was 15% flow rate B at 0.54 mL/min; at 10 minutes, 45% B at 0.6 mL/min; at 14 minutes, 42% B at 0.6 mL/min; and at 16 minutes, 100% B at 0.85 mL/min. The column was then washed with 100% B for a further 2 minutes at 0.85 mL/min before reequilibrating. The eluant was monitored at 426 nm. The limits of detection (LOD) and quantification (LOQ) for curcumin were consistent with previously published values of 0.5 ng and 1 ng on column. The LOD and LOQ for both plasma and urine were 1.25 and 2.5 ng/mL and for tissue, 125 and 500 ng/g, respectively.

Identification and quantification of curcumin metabolites in colonic tissue

LC/MS-MS characterization was conducted by coupling a Waters Xevo TQ to an Acquity UPLC using a 2:1 T-piece after the UV detector. LC/MS-MS was carried out with negative polarity and conditions consisting of capillary voltage 3 kV, cone voltage 30 V, source temperature 150°C, desolvation temperature 500°C, desolvation gas flow 1,200 L/h, and collision energy 20 eV. Dried supernatant was redissolved in methanol:water (1:1).

Curcuminoid sulfates and glucuronides were analyzed by UPLC-UV (Fig. 2A and B) and MS-MS (Fig. 2C) using selected reaction monitoring (SRM), which enabled identification of the parent compounds and major metabolites based on characteristic *m/z* transitions used previously (18). The *m/z* transitions for the dominant and most abundant compounds are shown in Fig. 2C with the corresponding LC/MS-MS chromatogram. Levels of curcumin glucuronides and sulfates were estimated through the use of the curcumin standard curve.

Statistical analysis

Statistical analysis was conducted with SPSS version 18 (Windows XP). *P* values less than 0.05 were considered significant. Data are presented as mean, range, and with

SEM. Comparison of paired postintervention tissues was made by the nonparametric Wilcoxon–Mann–Whitney test because of the small sample size. Correlations within tissue sample sets were analyzed using the Spearman coefficient with 2-tailed significance testing.

Results

Participants

Twenty-eight patients were recruited into this study (for demographics see Table 1), with 18 recruited at the UHL and 10 at SMH. Of the 28 participants, 26 commenced curcumin, 24 completed the prescribed course, and 23 met full-sampling requirements. Two participants did not start curcumin, 1 withdrew consent, and the other dropped out due to illness. Two participants withdrew after the course began due to adverse events. One participant completed the full course of curcumin, but the scheduled procedure was postponed and tissue was not obtained. Samples consisted of 12 pairs of matched left- and right-sided colonoscopic biopsies in duplicate, 6 quadruplicate left-sided sigmoidoscopic biopsies, and 5 quadruplicate full-thickness surgical biopsies from 4 left-sided and 1 right-sided colonic resections.

Participant's attitudes to curcumin

Pretrial questionnaires assessing the patients' knowledge of curcumin revealed that 21 (81%) participants had previously heard of turmeric but only 14 (54%) had ever knowingly used it in their diet. Only 1 participant had previously used turmeric or curcumin as a health food supplement although 10 people reported using other food supplements. Most (85%) had planned when best to take the capsules and how to integrate them with existing

Table 1. Demographics for the 28 recruited participants

Mean age, y	57.5 (35–82)
Gender	
Male	13
Female	15
Mean BMI, kg/m ²	31.2 (15.5–48.3)
WHO performance status	
0	22
1	6
Procedure (number completed/ number planned)	
Sigmoidoscopy	6/7
Colonoscopy	12/13
Right hemicolectomy	1/2
Left hemicolectomy or rectal resection	4/6
Participants completing curcumin course	24/28
Participants completing all procedures	23/24

Abbreviations: BMI, body mass index; F, female; M, male.
NOTE: Values in brackets show range.

medication, with the majority preferring a morning schedule. Three patients felt they may have problems remembering to take regular capsules, however, upon conclusion of the trial none reported this concern. Thirty-seven percent of responders felt they would not want to miss a dose of curcumin, and this number significantly increased ($P = 0.001$) to 91% by the end of the study. Although 46% of patients agreed they were worried that curcumin may produce unpleasant side effects at the start of the trial, 91% disagreed by the end. Upon completion of the course, 3 patients felt that 5 capsules may present a barrier to compliance, and 4 reported that the capsule size (00) used here may be problematic. Two patients commencing the course failed to finish it. Two thirds of patients would recommend curcumin as a long-term chemopreventive agent if proven to be effective.

Safety of curcumin

In total, 13 adverse events (NCI-CTC v3.0, grades 1–2) possibly or probably attributable to curcumin were recorded in 6 participants, all of which were gastrointestinal in nature (Table 2). Comparison of matched baseline (pre-curcumin) with posttrial questionnaires revealed a decrease in all self-reported gastrointestinal disturbances (constipation, diarrhea, flatulence, incontinence, and abdominal pain) following 2 weeks of curcumin administration (Table 3). There were no differences in the overall general health and activity of participants during the trial (data not shown). There were no serious adverse events (SAE) attributable to curcumin. Of the 24 participants that completed the course, 16 (67%) stated that they would regularly take curcumin as an adjunct to current medication if it were shown to be of benefit. Importantly, the number of patients stating the size or number of capsules to be swallowed may present a barrier to compliance before they started the course actually reduced slightly by the end of the trial.

Levels of curcuminoids in colonic mucosa

Curcuminoid concentrations were measured by UPLC-UV (Fig. 2B) in colorectal tissue biopsies ranging in mass

Table 2. Summary of adverse events deemed possibly or probably attributable to curcumin

Adverse event	Number of events	Grade	Number of patients
Total gastrointestinal events	13	1, 2	6
Abdominal pain	3	1, 2	3
Bloating	1	2	1
Diarrhea	3	1	3
Dyspepsia	1	1	1
Flatulence	1	1	1
Nausea	2	1, 2	2
Vomiting	2	1, 2	2

Table 3. Pre- and postcurcumin self-reported questionnaire assessments from 24 participants that completed the course of curcumin

	Responses (% participants)	
	Baseline	Posttrial
Thought capsule size would be difficult to swallow	26	17
Thought 5 capsules at once would be difficult to swallow	19	13
Experienced constipation	36	13
Experienced diarrhea	52	38
Experienced wind	70	58
Experienced abdominal pain	37	21
Experienced bowel incontinence	22	12

NOTE: Baseline responses refer to the period 3 months before commencing curcumin. Posttrial responses were completed at the end of the 2-week curcumin course and refer the period of administration.

from 0.5 to 6.75 mg (median 2.9 ± 0.25 mg). Parent compounds that eluted between 10.2 and 10.7 minutes, were present in the mucosa of every patient. To explore how much of the measured curcuminoids were loosely bound to the mucosa and how much had been absorbed into the tissue, volume-matched biopsy samples were subjected to a series of KCl washes. After 7 washes, curcumin levels detected in the wash buffer were typically below the LOQ (Fig. 3). Levels of curcuminoids remaining in washed tissues were between 17% (surgical) and 74% (endoscopic) of those measured in unwashed samples, with mean washed tissue levels of 18.85 ± 6.8 $\mu\text{g/g}$, 3.6 ± 1.4 $\mu\text{g/g}$, and 1.6 ± 1.0 $\mu\text{g/g}$ for curcumin, DMC, and BDMC, respectively (Table 4). There was a relationship between the curcumin levels of unwashed left-sided and right-sided biopsies taken from the same patient ($n = 12$ pairs; Spearman correlation coefficient 0.636; $P = 0.035$). The level of curcumin measured in individual unwashed samples seemed to reflect the amount found in the tissue from the same patient after washing ($n = 35$ pairs; Spearman correlation coefficient 0.421; $P = 0.013$). Unwashed tissue levels of curcumin ranged from 0.7 $\mu\text{g/g}$ (from a left-sided colonoscopic biopsy) to 681 $\mu\text{g/g}$ (from a flexible sigmoidoscopy biopsy), with a mean of 48.4 $\mu\text{g/g}$ (Table 4). In paired right- and left-sided endoscopic biopsy samples, mean prewash curcumin levels were 35.4 and 9.0 $\mu\text{g/g}$, respectively, and significantly different ($P = 0.021$). In contrast, there was no difference in levels after tissue had been washed ($P = 0.347$). DMC and BDMC were detectable in 27 of 35 mucosal samples (range, 0.2–83.9 $\mu\text{g/g}$; mean 7.1 $\mu\text{g/g}$) and in 15 of 35 (range, 0.03–2.8 $\mu\text{g/g}$; mean 0.7 $\mu\text{g/g}$) mucosal samples, respectively. Curcumin glucuronides (eluting between 7.7 and 8.5 minutes), which constituted the most abundant conjugates, were detected in 19 of 23 patients at levels that were

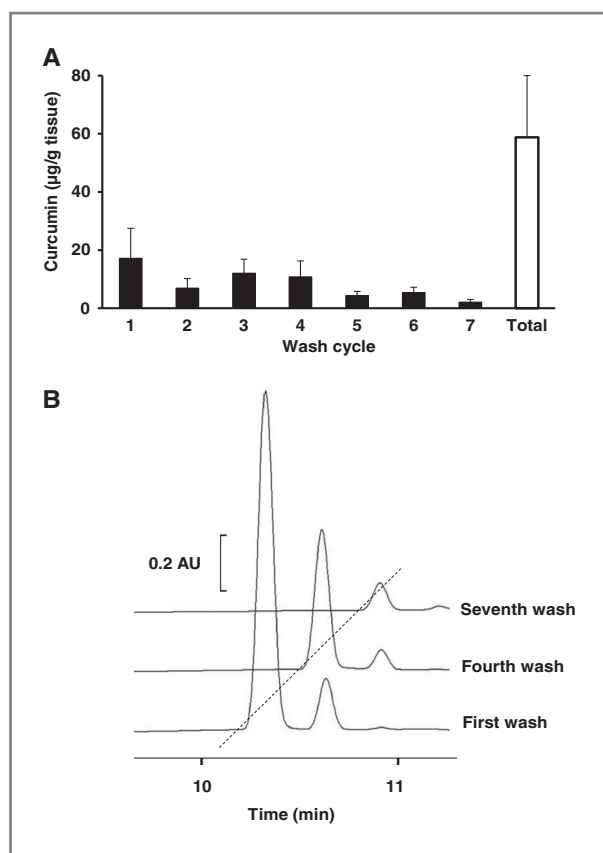


Figure 3. A, mean curcumin (expressed as $\mu\text{g/g}$ of tissue) present in consecutive washes from 1 right-sided, 4 left-sided, 6 sigmoidoscopy, 12 paired right-sided, and 12 paired left-sided biopsy samples. Black bars show mean data from each wash cycle. White bar shows mean cumulative curcumin removed during wash cycles ($n = 35$), \pm SEM. B, UPLC-UV chromatography of extracts from serial washes (using 1.15% KCl) conducted on colonic mucosa to remove surface Curcumin C3 complex. Up to 7 consecutive washes were conducted until excess surface curcumin had been removed and surface levels were at a steady state.

approximately one tenth of those seen for curcumin (range, 0.33–24.7 μg curcumin equivalents/g; mean 4.5 $\mu\text{g/g}$). Peaks with retention times corresponding to those of cur-

cuminoid sulfates were identified in the mucosa of 14 of 23 patients (range, 0.11–31.1 $\mu\text{g/g}$; mean 6.1 $\mu\text{g/g}$).

Levels of curcuminoids in plasma

Curcuminoids were identified in the plasma (Fig. 2B) obtained from 9 of 24 participants with parent compounds quantifiable in 4 participants (range for curcumin, 8.1–13.9 ng/mL; mean 12.2 ng/mL) and present in a further 2 at trace levels below the LOQ. Curcumin glucuronides were quantifiable in 7 participants (range, 0.33–29.4 ng curcumin equivalents/mL; mean 4.9 ng/mL) and were present in a further 8 at trace levels below the LOQ. In addition, analysis of plasma extracts from a further 15 participants produced peaks with retention times consistent with those of the parent compounds, but levels were below the LOD.

Levels of curcuminoids in urine

Curcuminoids were identified in the 24-hour urine collections (Fig. 2B) obtained from all participants with parent compounds quantifiable in 12 participants (range for curcumin, 0.67–503.3 ng/mL; mean 27.0 ng/mL) and present in a further 8 at trace levels below the LOQ. Analysis of urine extracts from 4 participants revealed peaks with retention times of the parent compounds, but levels were below the LOD. Curcumin glucuronides were quantifiable in all urine samples (range, 2.93–64.8 ng curcumin equivalents/mL; mean 17.6 ng/mL).

Discussion

This is one of the first studies to report the analysis of tissue curcumin levels in patients receiving repeat daily dosing. It is the first to assess the patient attitudes toward taking diet-derived chemopreventive agents.

Curcumin-induced adverse events observed in clinical trials in patients with cancer have been, almost exclusively, mild and self-resolving effects on the gastrointestinal tract (22, 23). Tolerability and acceptability of curcumin seems to decrease with increasing dose and also seems to be related to the number and size of capsules required to deliver high doses (22) although consistency of adverse event reporting varies considerably between countries (24). In the study

Table 4. Mucosal levels of parent compounds curcumin, DMC, and BDMC (mean concentration $\mu\text{g/g}$, \pm SEM) from colonic biopsies pre- and postwash cycles, quantified by UPLC-UV

Sample group	n	Curcumin		DMC		BDMC	
		Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
All biopsies	35	48.4 \pm 20.9	18.85 \pm 6.8	7.07 \pm 3.24	3.55 \pm 1.38	0.71 \pm 0.20	1.59 \pm 1.00
Surgical biopsies	5	75.32 \pm 39.71	12.50 \pm 3.07	10.42 \pm 6.52	1.27 \pm 0.42	1.18 \pm 0.15	0.21 \pm 0.04
Endoscopy biopsies	30	42.31 \pm 23.68	19.91 \pm 7.87	6.33 \pm 3.75	4.03 \pm 1.69	0.59 \pm 0.24	1.86 \pm 1.19

NOTE: There was a relationship between the curcumin levels of unwashed left-sided and right-sided biopsies taken from the same patient ($n = 12$ pairs, Spearman correlation coefficient 0.636, $P = 0.035$). The level of curcumin measured in each unwashed sample seemed to reflect the amount found in the tissue from the same patient after washing. ($n = 35$ pairs, Spearman correlation coefficient 0.421, $P = 0.013$.)

described here, 24 of 26 patients (92.3%) completed a 14-day course of daily oral curcuminoids (2.35 g). In contrast to the accrued adverse event data, only 1 participant felt that this regimen produced unpleasant side effects. The symptoms reported as adverse events, predominantly abdominal pain, bloating, nausea, and diarrhea may have been part of normal gastrointestinal behavior within this patient cohort. It is therefore conceivable that attribution of gastrointestinal adverse events in patients to the doses of curcumin described in this, and several past studies of similar patient cohorts (1, 15), may not accurately reflect the side-effect profiles of curcumin. Moreover, comparison of pre- and posttrial questionnaire data actually suggests a reduction in patient-reported bowel symptoms during the trial period. If these observations are confirmed in further trials, daily doses of 2 to 3 g of curcumin may be considered safe and well tolerated even during prolonged administration.

The novel method of pharmacokinetic analysis used in this study measures tissue levels of curcumin both before and after tissue has been washed copiously with an aqueous salt solution. The majority of curcumin present in a tissue sample is probably adherent to the surface and not intracellular. Reported studies of a similar nature (1, 13) have not attempted to quantify curcumin, which is bound to the surface and details about sample-cleaning processes before analysis are lacking. Direct comparison of human gastrointestinal tissue levels of curcumin between studies is therefore difficult. Daily administration of curcumin (2 g) for 30 days (1) resulted in mean concentrations of $8.2 \pm 2.9 \mu\text{g/g}$ curcumin in rectal mucosa in 5 of 21 patients and curcumin conjugates of $5.9 \pm 2.6 \mu\text{g/g}$ in 13 patients implying that the levels of curcumin and curcumin metabolites in the remaining patients were below the LOQ. In our study, curcumin was detectable in the gastrointestinal tract of all patients recruited after receiving a similar dose of curcumin daily for 14 days. Levels of the parent compound in tissue were $48.4 \pm 20.9 \mu\text{g/g}$ without prior washing, which has been standard practice for both clinical and *in vivo* studies (9, 13, 25). Levels in unwashed tissue were 5.7-fold higher than those in the previous study (1), however, washing the tissue reduced this difference to only 2-fold. Curcumin glucuronides, the major curcumin metabolites (25, 26) were semiquantitated in our study, whereas Carroll and colleagues (1) estimated total curcumin conjugates obtained by enzymatic hydrolysis in their study. Here, the mean level of curcuminoid glucuronides, when quantifiable, was 8.4 nmol/g ($4.54 \mu\text{g}$ curcumin equivalents/g) found in 27 of 35 (77%) samples. This value is similar to the amount of total curcumin conjugates reported by Carroll and colleagues, where metabolites were not precisely characterized. Therefore, despite the differences in levels of parent compound, similar levels of conjugates would suggest that intracellular curcumin levels available for metabolism were of a similar order of magnitude.

A clear disparity in curcumin tissue levels between unwashed paired right- and left-sided biopsies was apparent. A similar discrepancy in agent level has been observed in patients with colorectal cancer who received daily oral

resveratrol (21). Consistent with the explanation given for resveratrol (21), differences in gut content and fecal liquidity between the right and left sides may be responsible. The right-sided colonic mucosa is likely to be in contact with higher concentrations of fecal curcumin than the left. Curcumin is highly lipophilic and poorly soluble in aqueous media (6, 27) and seems to persist on the colon for a considerable length of time, and may therefore provide a period of sustained action to the mucosa. Consecutive washes of mucosa in paired right-sided biopsy samples to remove loosely bound material resulted in a 74% decrease in tissue curcumin. Topical levels of curcumin were higher on the right but tissue levels did not significantly differ between the left and right sides after washing. This finding implies that curcumin is similarly absorbed on both sides of the colon, which suggests that curcumin accesses gastrointestinal tissue mainly via the systemic circulation rather than the topical route. Bowel preparation techniques varied depending on the procedure being undertaken and this is likely to have had an effect on the levels of surface curcumin, particularly in unwashed mucosa. One participant undergoing right hemi-colectomy, and therefore receiving no bowel preparation of the biopsied area, had particularly high levels of tissue ($228.3 \mu\text{g/g}$) and surface curcumin ($551.1 \mu\text{g/g}$). Mucosal samples were biopsied up to 40 hours postcurcumin administration, indicating that despite iatrogenic bowel cleansing, high gastrointestinal tissue levels are likely to persist for several days. This finding suggests that less than once daily dosing regimens might generate curcumin levels in the gastrointestinal tract sufficient for activity in long-term polyp prevention trials. Dose scheduling has considerable implications in terms of participant compliance and study cost, with intermittent dosing schedules being associated with higher adherence than daily dosing regimens (28). Intermittent dose regimens may also decrease the incidence of adverse events. However, it is increasingly likely that systemic rather than topical curcumin may well be the major source of intramucosal agent and prolonged availability of intraluminal curcumin is perhaps not so important, which is in contention with using a less than once daily dose schedule.

Pharmacokinetic studies with healthy volunteers (16, 22, 29) and patients with colorectal cancer (13) reveal curcumin has a plasma T_{max} between 1 and 4 hours, with conjugates persisting for up to 36 hours after 10 g of curcumin (16). In this study, plasma levels of parent curcumin were quantifiable in only 4 participants (mean 12.2 ng/mL) and glucuronides in 7 participants (mean 4.9 ng/mL). Detection of the parent compound would only be expected at trace levels because blood sampling was conducted on average 24 hours postdose. This is important, because if curcumin is to be investigated in long-term polyp prevention regimens, evidence of systemic accumulation and rate of clearance should be considered when optimizing prescription schedules. Carroll and colleagues (1) did not detect curcuminoids in plasma of patients receiving 2 g daily oral curcumin, however, this disparity may be a result of the 4-fold greater LOQ of their methodology. Detectable

curcuminoid or curcuminoid conjugates were observed in the urine of all participants in this study, intimating that adherence monitoring for low-dose curcumin in future trials could use urine sampling as suggested previously (29).

The questionnaire responses provide a valuable, albeit preliminary, insight into the patient perspective of long-term use of curcumin, which is vital for the optimal design of future trials. The use of dietary agents by patients is reported to be in excess of 50% (30) with a tendency to increase following a cancer diagnosis (31), although the majority of usage seems to be with vitamins and minerals rather than plant extracts (32). Forty percent of recruits to this study had used health food supplements, although only 20% had heard of curcumin as compared with 81% of the participants who were acquainted with the spice turmeric, and none of these had previously used it. It has been suggested that large or numerous capsules required to deliver higher doses may present problems with compliance (22). By the end of this trial, only 3 patients felt that 5 capsules may present a barrier to compliance and 4 reported that the capsule size (00) used here may be problematic. Only 2 patients commencing the course failed to finish it. It is promising that two thirds of patients recommended curcumin as a long-term chemopreventive agent if proven to be effective.

We acknowledge there are limitations to this study, which include the selection of a single dose instead of several dose levels, the timing of the tissue sample collection *vis-à-vis* the time of the last dose administered, the lack of a control arm, the heterogeneous nature of the patient population, and the relatively short duration of the intervention. Several of these limitations are, at least to some extent, governed by aspects of clinical care pathways optimized for patients undergoing investigation or management of cancer and the availability of a sufficient number of participants with which to complete the study.

In summary, the analytic methodology described here provides robust and sensitive quantification of curcuminoids; such methods are vital in linking pharmacokinetic effects, on an individual basis, to target tissue concentrations in trials of chemopreventive agents. Pharmacologi-

cally active levels of curcumin were recovered from the bowel mucosa even after multiple tissue washes, and there was no systemic curcumin accumulation. These findings provide further support for the safety of curcumin and its potential usefulness in long-term colorectal cancer prevention strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Carroll RE, Benya RV, Turgeon DK, Vareed S, Neuman M, Rodriguez L, et al. Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. *Cancer Prev Res* 2011;4:354–64.
- Sugawara J, Akazawa N, Miyaki A, Choi Y, Tanabe Y, Imai T, et al. Effect of endurance exercise training and curcumin intake on central arterial hemodynamics in postmenopausal women: pilot study. *Am J Hypertens* 2012;25:651–6.
- Baum L, Lam CW, Cheung SK, Kwok T, Lui V, Tsoh J, et al. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. *J Clin Psychopharmacol* 2008;28:110–3.
- Epstein J, Sanderson IR, Macdonald TT. Curcumin as a therapeutic agent: the evidence from *in vitro*, animal and human studies. *Br J Nutr* 2010;103:1545–57.
- Ravindran J, Prasad S, Aggarwal BB. Curcumin and cancer cells: how many ways can curry kill tumor cells selectively? *AAPS J* 2009;11:495–510.
- Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. *Eur J Cancer* 2005;41:1955–68.
- Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* 1990;247:322–4.
- Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, et al. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis* 2000;21:921–7.
- Perkins S, Verschoyle RD, Hill K, Parveen I, Threadgill MD, Sharma RA, et al. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. *Cancer Epidemiol Biomarkers Prev* 2002;11:535–40.
- Tunstall RG, Sharma RA, Perkins S, Sale S, Singh R, Farmer PB, et al. Cyclooxygenase-2 expression and oxidative DNA adducts in murine intestinal adenomas: modification by dietary curcumin and implications for clinical trials. *Eur J Cancer* 2006;42:415–21.

11. Murphy EA, Davis JM, McClellan JL, Gordon BT, Carmichael MD. Curcumin's effect on intestinal inflammation and tumorigenesis in the ApcMin/+ mouse. *J Interferon Cytokine Res* 2011;31:219–26.
12. Irving GR, Karmokar A, Berry DP, Brown K, Steward WP. Curcumin: the potential for efficacy in gastrointestinal diseases. *Best Pract Res Clin Gastroenterol* 2011;25:519–34.
13. Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, et al. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiol Biomarkers Prev* 2005;14:120–5.
14. Garcea G, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, et al. Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br J Cancer* 2004;90:1011–5.
15. Sharma RA, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, et al. Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. *Clin Cancer Res* 2001;7:1894–900.
16. Vareed SK, Kakarala M, Ruffin MT, Crowell JA, Normolle DP, Djuric Z, et al. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev* 2008;17:1411–7.
17. Howells LM, Moiseeva EP, Neal CP, Foreman BE, Andreadi CK, Sun YY, et al. Predicting the physiological relevance of *in vitro* cancer preventive activities of phytochemicals. *Acta Pharmacol Sin* 2007;28:1274–304.
18. Marczylo TH, Steward WP, Gescher AJ. Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. *J Agric Food Chem* 2009;57:797–803.
19. Half E, Arber N. Colon cancer: preventive agents and the present status of chemoprevention. *Expert Opin Pharmacother* 2009;10:211–9.
20. U.S. Department of Health and Human Services NCI. Common terminology criteria for adverse events (CTCAE) version 3.0; 2006. [Cited 2006]. Available from: <http://ctep.cancer.gov/>.
21. Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, et al. Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res* 2010;70:7392–9.
22. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* 2001;21:2895–900.
23. Epelbaum R, Schaffer M, Vizel B, Badmaev V, Bar-Sela G. Curcumin and gemcitabine in patients with advanced pancreatic cancer. *Nutr Cancer* 2010;62:1137–41.
24. Joelson S, Joelson IB, Wallander MA. Geographical variation in adverse event reporting rates in clinical trials. *Pharmacoepidemiol Drug Saf* 1997;6(Suppl 3):S31–5.
25. Ireson CR, Jones DJ, Orr S, Coughtrie MW, Boocock DJ, Williams ML, et al. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol Biomarkers Prev* 2002;11:105–11.
26. Ireson C, Orr S, Jones DJ, Verschoyle R, Lim CK, Luo JL, et al. Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat *in vivo*, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res* 2001;61:1058–64.
27. Tonnesen HH. Chemistry of curcumin and curcuminoids. In: Ho C-T, Lee CY, Huang M-T, editors. *Phenolic compounds in food and their effects on health I*. Washington, DC: American Chemical Society; 1992. p. 143–53.
28. Kruk ME, Schwalbe N. The relation between intermittent dosing and adherence: preliminary insights. *Clin Ther* 2006;28:1989–95.
29. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, et al. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004;10:6847–54.
30. Block G, Jensen CD, Norkus EP, Dalvi TB, Wong LG, McManus JF, et al. Usage patterns, health, and nutritional status of long-term multiple dietary supplement users: a cross-sectional study. *Nutr J* 2007;6:30.
31. Velicer CM, Ulrich CM. Vitamin and mineral supplement use among US adults after cancer diagnosis: a systematic review. *J Clin Oncol* 2008;26:665–73.
32. Miller MF, Bellizzi KM, Sufian M, Ambis AH, Goldstein MS, Ballard-Barbash R. Dietary supplement use in individuals living with cancer and other chronic conditions: a population-based study. *J Am Diet Assoc* 2008;108:483–94.